# Fructose-Conditioned Flavor-Flavor Preferences in the Rat: Dopaminergic and Opioid Substrates in the Nucleus Accumbens and Amygdala

by

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## Abstract

Fructose-Conditioned Flavor-Flavor Preferences in the Rat: Dopaminergic and Opioid Substrates in the Nucleus Accumbens and Amygdala

by Sonia Y. Bernal

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Systemic dopamine (DA) D1 (SCH23390) and D2 (raclopride) receptor antagonists reduce acquisition and expression of fructose-conditioned flavor preferences (CFP) in rats. Given DA involvement in nucleus accumbens shell (NAcS) and amygdala (AMY) in learning of food reward, the first and second aims examined whether NAcS or AMY D1 or D2 antagonism altered acquisition and expression of fructose-CFP. In expression, food-restricted rats with bilateral NAcS or AMY cannulae were trained to drink a flavored fructose (8%) and saccharin (0.2%) solution or another flavored 0.2% saccharin solution. Two-bottle tests with both flavors in saccharin solutions occurred 10 min following NAcS or AMY doses of 0, 12, 24 or 48 nmol of SCH23390 or raclopride. CFP expression following vehicle (76-77%) was significantly reduced by SCH23390 (48 nmol: NAcS, 62%; AMY, 66%) and raclopride (NAcS: 24 nmol, 63%; 48 nmol, 68%). In acquisition, rats received 12 nmol of SCH23390 (D1) or raclopride (D2) in the NAcS or AMY 10 min prior to one-bottle training sessions. Yoked controls received vehicle with limited CS intakes, whereas untreated controls were not injected or limited. Two-bottle tests revealed initial CFP in all groups that remained stable in untreated and yoked controls, but were lost over six test sessions in the AMY D1 and NAcS D1 and D2 groups. Thus, D1 and D2 receptor blockade in the NAcS and AMY significantly attenuated expression, but not initial acquisition of fructose-CFP, and hastened extinction of fructose-CFP.

Systemic naltrexone (NTX), an opioid receptor antagonist, suppressed sweet intake, but failed to affect acquisition or expression of fructose-CFP. Because opioids in the NAc and AMY are implicated in food reward, the third and fourth aims examined whether NTX in these sites altered expression of fructose-CFP. Food-restricted rats with bilateral NAc or AMY cannulae were trained and tested in identical protocols using NTX doses of 0, 1, 25 or 50 ug. Significant CFP was observed following all NTX doses in all sites. Thus, DA, but not opioids modulate flavor-flavor conditioning through a regionally-distributed limbic brain network.

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I dedicate this dissertation to my dear family for their love, patience, and support all the way through.

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# Glossary of Abbreviations

AMY – Amygdala
CFP – Conditioned Flavor preference
CS – Conditioned Stimulus
DA - Dopamine
DAMGO - D-Ala2, N-Met-Phe4, Gly-ol5-enkephalin, mu agonist
GABA – gamma-aminobutyric acid
IG - Intragastric
LH – Lateral Hypothalamus
mPFC – Medial Prefrontal Cortex
NAc – Nucleus Accumbens
NAcC - Nucleus Accumbens Core
NAcS - Nucleus Accumbens Shell
NMDA - N-methyl-D-aspartate
NTX - Naltrexone
US – Unconditioned Stimulus
Veh – vehicle treated

# CHAPTER ONE: INTRODUCTION

## SIGNIFICANCE AND SPECIFIC AIMS

During the past decade, obesity and food-related illnesses, including diabetes, have become a major problem in the United States and other countries. Statistics show that being overweight along with poor health lifestyle account for almost one third of the total number of deaths in the United States, being just the second leading cause of death after tobacco-related disease (Mokdad et al., 2004). Food consumption is vital for maintaining metabolic activity, but either insufficient or excessive caloric ingestion can cause health problems. During the past 25 years, a trend of excessive caloric intake has been observed, particularly in developed countries where cheap calorically-dense food is more readily available and accessible. Hence, it is evident that eating habits are significantly responsible for overindulgence and weight gain (Dragone, 2009). Obesity is the result of an interaction of several biological and environmental factors including genetic dispositions, prenatal experiences, postnatal nutrition, and orosensory and postingestive signals that modulate eating and satiety processes, as well as neurochemical and hormonal influences (Smith, 2009). Although both innate and situational variables contribute to the prevalence of obesity, it is clear that changes in behavioral and learning patterns have increased the incidence of this condition during recent times. Thus, it is reasonable to believe that simple changes in behavior and lifestyle can considerably prevent and/or reduce obesity and its detrimental impact on health (Danaei et al., 2009). Humans, as well as other species, display inborn inclinations for certain foods; however, many feeding behaviors are also acquired through experience and learned associations between foods' flavors and their oral and post-oral effects.

It is now firmly established that animals 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 neurochemical systems that are also implicated in innate taste preferences and drug rewards. Although flavor preference learning involves multiple learning processes, it 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). Two processes have been identified: flavor-flavor and flavor-nutrient conditioning (Sclafani, 2004). Thus, flavor-flavor CFP refers 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). Flavor-nutrient CFP refers 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).

As detailed in the Background section below, the pharmacology of flavor-flavor and flavor-nutrient conditioning have begun to be elucidated with systemic studies examining the roles of dopamine D1 and D2 receptors on the one hand (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b), and opioid receptors on the other hand (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). In these studies, the respective roles of these neurochemical systems have been evaluated for the initial acquisition (learning) and the expression (maintenance) of these conditioned preferences. Whereas systemic dopamine (DA) D1, but not D2, receptor antagonists block the acquisition, but minimally the expression of flavor-nutrient conditioning by intragastric (IG) sucrose

(Azzara et al., 2001), systemic DA D1 and D2 receptor antagonists are involved in both the acquisition and expression of flavor-flavor conditioning induced by sucrose in sham-feeding rats (Yu et al., 2000a, 2000b) and by fructose in real-feeding rats (Baker et al., 2003). In contrast, systemic administration of the general opioid antagonist, naltrexone (NTX) failed to alter flavor-nutrient conditioning induced by IG sucrose (Azzara et al., 2000), flavor-flavor conditioning induced by sucrose in sham-feeding rats (Yu et al., 1999), or flavor-flavor conditioning induced by fructose in real-feeding rats (Baker et al., 2004). In addition to understanding the neuropharmacological mechanisms mediating flavor-flavor and flavor-nutrient conditioning, it is essential to identify the brain circuits underlying these forms of learning. Two major neuroanatomical sites are prime candidates for mediating these preference effects, the nucleus accumbens (NAC) shell and the amygdala (AMY). The present dissertation will focus on one of these two major types of learning, orosensory flavor-flavor preferences conditioned by the sweet taste of fructose, and examine the respective contributions of DA D1 and D2 as well as opioid systems in the NAC shell and AMY upon the expression and acquisition of fructose-CFP. The following four Specific Aims are designed to address these questions.

The **First Specific Aim** will examine whether DA D1 and D2 receptor signaling in the NAC shell can influence the expression (Specific Aim 1A) and acquisition (Specific Aim 1B) of fructose-CFP.

The **Second Specific Aim** will examine whether DA D1 and D2 receptor signaling in the AMY can influence the expression (Specific Aim 2A) and acquisition (Specific Aim 2B) of fructose-CFP.

The **Third Specific Aim** will examine whether general opioid receptor signaling in the NAC shell can influence the expression of fructose-CFP.

The **Fourth Specific Aim** will examine whether general opioid receptor signaling in the AMY can influence the expression of fructose-CFP.

The Background section examines relevant data related to the neuropharmacological and neuroanatomical substrates of CFP by reviewing: 1) the existence of food preferences in humans and animals; 2) the roles of sugars, starches and fats in the development of CFP; 3) the roles of orosensory (flavor-flavor) and postingestive (flavor-nutrient) processes in the development of CFP; 4) the underlying roles of DA, opioid and other neurochemical candidates in the mediation for CFP; 5) the specific systemic pharmacological studies on the acquisition and expression of flavor-flavor-mediated and flavor-nutrient-mediated CFP; 6) the underlying roles of the NAC shell and the AMY in food-related behaviors, learning and preference conditioning.

## Background

### 1. Food Preference

*Affective and Cognitive Factors:* Food is a potent unconditioned reinforcer because of its intrinsic incentive attributes. Several variables influence food reinforcement, including food access, food alternatives, and the costs of obtaining food, and these factors can promote overeating and obesity. For instance, nutrient intake, and consequently weight gain, was significantly increased in rats when more food sources were offered (Tordoff, 2002). Whereas one bottle of sucrose and five bottles of water were presented to one group of animals, the other group received five bottles of sucrose and one of water. Rats receiving more sucrose access displayed greater intakes, and

consequently gained more weight, thereby supporting the idea that animals, including humans, tend to overeat whenever more food is obtainable. However, a replication (Ackroff et al., 2007) failed to achieve the above results, and attributed effects to the bottle size in the original study that may have limited these rats' consumption. Although fundamental neuroanatomical and chemical substrates have been examined to explain basic spontaneous ingestive behaviors, one of the most persistent and challenging questions remains, namely, why do animals (humans and non-humans) eat certain foods?

The experience of eating appears to involve both affective and cognitive elements (Berridge et al., 2008). The affective aspect generally refers either to the acceptance (preference) or rejection (aversion) of food tastes (Sclafani et al., 2001). After all, it is well known that individuals differ on what they choose to eat, leaning more towards some foods and avoiding others. Although toxins and aversive stimuli are frequently detected by their orosensory properties (e.g., bitter taste) (Garcia et al., 1985), recent research demonstrated that poisonous molecules in the gut can also be identified by gastrointestinal receptors which play a role in flavor-nutrient conditioned aversions (Glendinning et al., 2008). The cognitive aspect of eating involves processing information regarding different food characteristics, such as quality and quantity, and using that information in higher processes like learning and memory (Yamamoto et al., 1998).

*"Liking", "Wanting" and "Learning":* Food reward has been conceptualized as an incentive process comprised by three functional elements that consciously or unconsciously determine what foods are worth to be ingested. These interrelated but distinct components are "liking," "wanting," and "learning" (e.g., Berridge, 1996, 2009).

"Liking" refers to the affective aspect of ingestive behavior involving the hedonic and pleasurable experiences of eating, often measured in rats using the taste-reactivity test. "Wanting" refers to the incentive salience or motivational disposition to eat, and is most often measured by a forced choice methodology (Finlayson et al., 2007). "Learning" involves the predictive associations between previously neutral stimuli on the one hand, and reward (the food itself) or reward-related cues (the rewarding ingestive situation). Although these three processes often happen simultaneously, they can become distinguishable both at a psychological and neurobiological level (Berridge, 1996, 2009). At the neural level, two interconnected brain pathways, the thalamo-cortical and the limbic paths, appear to underlie food reward processes. The thalamo-cortical path codes sensory aspects of taste quality. Sensory information about food stimuli is processed in the brainstem and travels from the nucleus of the solitary tract (NST) in the medulla to the gustatory cortex (GC), ascending through the parabrachial nucleus (PBN) of the pons and the ventro-postero-medial nucleus (VPM) of the thalamus. On the other hand, the limbic path appears to code hedonic aspects of food. This process begins in the brainstem and then reward information is integrated in limbic areas including the hypothalamus, the substantia innominata, and the AMY. However, the exact psychobiological mechanisms underlying the hedonic and incentive aspects of reward still need careful examination (Berridge, 1996).

More recently, very specific brain regions or "hotspots" appear to modulate different components of food reward. This limbic forebrain and brainstem network includes, but is not limited to, areas of the NAC and ventral pallidum (VP) (Smith, et al., 2009; Peciña et al., 2005). For instance, identification of a highly circumscribed (e.g.,

cubic-mm) area in the rostrodorsal part of the medial shell of NAC which enhances "liking" responses was elicited by receptor agonists of opioid and cannabinoid systems. Although "wanting" responses were also increased by opioid and cannabinoid activation in the NAC, this effect is more widespread rather that confined to a single spot in this brain region (Berridge, 2009). Further, central microinjections of mu-opioid agonists, such as DAMGO, in these hotspots elicit "double" hedonic responses to sweet taste qualities (e.g., sucrose), and suppression of aversive reactions to bitter taste qualities (e.g., quinine) (Peciña, 2008). Similarly, microinjections of anandamide, an endocannabinoid agonist in this NAC shell "hotspot" increased hedonic (positive facial) reactions to sucrose, but did not change negative facial responses to bitter quinine (Mahler, 2007). This neuroanatomical overlap for motivational and pleasurable responses to food reward may be indicative of an interaction between opioid and cannabinoid systems.

There are several measures of food reward in animals, including amount of actual intake, determination of food preference, and operant responses to work for food. The challenge with these approaches is that they appear to be measuring mostly, if not exclusively, "wanting" or incentive salience. Fortunately, based on evidence that humans and other species (e.g., monkeys, rats) display similar positive (e.g., tongue protrusions) and negative (e.g., gapes) facial reactions to different taste qualities, researchers have been able to study the "liking" or hedonic aspect of food reward by using taste reactivity tests (Berridge, 1989). Thus, individuals eat foods that they like, while avoid eating those that they dislike, but at the same time, they can be motivated to eat, obviously foods of which they are fond, but also foods that may have neutral or even aversive properties.

These processes may co-vary in different directions, and they are thought to be sustained by specific neural pathways (Berridge, 2008, 2009).

Hence, the relationship between food taste and its visceral effects can modify feeding behavior resulting in the development of preference or aversion. Such processes can occur very early in life as a result of experience and learning. For instance, increased hedonic responses have been observed in young rats (before weaning) when presented with sugars, carbohydrates, or fats. Associations between arbitrary flavors (either accepted or avoided) and positive or negative intragastric consequences can alter the original reaction to the flavor. Diets high in simple carbohydrates and fats are very appealing to humans (and other animals) because of both their flavor and their nutritional value (Myers et al, 2006a,b). Energy intake depends greatly on these two factors, and can lead to overeating, weight gain, and food-related problems. In particular, associating the taste of certain foods with a positive gastrointestinal sensation leads to a pleasurable experience. This observation is known as conditioned taste preference (CTP) (e.g., Capaldi et al., 2008). On the other hand, if the postingestive effects of food consumption are negative (e.g., pain, malaise, nausea, etc.), the resulting phenomenon is a conditioned taste aversion (CTA) (e.g., Parker et al., 2008). It is also possible to shift the natural positive response to rewarding stimuli (e.g., sweeteners) to aversion when these stimuli are associated with negative intragastric consequences (e.g., Glendinning et al., 2008). It is known that pleasant stimuli, including some psychoactive substances, produce opponent process effects in which the individual first feels gratification, but after the clearance of the substance, there is an aversive reaction. The purpose of this opponent process appears to be the resetting of a bodily homeostatic state. Thus, lesions placed in

the tegmental pedunculopontine nucleus (TPP) prevented rats from experiencing the rewarding consequences of morphine as well as its withdrawal symptoms, suggesting that the same dopaminergic neural substrate is involved in both incentive salience and withdrawal aversion (Vargas-Perez, 2007).

*Human Studies of Preferences:* Due to ethical and logistic issues, food preferences and aversions have been largely examined in laboratory animals; however, a limited number of human studies is also of interest. Both flavor-flavor and flavor-nutrient preferences have been reported for natural and artificial sweeteners in either hungry or sated conditions. In one study, individuals rated their preference for an arbitrary flavored solution slightly sweetened, either mixed with aspartame or sucrose. Although preferences were higher for the sucrose solutions consumed in a hungry state, increases in aspartame liking were also reported after participants had eaten a meal (Mobini et al., 2007). However, it has also been suggested that flavor-flavor learning in humans occurs only under hungry conditions. Thus, hungry participants reported strong preferences for a sweetened fruit tea whereas sated individuals did not show significant preferences (Brunstrom et al., 2008). In any event, modulations in flavor preference of different sweeteners (i.e., natural or artificial) have been observed after associating a previously preferred flavor with certain postoral consequences. Total intake and pleasantness for a sorbet were evaluated in different conditions (sucrose, aspartame, or maltodextrin). Participants reported higher preferences for the sucrose sorbet, but overall intake increased significantly for both the sucrose and the maltodextrin solutions. These results suggest that although preferences may be stronger for high-energy sweeteners, caloric

effects also stimulate consumption relative to sweet, but non-caloric, food stimuli (Yeomans et al., 2008).

Moreover, sham-feeding techniques have been modified in order to measure intake of sweeteners in humans based upon their orosensory properties. For example, a study required participants to sip a solution (sweetened or unsweetened) and then spit it out in order to minimize postoral consequences. As expected, intake and liking of sweet solutions were stimulated at higher concentrations of sucrose (Klein et al., 2006). A recent investigation evaluating food avoidance and aversion relative to age, gender, and educational variables found aversions to at least two types of food (vegetables and meats or fats) as well as greater aversions in women than men that correlated with educational level (Scott et al., 2007). The incentive value of food was measured by varying sweetness in yogurts and testing subjects on either a continuous or progressive ratio schedules. Men ate more than women when food was easily available, and palatability of sweet yogurt significantly increased intake during a continuous, but not a progressive schedule of reinforcement (Gondek-Brown, 2007). Although people may often not be able to describe the reasons why they want to eat certain foods, studies of obesity suggest that there are at least ten neurophysiological mechanisms underlying “subconscious” decisions that humans make to consume food. These mechanisms include innate preferences for sweets and fats, survival tactics, perceptual incapacity to judge nutritional value based on food appearance, firing of mirror neurons when observing another subject eating, and conditioned responses (Cohen, 2008). Thus, eating is influenced by the affective and motivational components of food reward, and the present series of studies are particularly

interested in conditioned preferences, the brain mechanisms mediating food preference, and impact of food rewards on feeding behavior.

*Conditioned Flavor Preference (CFP):* Although several chemicals can stimulate the gustatory system, four basic taste qualities had been classically identified in humans (salty, sour, bitter, and sweet). Later, it was discovered that people can also recognize the flavor of glutamate (Halpern, 2000), the taste of which is known as umami (Yamaguchi et al., 2000). Similar to other tastants, glutamate palatability increases at higher concentrations (Prescott, 2004) in that the hedonic value of glutamate was evaluated by associating different soups and varying amounts of monosodium L-glutamate (MSG). Although subjects showed stronger preferences for the flavor of soups high in MSG, nevertheless, it is conceivable that postoral consequences contributed to more liking of the soups. The development of food preference and aversion is determined by both innate and acquired factors, and can be measured by food acceptance, rejection, and amount ingested. Further, learned preferences rely upon different characteristics of food stimuli including concentration and their intrinsic aversive nature. For instance, conditioning with sodium chloride is more effective when using high (i.e., 0.9%) rather than low (0.45%) concentrations; and sweets are easier to condition than bitter or sour flavors (Ramirez, 1996)**.**

Associative learning is a decisive factor in the formation of most CFPs (Sclafani, 2001, Dwyer et al., 2008). Frequently, CFP is established by pairing a neutral flavor with an already preferred solution, producing a “positive” conditioned stimulus (CS+), while another neutral flavor is mixed with a less preferred solution, resulting in a “negative” conditioned stimulus (CS-) (Yiin et al., 2005). Often, researchers evaluate CFPs by

measuring intakes of two-bottle liquid solutions (CS+ vs. CS-) presented simultaneously (Sclafani, 2002). This testing technique provides a more preferred means of assessing preference because the two different options are presented simultaneously. In contrast, independent series of one-bottle tests measure the degree of “acceptance” of the single solution being offered. Similarly, operant tasks can be used to evaluate both a flavor preference as well as the enhancement of orosensory experiences as a result of their association with intragastric infusions of nutritive solutions. Thus, how hard rats are willing to work (e.g., number of licks per second), as measured by progressive ratio schedules, in order to receive access to a positively-flavored solution may be reinforced by positive postingestive consequences. Thus, rats exhibit greater preference and intake for a sweet flavored (e.g., saccharin) solution when it is associated with intragastric infusions of glucose, but not water, infusions (Sclafani et al., 2006).

Conditioned preferences can be established in food deprived situations which may further stimulate intake of the preferred solution without affecting learned associations with certain postingestive consequences. Food-restricted animals displayed higher intakes of the positively-flavored stimulus paired with intragastric glucose infusions, whereas free-fed animals showed smaller intakes under otherwise equal circumstances. However, both groups of animals showed comparable preferences (Yiin et al., 2005). Moreover, once a nutrient-driven CFP flavor preference has been acquired, it is quite difficult to be extinguished (Elizalde et al., 1990). Further, both classical and instrumental conditioning paradigms suggest that food provided immediately after CFP-extinction sessions does not affect maintenance of the preference, even when food is delayed 30 or 120 minutes (Albertella et al., 2006). Nevertheless, an extinction model can debilitate associations

between the conditioned and unconditioned stimuli after a CFP had been established (Delamater, 2007). Because healthy aging affects different neurochemical systems (e.g., dopaminergic), causing deficits in learning, memory, and other processes involved in decision making (Marschner et al., 2005), a recent study revealed that CFP decreases with age. Comparisons between 7- and 24- month old rats showed that the younger animals easily developed learned preferences for a positive flavor stimulus, whereas older rats showed no preference for the positive or negative stimuli after training, suggesting that the older rats' inability to develop strong preferences is related to a decreased learning capability, that is, to impaired facility to form associations between the conditioned and unconditioned stimuli (Renteria, 2008).

In addition, gender differences have been observed in both human and animal food preferences. For example, female rats display changes in palatability across the estrous cycle, suggesting that gonadal hormonal effects are responsible for these differences (Geary, 2001). Despite the fact that flavor preferences can be conditioned by fructose in male and female rats, males display greater responses to this natural sweetener (Ackroff, 2004). Further, female rats display different responses to the artificial sweetener, sucralose, such that they prefer such sweeteners as saccharin or sucrose to sucralose, but display mixed preferences for sucralose paired with other sugars (Sclafani et al., 2004). These studies indicate that gender differences exist in regards to flavor preferences and learning, and that those differences may be explained at least partially by gonadal hormonal influences. Therefore, in ascertaining the neuroanatomical and neurochemical substrates of CFP, a more consistent strategy would be to employ male

animals as experimental subjects to avoid potential interactions with circulating gonadal hormone influences.

**2. The Roles of Sugars, Starches and Fats in the Development of CFP**

It is unknown whether the same neurochemical and neuroanatomical pathways are involved in flavor preferences conditioned by the three different nutrients, sugars, starches, and fats. It is important to consider the following point made by Berridge (2009). “We may be used to thinking of sweet tastes as innately pleasant, but their pleasure is not contained in the intrinsic detail of their sensation but rather in their evolved ability to act as keys that unlock activation of brain ‘liking’ systems. This is evident by considering that if the ability to unlock hedonic brain systems is lost, a sweet taste loses its pleasure while remaining sweet as ever.”

*Sugars:* Although food preference has been studied on the basis of different tastants, the sweet taste of sugar has been most examined in laboratory animals due to its intrinsic rewarding properties. Foods high in sugars are greatly attractive, for both their taste and nutritional value, encouraging overeating. Due to orosensory and postingestive associations, it is thought that individuals may expect to obtain a certain amount of energy value from sweet foods based on their orosensory characteristics. Specific sweet taste receptors (T1R2 and T1R3) have been identified in humans and other animals as well (Montmayeur et al., 2001). Studies in normal and knockout rodents have shown that stimulation of these receptors activates peripheral nerves in the gut and subsequently, the gustatory neural network by increasing the release of hormones that prompt glucose absorption (Nelson et al, 2001). However, not all sweeteners have the same palatability or work equally in establishing CFPs. For example, increasing the concentrations of sucrose

continue to increase stimulation of intake in sham-fed, but not necessarily in free-fed, animals probably because of its satiation consequences in the latter group. In contrast, saccharin solutions are preferred at low (0.05-0.2%) concentrations, whereas higher saccharin concentrations reduce intake apparently due to its bitter taste (Smith et al., 2002).

Moreover, fructose has minimal post-oral effects in short-test sessions when compared to glucose which appears to be highly rewarding following intragastric administration (Ackroff, 2004). Nevertheless, it is possible to elicit flavor preferences, comparable to those conditioned by glucose, with intragastric infusions of fructose solutions over long-daily training sessions (Ackroff et al, 2001). Therefore, flavor-flavor learning studies using sucrose or glucose as the unconditioned stimulus require sham-fed preparations in order to minimize the post-oral consequences of these sugars. Fructose, on the other hand, is frequently used in short-term real-feeding conditioning experiments due to its negligible postingestive actions. Moreover, consumption of artificial sweeteners may actually increase intake, and consequently weight gain, due to differences in signaling between the mouth and the gut. Rats received daily yogurt regimens sweetened with either glucose or saccharin with higher intakes and weight observed in the saccharin relative to the glucose condition. The expectation that oral cavity receptors for sweet taste modulate intake by sending information to gastrointestinal receptors about the caloric value of food being consumed was not confirmed because of the actual energy value of artificial sugars (Swithers at al., 2008). Finally, female ingestive behavior is modulated by estrous cycle hormones such that estrogen mediates appetite for sweeteners by

impairing the ability to distinguish low sucrose concentrations from water, resulting in lower sucrose consumption at these concentrations (Curtis et al., 2005).

*Starches:* Rats not only can identify the five basic taste qualities, but can also discriminate the taste of polysaccharides derived from starches (Sclafani, 2004). Starch derivatives used in the study of flavor-nutrient learning demonstrated that the reinforcing nutritional consequences of IG polycose infusions increased as the concentration of this form of starch increased. However, a very large (32%) polycose concentration of polycose decreased flavor-nutrient preferences (Lucas et al., 1998). Hence, conditioned preference studies show that intragastric infusions of either carbohydrates (e.g., polycose) or fats (e.g., corn oil) have strong rewarding effects, although, the former appear to be more satiating (Lucas et., 1999). Infusions of pure or mixed (e.g., starch + fat) intragastric solutions are expected to stimulate intake and conditioned preferences that may also vary in magnitude depending on the time (short vs. long) of training (Lucas et al., 1999).

*Fats and other nutrient-related stimuli:* Fats appear to be very palatable and have positive effects in the gut, leading to overconsumption. The orosensory properties of fats were sufficient to stimulate consumption in sham-fed obese Zucker rats, and positive associations with intragastric infusions of oils were also noted (Greenberg et al., 1996). Brain areas involved in reward release neuropeptides (e.g., substance P, NPY, AGRP, opioid peptides) and DA when fats are placed on the tongue. Although the specific mechanism has not been elucidated, it has been suggested that identification of long-chain fatty acids in the tongue is crucial for this signaling system (Mizushige et al., 2007). Research indicates that, through conditioned taste aversion (CTA) paradigms, rats are able to recognize and differentiate linoleic and oleic acid, two components of corn oil,

which appear to have similar orosensory attributes. In fact, CTA to linoleic acid generalizes to avoidance of oleic acid stimuli and vice-versa, but generalization does not occur with other avoidant stimuli such as ethanol or sodium chloride (McCormack et al., 2006). Intragastric infusions of different types of fats (e.g., corn oil and safflower oil) strongly condition flavor preferences in rats, although less saturated fats (e.g., oleic acid and linoleic acid) seem to be more satisfying (Ackroff, 2005). Also, intragastric infusions of high-fat relative to high-carbohydrate solutions appear to be more effective in producing learned flavor preferences due to less satiety effects and a high nutritional value (Ackroff et al., 2006).

Other nutrient-related stimuli have been examined in the context of CFP. For instance, alcohol consumption has been positively correlated with ingestion of foods high in fat, carbohydrate, or sugar content. Rats develop flavor preferences for flavored solutions that are associated with intragastric infusions of ethanol, sugars, or corn oil, yet the conditioning effects of ethanol do not appear to be as strong as those of sugars and fats (Ackroff et al., 2004). Further, administration of opioid agonists (e.g., morphine) increases alcohol ingestion in a similar fashion to food intake stimulated by opioids (Reid, 1996).

### 3. Roles of the Orosensory and Postingestive Factors in Preference Conditioning

Different neural and chemical substrates appear to exist for the sensory and hedonic components of food intake and the development of preferences. The orosensory aspect involves the perception of several food properties (smell, taste, texture) experienced when food enters through the mouth, while the postingestive component

refers to the positive or negative consequences of food consumption. Several methods have been used to examine separately the oral and postoral effects of foods on ingestive behavior and reward. For instance, animals can be surgically implanted with an intragastric fistula that is opened prior to liquid food reaching the stomach to prevent nutrients from reaching the intestine; thus, the orosensory properties of the food are studied largely in isolation (sham-feeding procedure). Also, direct intragastric injections of nutritive or non-nutritive solutions allow examination of postingestive consequences without the oral effects (Sclafani, 2004, Ackroff, 2008).

Interactions between the flavor and nutritional value of palatable foods act upon preference, and may lead to overconsumption. Both orosensory and gastrointestinal (GI) stimuli can both positively and negatively affect food intake (Sclafani, 2001), and that the concentration of the solutions used during real- or sham- feed procedures modulate reward (Sclafani at al., 2004). Distinct aspects of food reward have been examined based on flavor cues and/or postingestive consequences. In addition, learning has been found to exert a crucial role in food preferences. Based on classical conditioning theories, animals learn to associate the excitatory or inhibitory oral and postoral effects of food stimuli, and these associations alter original preferences (Sclafani et al., 2004). This dissertation proposal focuses particularly on two types of learning: flavor-flavor and flavor-nutrient conditioning.

*Flavor-Flavor Conditioning:* This type of learning refers to the development of food preference for a neutral flavor based on connections with orosensory (taste, texture, smell) attributes of an already preferred flavor. Most commonly, an innately preferred taste (e.g., fructose) is mixed with a neutral flavor that eventually comes to be preferred

(CS+) while a less-preferred taste (e.g., saccharin) is mixed with another neutral flavor that eventually becomes less preferred (CS-) as compared to the CS+ solution (Baker et al., 2003, 2004). However, other procedures for examining flavor-flavor conditioning have also been employed. For instance, in sham-feeding, animals are allowed to drink the solutions, but they are drained out from the gut to reduce postingestive consequences (Yu et al., 1999, 2000a, 2000b; Klein et al., 2006). Different rewarding stimuli have been paired with neutral flavors in order to study their effects on conditioned preferences. Sugars have been found to be extremely potent in establishing flavor-flavor learning. Although starches have strong postingestive effects and their taste appears to be attractive to rats, they fail to develop strong CFPs (Bonacchi et al., 2008). The pairing of a novel flavor with polycose relative to another novel flavor mixed in water failed to produce significant preferences even when the flavored polycose solution was added to saccharin. The difference in conditioning potency between sugars and starches is not clear yet, but activation of separate neurotransmitters systems is suspected (Bonacchi et al., 2008).

*Flavor-Nutrient Conditioning:* This type of learning refers to the development of food preferences based on connections between a flavor and its postingestive (positive or negative) effects. IG infusions of neutral (e.g., water), positive (e.g., sugars), or negative (e.g., bitter solutions) stimuli lead to associations with the flavor that is being consumed orally at the time and result in either acceptance or rejection of that flavor (Ackroff, 2008; Delamater, 2006). It is noteworthy that IG infusions apparently do not possess incentive value on their own, but rather they need to be linked to orosensory stimuli (e.g., uniquely flavored oral saccharin solutions) in order to elicit CFP (Sclafani, 2004).

Furthermore, nutrients vary in their potentiating effects, that is, some of them produce stronger associations with flavors ingested orally; for instance, glucose is more effective than fructose, fat, and ethanol (Ackroff, 2008). Flavor-nutrient preferences can be well established in both food restricted and free-fed animals such that in training rats to develop a preference for an intragastric nutritive infusion (e.g., maltodextrin, casein, or corn oil) paired with a neutral flavor, the nutritive solutions ubiquitously condition preferences regardless of food availability (Yiin et al., 2005). Moreover, it is possible to switch innate preferences for a flavor when it is associated with the negative consequences of an intragastrically infused solution that would be usually avoided (e.g., a bitter solution) (Glendinning et al, 2008). Finally, the post-ingestive effects of some stimuli (e.g., glucose) are strong enough that it is possible to establish CFPs after just one intragastric infusion session of the rewarding solution (Myers, 2007).

**4. Roles of DA, opioid and other neurochemical candidates in the mediation of CFP**

The following neurotransmitter receptor systems have been examined in relation to palatability: DA, opioid, glutamate, cannabinoid and GABAergic systems. There is substantial evidence that brain DA systems are involved in food reward and motivation (see reviews by Berridge and Robinson, 1998; Smith, 2004; Wise, 2008). Opioids, for instance, have been studied over the past three decades because of their involvement in the hedonic aspect of feeding  with general and selective opioid receptor antagonists suppressing food and fluid intake whereas opioid agonists increase food and fluid intakes in a variety of situations (Bodnar, 2004; Cooper, 2007; Levine, 2006). Research about the

involvement of other neurochemical systems in feeding and taste learning is limited but also includes the glutamatergic, cholinergic, and cannabinoid systems.

*DA System:* DA plays an important role in food reward, particularly in specific functions related to the modulation (Berridge, 2007) and wanting of food (Barbano et al., 2007), including the regulation of the incentive motivation of food reward (Epstein et al., 2006). In particular, the D1 DA receptor appears to be vital for establishing conditioned reward behaviors (Sutton et al., 1999). DA release is increased in different brain regions implicated in reward by food ingestion, mainly by sucrose solutions (Smith, 2004). 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).

*Opioid System:* The opioid system is pivotal for the consumption of palatable stimuli such that administration of general opioid agonists dose-dependently increases food and liquid intake, whereas administration of general opioid antagonists decrease intake (see reviews: Bodnar, 2004; Kelley et al., 2004). Opioid agonists and antagonists

are more effective when injected directly into the brain than systemically. For example, intracranial administration of opioid agonists into the nucleus accumbens increases ingestion of saccharin (Zhang et al., 2002). In general, opioids appear to be involved in the pleasurable experience of food palatability, and particularly with the maintenance of eating behavior (Frisina el at., 2002; Taha et al., 2006). NTX's effects on the hedonic responses of rats to sucrose-saccharin solutions involved reduction of intake but no effects on initial licking responses (Frisina et al., 2002). Further, NTX selectively suppressed intake for more appetizing food stimuli (e.g., varying degrees of sucrose) (Taha et al., 2006). Moreover, 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), 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). Furthermore, the effects of opioid antagonists (e.g., naloxone) persisted under free-feeding, food-restricted, or sham-feeding conditions (Shabir et al., 1999). Principally, μ- and κ-opioid receptor antagonists appear to decrease the hedonic effects of sucrose (Beczkowska at al., 1992). Apparently, opioids facilitate more the consumption of highly-palatable foods (e.g., sugars) than less preferred ones (e.g., plain water), suggesting an opiate modulation of the liking aspect of food reward. Thus, NTX dose-dependently inhibits rats' intakes when evaluated with solutions of increasing sucrose concentration (Cleary et al., 1996), and opioid antagonism appears to reduce food intake by diminishing the pleasure experienced when consuming appetizing foods (Tallet et al., 2008).Whereas systemic injections of low doses of

morphine stimulates intake of sucrose, and to a lesser extent, ethanol relative to water (Stromberg et al., 1997), NTX failed to decrease ethanol consumption in a study in which with rats had free, constant access to alcohol (Goodwin et al., 2001). In addition, endogenous opioids modulate the facilitatory effects of benzodiazepine on ingestion, suggesting an interaction between these two systems (Cooper, 1983).

**5. Systemic Pharmacological Studies and CFP**

*Systemic Opioid Antagonism and Flavor-Flavor and Flavor-Nutrient CFP:* The previous section would 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. Further studies (Yu et al., 1999; Azzara et al., 2000) were conducted in which NTX was paired with both CS+ and CS- flavors during training to determine systemic drug effects on the flavor-flavor and flavor-nutrient conditioning effects of sugars.

*Systemic Opioid antagonism fails to disrupt the acquisition or expression of flavor-flavor learning:* The effects of NTX on the expression of a flavor preference conditioned by the sweet taste of sham-fed sucrose were examined (Yu et al., 1999).

Following one-bottle training of a CS+ flavor paired with 16% sucrose and a CS- flavor paired with 0.2% saccharin, the rats significantly preferred the CS+ to CS- flavor in two-bottle tests containing a combined 8% sucrose and 0.1% saccharin solution. Treating the rats with a 0.1 to 10 mg/kg dose range of NTX prior to the choice tests failed to block the CS+ preference. This systemic dose range of NTX has been shown to effectively reduce intakes of sugars and saccharin (Bodnar, 2004). In an acquisition study, rats were injected with NTX (0.1 mg/kg) or saline prior to CS+ and CS- training sessions. Although NTX 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%). A subsequent study (Baker et al., 2004) determined the effects of an expanded NTX dose range (0.1, 1 and 5 mg/kg) on preference conditioning by the sweet taste of fructose. Although NTX treatment dose-dependently reduced CS intakes during one-bottle training, it did not prevent flavor preference conditioning. The CS+ preferences of the three NTX groups (72-86%) did not significantly differ from that of the saline-treated group (78%). In addition, NTX 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.

*Systemic Opioid antagonism fails to disrupt the acquisition or expression of flavor-nutrient learning:* The effects of systemic NTX (0.1 or 1 mg/kg) treatment on flavor conditioning by IG sucrose infusions was also investigated (Azzara et al., 2000). Although NTX 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 of NTX 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 NTX (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. However, opioid modulation appears to act on the maintenance but not on the acquisition of conditioned preferences stimulated by sucrose, as observed seen in place-conditioned preference experiments (Delamater et al., 2000). NTX reduced, but did not block, the expression of sucrose preferences previously conditioned through a place-preference paradigm. Acquisition of these preferences was not impaired.

*Systemic DA Antagonism and Flavor-Flavor and Flavor-Nutrient CFP:* The previous section would predict that DA antagonists should reduce flavor preference conditioning by the sweet taste of sugars. 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 DA D2-like receptor antagonism reduced the reward potency of sweet taste.

*DA antagonism attenuates the acquisition and expression of flavor-flavor learning:* A role for DA in flavor-flavor learning was examined 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 two-bottle choice test, the CS+ flavor was preferred to the CS- flavor following saline treatment. Systemic treatment with DA D1-like (SCH23390) or D2-like (raclopride) receptor antagonists (50-800 nmol/kg) prior to the choice tests significantly attenuated the expression of the CS+ flavor preference at lower doses and completely blocked it at higher doses (400-800 nmol: raclopride, 200-800 nmol: SCH23390) . This systemic DA 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). It should be noted 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. A follow-up study (Baker et al., 2003) investigated the role of DA in flavor-flavor conditioning using the fructose real-feeding paradigm. 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 DA 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 DA D1 and to a lesser degree D2 signaling.

*DA antagonism attenuates the acquisition but not expression of flavor-nutrient Learning:* The investigation of systemic DA antagonist effects on flavor-nutrient learning involved training of 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 findings indicate that, unlike flavor-flavor learning, flavor-nutrient learning with sugars is critically dependent only on DA D1-like receptor signaling. In this

respect, flavor-nutrient learning is similar to flavor-toxicosis learning (with lithium chloride), which is also disrupted by DA D1-like but not D2-like receptor antagonists (Fenu et al., 2001). DA 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).

*Glutamate:* 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.

*Cannabinoids:* An abundant literature exists 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). The involvement of cannabinoid signaling in flavor conditioning was determined by examining determining the effects of CB-1 receptor antagonism with the inverse agonist, 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 doses of 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.

*Benzodiazepines:* Another neurotransmitter receptor implicated in flavor palatability is the benzodiazepine receptor, a part of the GABA-A receptor complex (Cooper, 2004, 2005). 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).

### 6. Putative Sites of Action of DA and Opioid Effects upon Fructose-CFP

Two separate but interrelated neuroanatomical pathways have been described to underlie the taste and reward systems (Sewards, 2004). The taste system consists of chemical information conveyed to the nucleus tractus solitarius (NTS) by afferent fibers from the facial, glosso-pharyngeal, and vagus cranial nerves which synapse with receptor

cells in the taste buds of the tongue. This information relays in the parabrachial nuclei (PBN) in the pons, and continues ascending to the ventral posteromedial parvocellular component of the thalamus (VPMpc). Then, these fibers project to the cortical gustatory area (CGA) of the insular cortex (IC). On the other hand, the reward system arises from DA neurons located in the ventral tegmental area (VTA). These cells reach the NAC which then projects directly as well as indirectly to the lateral hypothalamus (LH) through the ventral pallidum (VP). The PFC and the AMY appear to interconnect these two systems. The PFC conveys information from the IC and projects to the NAC. Similarly, the AMY receives information from the PBN, the VPMpc, and the IC and then projects to the VTA, the NAC, and the LH (Yamamoto et al., 2006).

Pharmacological systemic studies have demonstrated the involvement of opioids, DA, and other chemical systems in feeding. Therefore, it is of great interest to evaluate specific sites of action in the brain for these pharmacological actions. Intracranial injections of neuroactive reward-related chemicals have demonstrated more potent effects than when administered systemically. Principally, central injections of DA- and opioid-agonists and antagonists significantly alter eating behavior. Although the exact mechanisms and neural substrates are not well understood, the respective roles of the NAC and AMY on CFP are of particular interest.

*Nucleus Accumbens (NAC):* The NAC is a critical structure located in the ventral striatum, and is sub-divided into two regions known as the core (NAC/c) and the shell (NAC/s). The NAC receives DA inputs from the VTA through the mesolimbic system, a crucial pathway in food reward. In fact, increases in NAC DA levels have been registered after presentation of various motivational agents (Roitman et al., 2004). The NAC is a

site in which palatable foods and sweet solutions, in particular, stimulate DA efflux (Bassareo & Di Chiara, 1997; Bassareo & Di Chiara, 1999; Genn et al., 2004). Recent findings further indicate that the post-ingestive effects of glucose promote NAc DA release (De Araujo et al., 2008; Ren et al., 2009). In addition, intake of a CS+ solution previously paired with IG carbohydrate, but not a CS- solution, increased NAC DA efflux (Mark et al., 1991). Further evidence for Pavlovian learning in the NAC was demonstrated by increased DA release in this brain area after rats consumed a slightly bitter-sucrose solution that was paired with intragastric infusions of polycose during training (Mark et al., 1994). DA receptor antagonism in the NAC impairs learning in several paradigms such as Pavlovian approach conditioning to a CS paired with sugar (Parkinson et al., 1999), operant responding for sugar (Parkinson et al., 1999; Smith-Roe & Kelley, 2000), and conditioned avoidance of sweet taste (Fenu et al., 2001). Further, increased DA release has been observed after NAC self-stimulation, and this behavior is reduced following microinjections of DA D1 receptor antagonists (Cheer et al., 2007). Finally, DA and glutamatergic interactions in the NAC have also been examined in the context of feeding-related instrumental behavior. Central administration of different glutamate receptors antagonists (AMPA/KA, NMDA) as well as a DA D1 receptor antagonist blocked the acquisition but not the expression of instrumental learning (Hernandez et al., 2005).

Further research has shown a strong relationship of opioid control within the NAC. Intracerebral injections of the opioid agonist, morphine, into the NAC dose-dependently stimulated palatability and conditioned feeding (Kelley et al., 2000). It has been suggested in central antagonist studies that μ- and κ- opioid receptors in the NAC

modulate food intake under a variety of situations including spontaneous, deprivation, glucoprivic, and sucrose feeding contingencies (Bodnar et al., 1995). Although specific pathways for palatability and incentive motivation are not described, it has been suggested that activation of μ-opioid agonists (et al, DAMGO) in a 1-milimiter spot of the medial region of the NAC/s mediates the hedonic aspect of food reward. Moreover, incentive motivation appears to be enhanced by μ-opioid agonists distributed throughout the entire shell (Peciña, 2008). In addition, interactions between endogenous opioids and ethanol have been suggested to occur at the level of the NAC. In rats, activation of μ-opioid receptors by central injections of the opioid agonist DAMGO into the NAC significantly stimulated alcohol intake. This finding implied that food and alcohol consumption may be regulated by the same brain pathways (Zhang, et al., 2002). Interactions between NAC opioid and DA systems have also been examined in terms of the mediation of feeding by opioid receptor subtypes in the NAC. Whereas only NAC DA D1 antagonism was effective in reducing intake stimulated by DAMGO, neither DA D1 nor D2 receptor antagonists in the NAC significantly reduced feeding stimulated by Deltorphin (Ragnauth et al., 2000a, b).

*Amygdala (AMY):* The AMY has been implicated in learning related to flavor aversion (Gallo et al.1991), flavor preference (Gilbert et al., 2003; Touzani & Sclafani, 2005), and Pavlovian and instrumental reward (Cardinal et al., 2002; Baxter & Murray, 2002). Feeding and gastric nutrient infusions increased AMY DA turnover or efflux (Heffner et al., 1980; Hajnal & Lenard, 1997), and a Pavlovian CS for food elicited AMY DA efflux (Harmer & Phillips, 1999). Further, AMY microinfusions of amphetamine facilitated learning to respond to a food-related CS (Hitchcott et al., 1997), whereas AMY

inactivation modulated feeding-stimulated DA efflux in the NAc (Ahn and Phillips, 2002).

### 7. Rationale and Specific Aims of the Present Study

The foregoing sections presented a series of studies that evaluated the role of the DA and opioid systems in the acquisition and expression of flavor-flavor preferences conditioned by fructose. In particular, systemic administration of the selective DA D1 receptor antagonist SCH23390, the selective DA D2 receptor antagonist raclopride, and the general opioid antagonist NTX were used to examine fructose-CFP in real-feeding rats. This section will focus on the main characteristics and methodology involved in all the experiments performed, followed by a description of the specific aims of this study.

*Acquisition and Expression of CFP:* Flavor-flavor conditioning is comprised of two phases, acquisition and expression. In the acquisition phase, rats are trained to associate the taste of fructose with an artificial flavor. During the expression phase, rats show and maintain any learned preference. When evaluating the pharmacological effects of DA or opioid antagonists on the expression of CFP, rats will be trained to drink different training solutions in a series of one-bottle daily sessions. Then, learned preferences will be assessed in a series of two-bottle choice tests preceded by either vehicle of drug central microinjections. On the other hand, when evaluating the pharmacological effects of DA antagonists on the acquisition of CFP, rats will be administered either vehicle of drug microinjections centrally and then trained to drink the training solutions in a series of one-bottle daily sessions. Any learned preferences will be assessed in a series of two-bottle choice tests. In the acquisition studies, two additional groups are tested. A Yoked Control group receives vehicle injections throughout one-

bottle training, and their exposure to the CS+/Fs and CS-/s solutions will be limited to the mean 60-min intakes of the D1 and D2 groups. An unoperated Control group is trained as above except without injections and with their CS+/Fs and CS-/s intakes limited to 16 ml/session; the purpose of this group will be to evaluate the effectiveness of the training procedure.

*Food Restriction:* In all of the studies, animals will be food-restricted to 85-90% of their body weight in order to motivate them to sample the solutions with short latencies. In all studies, the rats will initially be trained to drink unflavored 0.2% saccharin solution from calibrated sipper tubes (100 ml, 1 ml gradations) during daily 1 h sessions. The sipper tube will be mounted on the front of the cage held by a taut steel spring, and positioned 3-6 cm above the cage floor. This training procedure will be repeated daily until all rats approached the sipper tubes with short (< 1 min) latency, typically within three days. The limited food rations will be given 1 h after each training session.

*Training and Testing Solutions:* The training solutions will consist of an 8% fructose (Sigma Chemical Co., St. Louis, MO) and 0.2% sodium saccharin (Sigma) mixture or a 0.2% sodium saccharin solution; the solutions will be flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft Foods, White Plains, NY). A combined fructose and saccharin solution is employed for two reasons as introduced in previous sections. The saccharin solution enhances the sweet taste of fructose, and the fructose as a sugar drives the stimulation of DA efflux from the NAc and AMY. Finally, although sucrose, glucose and sucrose all possess post-ingestive nutritive effects, the first two (sucrose and glucose) elicit powerful flavor-nutrient preferences, whereas the last

(fructose) fails to produce flavor-nutrient conditioning. Thus, in each paradigm, half of the rats in each group will have the cherry flavor added to the fructose+saccharin solution, and the grape flavor added to the saccharin only solution; the flavors will be reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors will be 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 (Baker et al., 2003, 2004). CS+/Fs refers to the flavored fructose+saccharin solution used in training, and CS+/s refers to the same flavor presented in saccharin-only during choice testing. The CS-/s refers to the flavored saccharin solution used in training and testing. All testing will take place in the rats' home cage during the mid-light phase of the light:dark cycle. The position of the CS and water sipper tubes during training and the CS+/s and CS-/s sipper tubes during testing will vary across days using a left-right-right-left pattern. The rats will be tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions to control for any position effects.

*Equimolar Dose Response Curves in Dopamine Antagonist Studies:* In order to directly assess the potency of DA D1 and D2 antagonist effects, equimolar dose curves will be used in all central studies with bilateral test doses of 12, 24 and 48 nmol. Half of the rats will be tested with an ascending dose order and the remaining rats will be tested in a descending dose order.

*Dependent Measures:* In the expression studies, training intakes will be averaged over the five CS+/Fs and five CS+/s sessions and evaluated with a t-test. Intakes during the preference tests will be 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 will evaluate total intakes and percent CS+/s intakes as a function of dose for the two groups.

In the acquisition studies, training intakes will be averaged over the four CS+/Fs and four CS-/s sessions and will be analyzed with a two-way ANOVA (CS conditions x Groups). Intakes during the preference tests will be 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 ANOVA will compare the CS intakes of D1, D2 and control groups (Group x CS x Test). Separate two-way ANOVAs will evaluate total CS intakes and percent CS+/s intakes of the four groups. When main or interaction effects are found, Bonferroni corrected comparisons ($p<0.05$) will be used to detect significant effects.

***Specific Aims and Hypotheses:***

**Specific Aim 1A:** D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **NAcS** will dose-dependently decrease the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is based upon the ability of DA D1 and, to a lesser extent, D2 receptor antagonists administered systemically to significantly reduce the expression of fructose-conditioned flavor-flavor preferences in free-feeding rats (Baker et al., 2003) and of sucrose-conditioned flavor-flavor preferences in sham-feeding rats (Yu et al., 2000a, 2000b).

**Specific Aim 1B:** D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **NAcS** will dose-dependently alter the **acquisition** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is based upon the ability of DA D1 and, to a lesser extent, D2 receptor antagonists administered

systemically to block the acquisition of fructose-conditioned flavor-flavor preferences in real-feeding rats (Baker et al., 2003) and of sucrose-conditioned flavor-flavor preferences in sham-feeding rats (Yu et al., 2000a, 2000b).

**Specific Aim 2A**: D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **AMY** will dose-dependently reduce the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is based on the important role of the AMY in flavor preference learning (Gilbert et al., 2003; Touzani et al., 2005) as well as on the ability of D1 and, to a lesser extent, D2 DA receptor antagonists administered systemically to significantly reduce the expression of sucrose-conditioned flavor-flavor preferences in sham-feeding rats (Yu et al., 2000a, 2000b) and fructose-conditioned flavor preferences in real-feeding rats (Baker et al., 2003).

**Specific Aim 2B**: D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **AMY** will dose-dependently alter the **acquisition** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is based on the important role of the AMY in flavor preference learning (Gilbert et al., 2003; Touzani et al., 2005) as well as on the ability of D1 and, to a lesser extent, D2 DA receptor antagonists administered systemically to significantly reduce the acquisition of fructose-conditioned flavor preferences in real-feeding rats (Baker et al., 2003) and sucrose-conditioned flavor-flavor preferences in sham-feeding rats (Yu et al., 2000a, 2000b).

**Specific Aim 3**: The opioid receptor antagonist, NTX, injected bilaterally into the **NAc** will dose-dependently alter the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is proposed despite the inability of

systemic NTX to affect the acquisition or expression of either sucrose-conditioned preferences in sham-feeding rats (Yu et al., 1999) or fructose-conditioned preferences in real-feeding rats (Baker et al., 2004). A recent study by Wooley et al. (2006) indicates that the route of drug administration can influence the effect of NTX on food preference. Rats were given choice tests with two differently-flavored (chocolate and banana) but nutritionally-identical food pellets. Most animals displayed a mild preference (presumably unlearned) for the chocolate-flavored food. Systemic NTX treatment did not alter this preference but rather reduced the intakes of both flavored foods. However, NTX microinfusions into the NAc) selectively reduced the intake of the preferred chocolate-flavored food. The NAc is recognized as a critical site of opioid action on food intake and reward (see reviews: Kelley et al., 2002; Peciña, 2008). Thus, it is not surprising that NAc and systemic administration of opioid drugs might have different effects on food preferences based on the role of opioid signaling in the NAc on food intake and reward (Woolley et al., 2006).

**Specific Aim 4:** The opioid receptor antagonist, NTX, injected bilaterally into the **AMY** will dose-dependently alter the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats. This hypothesis is proposed despite the inability of systemic NTX to affect the acquisition or expression of either sucrose-conditioned preferences in sham-feeding rats (Yu et al., 1999) or fructose-conditioned preferences in real-feeding rats (Baker et al., 2004). This hypothesis is based on the previous rationale described in light of the Wooley (2006) study.

## CHAPTER TWO: GENERAL METHODS

**Subjects**: Adult male Sprague-Dawley rats (260-300 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages in the Queens College vivarium. Rats were maintained at 21 °C under a 12:12 h light:dark cycle with chow (5001, PMI Nutrition International, Brentwood, MO) and tap water available ad libitum, except as noted below. All experimental protocols were approved by the Queens College Institutional Animal Care and Use Committee (Protocol 69) certifying that all subjects and procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

**Surgery**: 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 a range of bilateral placements using the following coordinates: For the NAc incisor bar (+5 mm), 3.1-3.5 mm anterior to the bregma suture, 1.7-1.8 mm (shell) or 2.7-2.8 (core) and angled 10° towards each side of the sagittal suture, and 6.5-6.8 mm from the top of the skull. For the AMY incisor bar (-3.3 mm), 2.8 mm anterior to the bregma suture, 4.1-4.5 mm lateral to the sagittal suture, and 8.0-8.4 mm from the top of the skull to sample the full extent of the AMY across placements and conditions. 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 begins.

**Drugs and Microinjections**: In experiments 1 and 2, the selective D1 antagonist, SHH23390 (Sigma Chemical Co.) and the selective D2 antagonist, Raclopride (Sigma

Chemical Co.) were dissolved in 0.9% normal saline. In experiments 3 and 4, the opioid antagonist NTX (Sigma Chemical Co.) was also dissolved in 0.9% normal saline. The drugs and vehicle (0.9% saline) were administered at a volume of 0.5 µl/side. Microinjections were performed bilaterally through a 33-gauge stainless steel internal cannula (Plastics One) connected to a 2-µl Hamilton microsyringe (Hamilton Company Reno, NV, USA) by polyethylene tubing. Each rat was held gently, the stylet was removed, and the internal cannula inserted. The tip of the internal cannula extended 1.0 mm beyond the tip of the guide cannula. The usual caveats about the specificity of the injection site must be raised as a 0.5 ul injection probably affects a 0.5 $mm^3$ area. However, even with that proviso, it appeared that the NAcS injections in Specific Aims 1 and 3 would not extend much further than the NAcS area itself. However, in Specific Aims 2 and 4, it would appear that injections into the baso-lateral AMY would extend into the central AMY, and vice-versa. For this reason, we typically describe NAcS effects with a great deal of specificity, whereas descriptions of AMY effects implicate the general AMY region.

**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, 2004). CS+/Fs refers to the flavored fructose+saccharin solution used in training, and CS+/s refers to the same flavor presented in saccharin only during choice testing. The CS-/s refers to the flavored saccharin solution used in training and testing. All solutions were given to the rats in sipper tubes mounted on the front of the cage held by a taut steel spring, and were positioned 3-6 cm above the cage floor. Solution intakes were measured to the nearest 0.1 g.

**Pre-Training Procedure**: Training and testing protocols took place in the rat's home cage during the mid-light phase of the light:dark cycle. Two weeks before pre-training, the rats were placed on a food restriction schedule that maintained their body weights at 85-90% of their ad libitum level. In order to familiarize the rats with the containing-solution bottles, they were trained to drink an unflavored 0.2% saccharin solution from sipper tubes during daily 1-h sessions. This pre-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.

**One-Bottle Training**: Rats were given ten one-bottle training sessions with 16 ml of the CS+/Fs solution presented on odd-numbered days, and 16 ml of the CS-/s solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests. One-bottle training intakes were limited to 16 ml/session to minimize the difference between CS+/Fs and CS-/s intakes. The left-right position of the CS and water sipper tubes was counterbalanced over the two days.

Solution intakes were measured by weighing (0.1 g) the bottles before and after the one-bottle training sessions.

**Two-Bottle Testing**: Following training, the rats were given eight two-bottle choice test sessions with unlimited (50 ml) access to the CS+/s and CS-/s solutions. The left-right position of the CS+/s and CS-/s sipper tubes was counterbalanced over the eight days. Solution intakes were measured by weighing (0.1 g) the bottles before and after the two-bottle choice sessions.

**Histological Analysis**: At the end of the experiments, 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. The brains were removed and soaked in a 10% formalin solution. Coronal 40-μm sections, stained with Cresyl violet, were examined by light microscopy by an observer unfamiliar with the behavioral data, and reconstructed on the appropriate frontal planes of the Paxinos and Watson's rat brain atlas. All animals with confirmed cannula placements were included in the data analysis.

**Statistical Analyses**: In the expression studies, training intakes were averaged over the five CS+/Fs and five CS+/s 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 drug groups, respectively. Separate ANOVAs evaluated total intakes and percent CS+/s intakes as a function of dose for the groups. In the acquisition studies, training intakes were averaged over the 4 CS+/Fs and 4 CS-/s sessions and were analyzed with a two-way randomized-blocks ANOVA (CS conditions x Groups). 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 drugs and control groups (Group x CS x Test). Separate two-way ANOVAs evaluated total CS intakes and percent CS+/s intakes of all the groups. When main or interaction effects were found, Bonferroni corrected comparisons ($p<0.05$) detected significant effects.

# CHAPTER THREE: DOPAMINE RECEPTOR ANTAGONISM IN THE NUCLEUS ACCUMBENS SHELL AND FRUCTOSE-CONDITIONED FLAVOR PREFERENCES

## Introduction

The neurochemical DA is involved in the reward value of sweet taste; particularly, sweet taste stimulates mesolimbic DA pathways that are implicated in the regulation of both natural and drug rewards (Genn et al., 2004). DA receptor antagonists reduce the intake of sweet solutions in rats (Salamone et al., 1997), probably through a reduction of the hedonic value of sweet taste (Smith, 2004) or other reasons such as changes in incentive salience (Berridge, 2007). DA antagonists also modify the ability of sweet solutions to strengthen the preference for other flavors. For instance, administration of raclopride, the selective D2 antagonist, reduced the preference for a flavored 10% sucrose solution paired with treatment relative to a differently-flavored sucrose solution paired with vehicle treatment (Hsiao et al., 1995). Sucrose can reinforce flavor preferences based on both its sweet taste (flavor-flavor conditioning) and its post-ingestive effects (flavor-nutrient conditioning) (Sclafani, 2002).

Different training procedures have been used to examine separately flavor-flavor and flavor-nutrient conditioning. Flavor-nutrient learning was studied in rats trained to drink flavors paired with IG infusions of sucrose and water. Systemic administration of a D1 antagonist (SCH23390), but not a D2 antagonist (raclopride), blocked flavor conditioning by IG sucrose infusions (Azzara, 2001). Neither drug showed much effect on the expression of a previously acquired flavor preference. Flavor-flavor learning was initially examined using a sham-feeding procedure in which rats fitted with a gastric

cannula were trained to drink a flavored sucrose solution and a less preferred flavored saccharin solution. Since gastric sham-feeding significantly reduces the post-ingestive actions of sucrose, a preference for the sucrose-mixed flavor over the saccharin-mixed flavor is believed to result from the sucrose's more palatable taste. Rats injected systemically with D1 (SCH23390) or D2 (raclopride) receptor antagonists during sham-feeding training sessions displayed preferences for the sucrose-mixed flavor similar to control rats (Yu et al., 2000b). However, both antagonists dose-dependently reduced the preference for the flavor originally mixed with sucrose when administered before the choice test, suggesting that D1 and D2 signaling are implicated in the expression of a conditioned preference (Yu et al., 2000a,b).

A restraint of the sham-feeding procedure was that the rats consumed substantially more of the flavored sucrose solution than the flavored saccharin solution during training and therefore were more familiar with the sucrose-mixed flavor. Therefore, a subsequent study examined flavor-flavor conditioning by training rats to real-feed similar amounts of a flavored fructose+saccharin solution and a less preferred saccharin solution. Again, fructose was used instead of other sugars (i.e., sucrose or glucose) because of its minimal post-ingestive flavor conditioning effects (Sclafani et al., 1993). Thus a preference for a flavor mixed into a fructose solution is believed to result from flavor-flavor conditioning. Using this training procedure, Baker et al., (2003), found that systemic treatment with SCH23390 and, to a lesser extent, raclopride blocked acquisition of flavor-flavor conditioning induced by the fructose+saccharin solution. Both drugs also significantly reduced the expression of a flavor preference previously conditioned by fructose.

The central anatomical sites of action for DA modulation of fructose-conditioned flavor-flavor preferences are unknown. Sweet taste activates DA efflux in the nucleus accumbens shell (NAcS) (Genn et al., 2004) while DA antagonists suppress lithium chloride-conditioned saccharin aversions when injected in this area (Fenu et al., 2001).

In view of these considerations, the next two experiments examined whether DA D1 (SCH23390) or D2 (raclopride) antagonists administered bilaterally into the NAcS dose-dependently altered the acquisition and/or expression of fructose-conditioned flavor-flavor preferences. This work has been published in the journal, Behavioural Brain Research (Bernal et al., 2008) and presented at Society for Neuroscience meetings (Bernal et al., 2007; Dostova et al., 2006).

## Methods

**Experiment 1A: Expression Procedure**: The first experiment determined whether D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **NAcS** dose-dependently altered the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the general Methods Section.

Training: Rats were given ten one-bottle training sessions (30 min/day) with 16 ml of the CS+/Fs solution presented on odd-numbered days, and 16 ml of the CS-/s solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests. Training intakes were limited to 16 ml/session to minimize the difference between CS+/Fs and CS-/s intakes. The position of the CS and

water sipper tubes varied across days using a left-right-right-left pattern. Following training, the rats were given eight two-bottle choice test sessions (30 min/day) with unlimited (50 ml) access to the CS+/s and CS-/s solutions. The position of the two sipper tubes was counterbalanced as described above. Solution intakes during the training and testing were measured by weighing (0.1 g) the bottles before and after the 30-min sessions.

Testing: Ten min prior to the two-bottle test sessions the rats were given bilateral injections (0.5 μl/side) through a stainless steel internal cannula (33-gauge, Plastics One) that extended 1 mm past the guide cannula. This was accomplished using a Hamilton microsyringe that was connected by polyethylene tubing to the internal cannula. For the first two sessions of two-bottle tests, all rats were given a vehicle (0.9% saline) injection. Based on their CS+/s and CS-/s intakes in these tests, the rats were divided into two matched groups. The D1 group was treated with the D1 antagonist, SCH23390 (Sigma Chemical Co.) at total doses of 12 (6 nmol/side), 24 (12 nmol/side) and 48 (24 nmol/side) nmol administered into the NAcS. Half of the rats were tested with an ascending dose order and the remaining rats were tested in a descending dose order. The D2 group was similarly tested, but with microinfusions of the D2 antagonist, raclopride (Sigma Chemical Co.) at total doses of 12, 24, and 48 nmol. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups.

**Experiment 1B: Acquisition Procedure**: The second experiment determined whether D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally

into the **NAcS**, dose-dependently altered the **acquisition** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the General Methods Section.

Training and Testing: Four groups of rats were matched for their intakes of an unflavored 0.2% saccharin solution prior to training. The rats were given eight one-bottle training sessions (60 min/day) with the CS+/Fs solution presented on odd-numbered sessions, and the CS-/s 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 NAcS injections. Rats in the D1 and D2 groups were given bilateral injections of the D1 antagonist, SCH23390 (12 nmol, 6 nmol/side) and the D2 antagonist, raclopride (12 nmol, 6 nmol/side), respectively, into the NAcS 10 min prior to each one-bottle training session. A third group (Yoked Control) received vehicle injections throughout one-bottle training, and their intakes of the CS+/Fs and CS-/s solutions were limited to the mean 60-min intakes of the D1 and D2 groups. A fourth group of unoperated rats (Control) was trained as above except without injections and with their CS+/Fs and CS-/s intakes limited to 16 ml/session; the purpose of this group was to evaluate the effectiveness of the training procedure. Following training, all groups were given six daily two-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. The positions of the CS+/s and CS-/s solutions were counterbalanced across sessions, and the results were analyzed as mean 60-min intakes during successive pairs of sessions (referred to as Tests 1, 2, and 3).

## Results

*Histological Verification*: Figure 1 is a schematic representation (Paxinos and Watson, 1998) of the bilateral cannula placements of all 56 animals in the expression (SCH23390 [SE, n=12]; raclopride [RE, n=12]) and acquisition (SCH23390 [SA, n=7]; raclopride [RA, n=6]; yoked [YA, n=19]) experiments. The bilateral cannulae were localized within the shell region of the NAcS, or bordered the core and shell regions of the NAc, or bordered the NAcS and immediately adjacent ventral diagonal band area. Multiple animals had highly similar cannula placements, and there was considerable overlap of placements for the animals involved in the five different groups, those receiving SCH23390 or raclopride in the Expression experiment, and those receiving SCH23390, raclopride or vehicle (Yoked Control) in the Acquisition experiment.

*Experiment 1. Effects of DA antagonism in the NAcS on the expression of fructose-CFP.* The mean one-bottle training intake of the CS+/Fs solution (11.2 ml) significantly ($t(23)= 4.75$, $p<0.0001$) exceeded that of the CS-/s solution (8.4 ml). In the two-bottle preference tests, overall, the D1 rats consumed significantly more CS+/s than CS-/s ($F(1,44)= 80.62$, $p<0.0001$), but total intake significantly varied as a function of drug dose ($F(3,44)= 4.55$, $p<0.007$) and there was a significant CS x Dose interaction ($F(3,44)= 5.57$, $p<0.003$). CS+/s intakes significantly exceeded CS-/s intakes at the 0 (vehicle) and 12 nmol dose of SCH23390 but not at the 24 and 48 nmol SCH23390 doses (Figure 2A). The rats consumed significantly less CS+/s at the 24 and 48 nmol SCH23390 doses as compared to vehicle; CS-/s intakes failed to differ as a function of SCH23390 dose (Figure 2A). Significant differences in the percent CS+/s intakes were observed ($F(3,33)= 4.68$, $p<0.008$) and the preference (62%) at the 48 nmol SCH23390

dose was significantly lower than the preference (76%) following vehicle (Figure 2A). Preferences at the 12 (68%) and 24 (63%) nmol SCH23390 doses were intermediate but failed to significantly differ from the vehicle test. Differences in total CS intakes were observed across doses ($F(3,33)= 7.48$, $p<0.0006$) and the rats consumed significantly less at the 24 (11.1 ml) and 48 (10 ml), but not 12 (13.3 ml) nmol SCH23390 doses relative to vehicle (15 ml).

In the two-bottle preference tests, overall, the D2 rats consumed significantly more CS+/s than CS-/s overall ($F(1,42)= 111.53$, $p<0.0001$), but total intake significantly varied as a function of drug dose ($F(3,42)= 5.38$, $p<0.003$) and there was a significant CS x Dose interaction ($F(3,42)= 4.53$, $p<0.008$). CS+/s intake was significantly higher than CS-/s intake at the 0, 12 and 48 nmol, but not the 24 nmol doses of raclopride (Figure 2B). The rats consumed significantly less CS+/s at the 24 and 48 nmol raclopride doses as compared to vehicle; CS-/s intakes failed to differ among raclopride doses (Figure 2B). There were significant differences in the percent CS+/s intakes ($F(3,33)= 5.33$, $p<0.004$) with the preference (63%) at the 24 nmol raclopride dose significantly lower than the preference (75%) following vehicle (Figure 2B). Preferences following the 12 (71%) and 48 (69%) nmol raclopride doses failed to differ significantly from vehicle. Significant differences in total intake were observed across doses ($F(3,33)= 6.18$, $p<0.002$) with total CS intakes at the 12 (11.8 ml), 24 (12.4 ml) and 48 (11.9 ml) nmol raclopride doses significantly lower than following vehicle (15.4 ml).

Figure 12

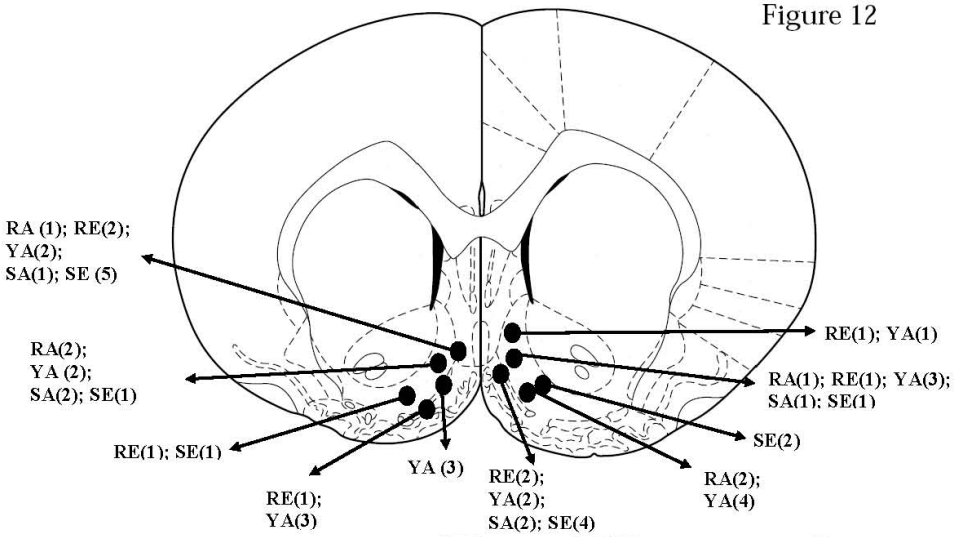



Figure 13

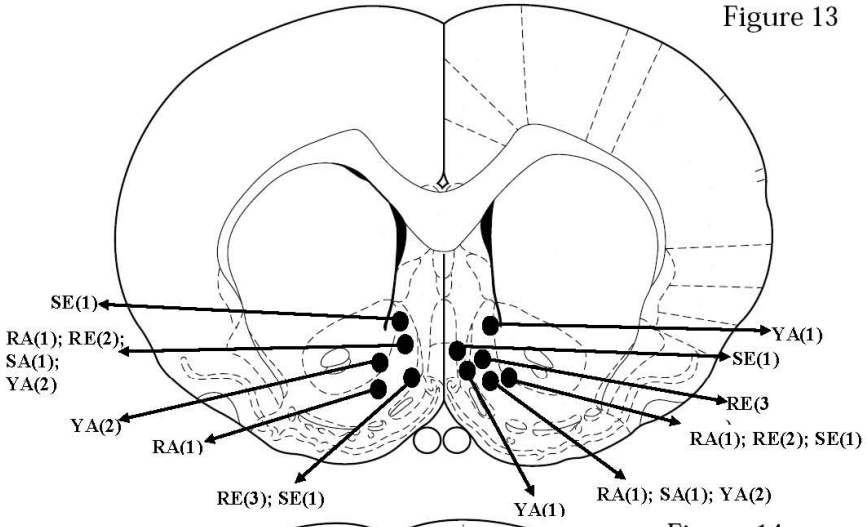



Figure 14

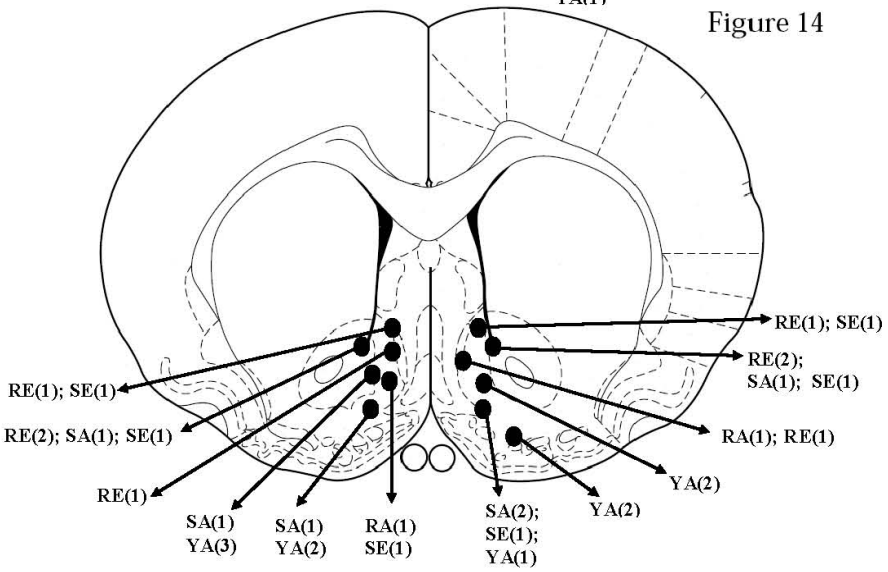
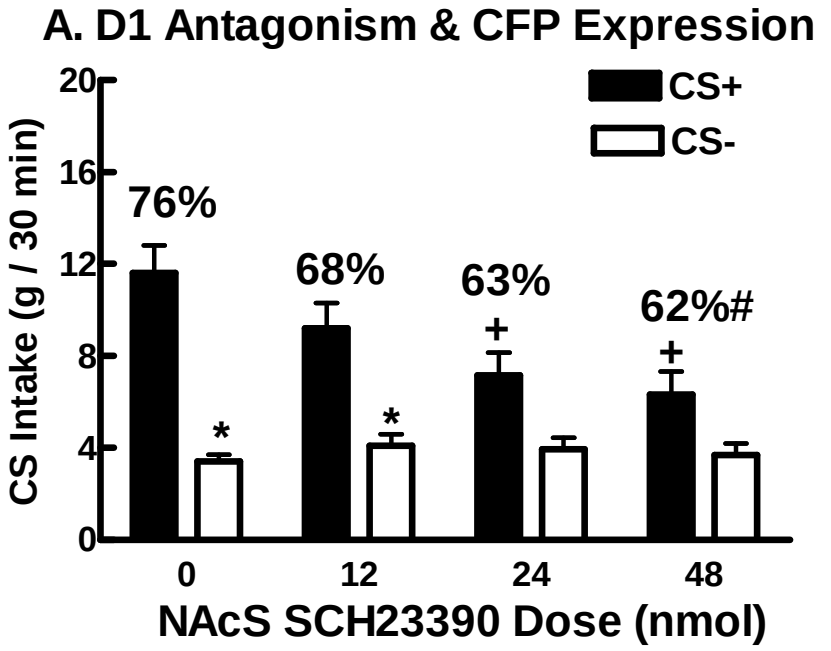


**Figure 1.** Bilateral representation of cannula sites aimed at the nucleus accumbens shell (NAcS) region of 56 rats using Figures 12, 13 and 14 of the stereotaxic atlas of Paxinos and Watson based on a scale of 1.6 cm of the depicted structures equaling 1 mm of the actual distance between structures. The bilateral cannulae were localized within the shell region of the NAcS, or bordered the core and shell regions of the NAc, or bordered the NAcS and immediately adjacent ventral diagonal band area. Multiple animals had highly similar cannula placements, and there was considerable overlap of placements for the animals receiving SCH23390 (SE, n=12) and raclopride (RE, n=12) in the Expression paradigm, and for the animals receiving SCH23390 (SA, n=7), raclopride (RA, n=6) and vehicle (Yoked Control, YA, n=19) in the Acquisition paradigm.

## Figure 2


A. D1 Antagonism & CFP Expression
CS+
CS-
CS Intake (g / 30 min)
20
16
12
8
4
0
76%
68%
63%
+
62%#
+
*
*
0
12
24
48
NAcS SCH23390 Dose (nmol)

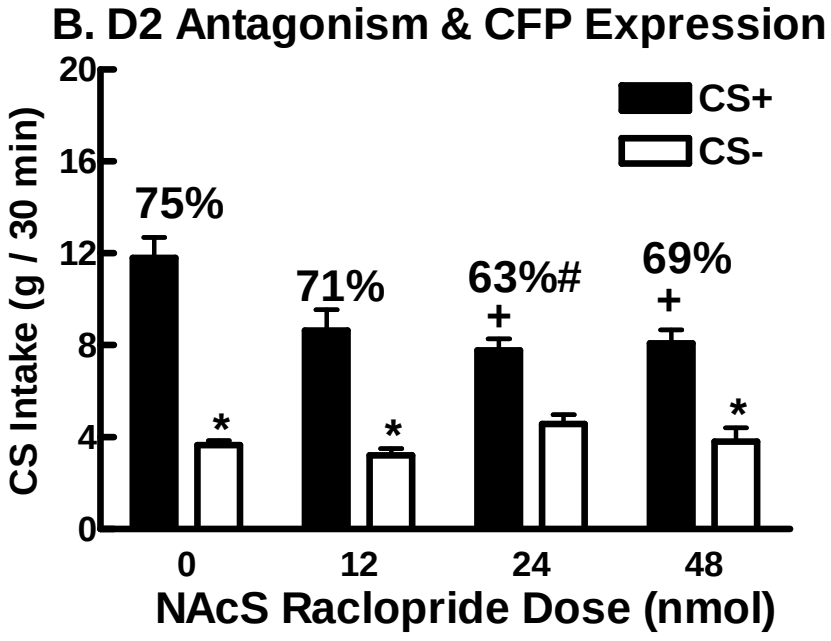

B. D2 Antagonism & CFP Expression
CS+
CS-
CS Intake (g / 30 min)
20
16
12
8
4
0
75%
71%
63%#
+
69%
+
*
*
*
0
12
24
48
NAcS Raclopride Dose (nmol)

**Figure 2 (Exp 1A. Expression Procedure).** Intakes (mean ±SEM, 0.5 h) of CS+/s and CS-/s solutions in two-bottle tests in animals receiving bilateral NAcS injections of the D1 dopamine antagonist, SCH23390 (upper panel) or the D2 dopamine antagonist, raclopride (lower panel) at total doses of 0, 12, 24 or 48 nmol 10 min prior to testing. Significant differences are denoted between CS+/s and CS-/s intake within an injection condition (*) and between CS+/s intake following a drug dose relative to the vehicle treatment (+). The percentages of CS+/s intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (#) noted.

*Experiment 1B. Effects of DA antagonism in the NAcS on the acquisition of fructose-CFP*. In the one-bottle training intakes, overall CS+/Fs intake (11.9 ml) significantly ($F(1,18)= 76.82$, $p<0.0001$) exceeded CS-/s intake (10.1 ml) with the four groups displaying significant main ($F(3,54)= 13.1$, $p<0.0001$) and interaction ($F(3,54)= 8.29$, $p<0.0001$) effects with CS condition. Control rats displayed significantly greater CS+/Fs than CS-/s (13.4 vs. 10.6 ml) intakes; similarly, the Yoked Control rats consumed more CS+/Fs than CS-/s (10.4 vs. 7.8 ml). In contrast, significant CS+Fs vs. CS+/s differences failed to occur in D1 (11.5 vs. 9.9 ml) and D2 (12.3 vs. 12.1 ml) groups. CS+/Fs intake was significantly higher in Control rats relative to Yoked Control animals, and CS-/s intakes were significantly higher in D1, D2, and Control groups relative to the Yoked Control group. This occurred because some of the yoked control rats did not consume their entire allotted ration in every session.

Following training, the rats were given three consecutive series of preference tests (2 sessions each) without drug treatment. Significant differences in intake were observed between the CS+/s and CS-/s solutions ($F(1,18)= 219.24$, $p<0.0001$), among the four training groups ($F(3,54)= 10.27$, $p<0.0001$), and among the three tests ($F(2,36)= 23.04$, $p<0.0001$). In addition there were interactions between groups and tests ($F(6,108)= 116.65$, $p<0.0001$), between CS and tests ($F(2,36)= 39.69$, $p<0.0001$), and among groups, CS, and tests ($F(6,108)= 17.82$, $p<0.0001$). Within group comparisons revealed that the Yoked Control and Control groups (Figures 3C, 3D) consumed significantly more CS+/s than CS-/s in all three Tests. The D1 and D2 groups, in contrast, consumed significantly more CS+/s than CS-/s in Tests 1 and 2 but not in Test 3 indicating extinction of the CS+/s preference (Figures 3A, 3B). This loss of preference was due to a selective and

significant reduction in CS+/s intakes from Test 1 to 3 in the D1 and D2 groups. The CS+/s intakes of the D1 and D2 groups significantly exceeded those of the Yoked Control in Tests 1 and 2, but were significantly below both control levels in Test 3. CS-/s intakes failed to change over testing in any group. Analysis of the percent CS+/s data failed to reveal any significant overall group difference but there was an interaction between group and tests ($F(6,108)= 7.01$, $p<0.0001$). Whereas percent CS+/s intakes remained stable across the Tests in both Control groups (Figures 3C, 3D), the percent intakes significantly decreased in the D1 and D2 groups with scores significantly lower in Test 3 than in Test 1. Significant differences in total CS intakes were observed among the four groups ($F(3,54)= 10.27$, $p<0.0001$), among the three tests ($F(2,36)= 22.04$, $p<0.0001$), and for the interaction between groups and tests ($F(6,108)= 116.65$, $p<0.0001$). Total CS intakes significantly increased from the first to third Tests in the Yoked (14.0 to 18.7 ml) and Control (13.3 to 15.8 ml) groups. In contrast, total CS intakes significantly decreased from the first to third Test in the D1 (20.2 to 15.1 ml) and D2 groups (19.0 to 13.4 ml). In Test 3, total CS intake in the D1 group were significantly lower than that of the Yoked group, and the total CS intake of the D2 group was significantly lower than that of the Yoked and Control groups. Finally, total CS intakes in the Yoked control group were significantly higher than those of the Control group in Tests 1 and 3.

# Figure 3

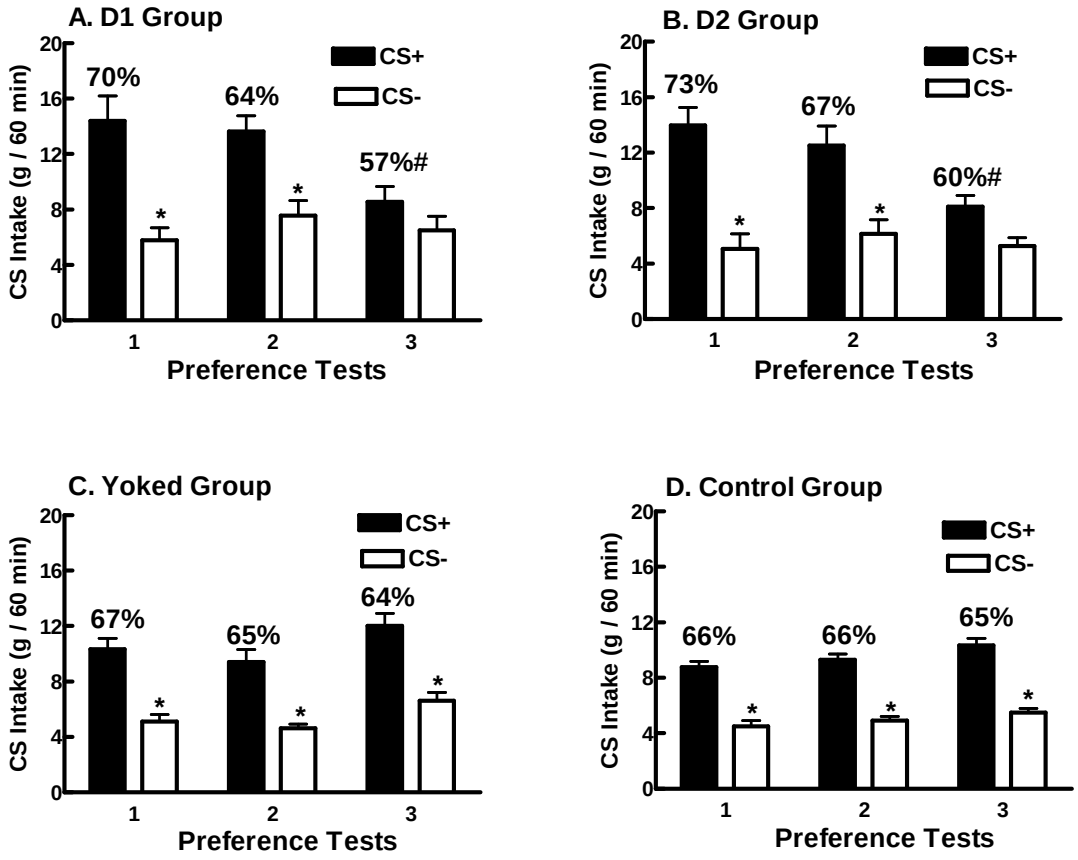

A. D1 Group
CS+
CS-
70%
64%
57%#
*
CS Intake (g / 60 min)
Preference Tests
B. D2 Group
73%
67%
60%#
C. Yoked Group
67%
65%
64%
D. Control Group
66%
66%
65%

**Figure 3 (Acquisition Study).** Intakes (mean ±SEM, 1 h) of three pairs of CS+/s and CS-/s solutions during three pairs of two-bottle tests. During training, the D1 group received NAcS injections of SCH23390 (12 nmol, Panel A) and the D2 group received NAcS injections of raclopride (12 nmol, Panel B); the Yoked Control group received NAcS vehicle injections (Panel C) while the Control group (Panel D) received no injections during training. Significant differences are denoted between CS+/s and CS-/s intake within each test are denoted (*) as are significant differences in the percentage of CS+/s intake over total intake (#).

*Differences in the Magnitude of Fructose-Conditioned Flavor Preferences in the Acquisition and Expression Paradigms.* Compared to the training procedure used in the expression paradigm (10 days of one-bottle training, 5 with the CS+/Fs and 5 with the CS-/s in 30 min sessions), training procedure in the acquisition paradigm was modified so as to reduce the impact of repeated NAcS microinjections by decreasing the number of training sessions from 10 to 8, by introducing a rest day between each of the pairs of training sessions, but also lengthening the training session from 30 to 60 min. To evaluate whether modifications in the training procedures affected fructose-conditioned preferences, combined two-day vehicle preference values of the SCH23390-tested and raclopride-tested rats in the expression paradigm were compared with the preference score (Test 1) of the untreated control group in the acquisition paradigm. Significant differences in intake were observed between acquisition and expression paradigms ($F(1,23)= 4.97$, $p<0.036$), between CS+/s and CS-/s solutions ($F(1,23)= 218.49$, $p<0.0001$), and for the interaction between paradigms and solutions ($F(1,23)= 26.50$, $p<0.0001$). Whereas the acquisition and expression training both resulted in significant respective increases in CS+/s intake (acquisition: 8.8 ml; expression: 11.7 ml) over corresponding CS-/s intake (acquisition: 4.5 ml; expression: 3.5 ml), the CS+/s intake of the expression group was significantly higher than the CS+/s intake of the acquisition group. CS-/s intakes failed to differ between the two groups. Correspondingly, the percentage of CS+/s intake over total intake was significantly ($t(34)= 4.34$, $p<0.0002$) higher in the expression paradigm (75.8%) than in the acquisition paradigm (66.4%).

## Discussion

The present study demonstrated that direct bilateral administration into the NAcS of either the D1 receptor antagonist SCH23390 or the D2 receptor antagonist raclopride significantly attenuated the expression of a preference for a flavor paired with a frucrose/saccharin solution. These data are in agreement with the previous reports that systemic administration of D1 and D2 antagonists eliminated the expression of fructose-conditioned flavor preferences in real-feeding rats (Baker et al., 2003), and reduced the expression of sucrose-conditioned flavor preferences in sham-feeding rats (Yu et al., 2000a, b). Taken together, these findings indicate that the NAcS is a central site of action at which dopamine antagonists interfere with the expression of sugar-conditioned flavor-flavor preferences.

It is important to note, however, that the respective effects of systemic and NAcS administration differed in magnitude. SCH23390 administered into the NAcS reduced the fructose-conditioned CS+ preference from 76% (0 nmol dose) to 62% (48 nmol dose). In contrast, systemic SCH23390 eliminated the CS+ preference (39-55%) at all doses tested (50–800 nmol/kg) compared to the 77% preference observed with vehicle treatment (Baker et al., 2003). The ability of D2 antagonism using raclopride in the NAcS to reduce the fructose-conditioned CS+ preference (75% for vehicle, 63% for the 24 nmol raclopride dose) was comparable to SCH23390, but not as dose-dependent. Similarly, systemic administration of raclopride significantly reduced the expression of the CS+ preference, but this effect was less profound and dose-dependent than that observed with systemic SCH23390 treatment. The data obtained with NAcS and systemic administration of SCH23390 suggests that the NAcS is one, but not the only site that

participates in the dopaminergic mediation of the expression of fructose-conditioned flavor preferences. Consistent with this view, our laboratory (Bernal et al., 2006) recently found that D1 and D2 antagonists administered into the amygdala significantly reduced the expression of fructose-conditioned flavor preferences, suggesting a regional network of sites mediating this type of flavor-flavor conditioning. Such regional networks of interacting brain sites have been proposed in other feeding paradigms (e.g., Baldo et al., 2007; Bodnar et al., 2008; Stratford et al., 1999; Will et al., 2003).

In addition to attenuating the expression of the CS+ preference, injection of SCH23390 or raclopride into the NAcS during training influenced the acquisition of fructose-conditioned CS+ preference, albeit in a subtle way. Thus, D1 and D2 groups displayed a significant preference for the CS+ flavor in the initial pair of two-bottle tests (70–73%), but this preference declined to 57–60% in Test 3, and were no longer significant. In contrast, the rats in the Yoked and Control groups displayed a stable preference of about 65% from the first through the third pairs of tests. The Control group data agrees with our original study showing that fructose-conditioned flavor preferences are relatively stable (i.e., resistant to extinction) with repeated testing (Ackroff et al., 2004). However, subsequent research revealed that the stability of such a preference varies as a function of the amount of CS solutions (CS+/Fs and CS-/s) the animals are given during one-bottle training sessions (Yiin et al., unpublished observations, 2005). Thus, animals trained with 10 ml rations of the CS+/Fs and CS-/s during one-bottle sessions tended to reduce their CS+ preference during repeated two-bottle testing in contrast to animals trained with CS rations of 15 ml or greater. In the present study, the D1 and D2 rats were limited to 16 ml of CS solutions during training although their

average training intake was 10-12 ml/session. Thus, in this and the subsequent AMY study, any impairments of the D1 or D2 antagonists were minimal, and therefore strong behavioral impairments were not observed. Although not formally measured, the latency of D1-treated or D2-treated animals approaching the single bottle during training days did not appear to differ from untreated controls or vehicle-treated yoked controls. Despite this, the paradigms employed Yoked Control groups. Indeed, the Yoked Control group consumed slightly less because of the yoking procedure limiting them to a 12 ml ration at paradigm onset, and indeed consumed significantly less CS+/Fs and CS-/s solutions during training relative to the Control group. Moreover, the methodological differences in the expression and acquisition training paradigms produced significantly greater preferences for the expression (76%: ten sessions) relative to the acquisition (65%: eight sessions). However, differences in training intakes among the drug and control groups do not readily explain why the D1 and D2 rats, unlike the control rats, lost their CS+ preference with repeated two-bottle testing. It may be, however, that the salience of the CS+/Fs solution was reduced by the SCH23390 and raclopride treatment during training such that the D1 and D2 rats behaved as if they consumed less CS+/Fs than did the control rats during training. This explanation is supported by a proposed role for incentive salience in dopaminergic control of food intake and reward (e.g., Berridge, 2007; Berridge et al., 1998). According to this analysis, increasing the salience of the CS+/Fs solution during training (e.g., by increasing the sugar concentration) may prevent the extinction of CS+ preference observed in rats treated with DA receptor antagonists in the NAcS.

A second possibility for differences in initial preferences observed in the expression (76%) and acquisition (65%) studies was that the former received vehicle before testing, whereas the latter received no injection treatment before testing. However, the observed greater effects of expression-elicited CFPs over acquisition-elicited CFPs is counter-intuitive to any hypothesis suggesting that the "stress" of a vehicle injection would impair subsequent behavior relative to no intervention before testing.

In contrast to the present results, systemic treatment with SCH23390 or raclopride (200 nmol/kg) completely prevented the acquisition of a fructose-conditioned CS+ flavor-nutrient preference (Baker et al., 2003). Animals given systemic injections of the D1 and D2 antagonists displayed percent CS+ intakes of 46-56% (i.e., no preference) in flavor-nutrient conditioning compared to Yoked Control rats that had percent intakes of 66%. Taken together, these results indicate that the NAcS is not the critical site for the acquisition of a fructose-conditioned flavor preference. The involvement of the NAc core, which is implicated in flavor-nutrient learning (Touzani et al., 2006), as well as other brain sites involved in flavor-flavor learning, requires further investigation.

Accumbens dopamine signaling is implicated in other forms of flavor learning. As noted previously, systemic D1, but not D2 antagonism blocks the acquisition of a flavor preference conditioned by the post-oral actions of sucrose (Azzara et al., 2001). More recent findings demonstrate that injection of SCH23390 into the NAcS blocked flavor preference conditioning by IG infusions of glucose (Touzani et al., 2006). The effects of NAc injections of raclopride on flavor-nutrient learning were not investigated given the lack of effect obtained with systemic injections of the D2 antagonist. The NAc is a site at which sweet solutions in the mouth stimulate dopamine efflux (e.g., Cheng et al., 2006;

Genn et al., 2004; Hajnal et al., 2003). Whether IG sugar infusions also promote dopamine release in the NAc is not known. If the post-oral actions of sugar do not promote dopamine release, this could explain why D2 receptor signaling is involved in flavor-flavor but not flavor-nutrient learning. That is, the D2 antagonist may act to reduce the reward value of an oral US but not a post-oral US. Interestingly, NAcS injection of a D1, but not a D2, antagonist was observed to block LiCl-induced saccharin taste aversions (Fenu et al., 2001). These findings along with the IG sugar data suggest only D1 receptors mediate flavor learning that involves post-oral negative as well as positive unconditioned stimuli. Flavor aversions are produced by combining a neutral CS flavor with an unpalatable flavor (e.g., bitter taste; (Fanselow et al., 1982)). Whether flavor-flavor aversion conditioning, like flavor-flavor preference conditioning, involves both D1 and D2 receptor signaling, remains to be investigated.

Given the partial reductions in the expression of fructose-CFP by DA D1 and D2 antagonists in the NacS, and the failure to affect initial acquisition, but hasten subsequent extinction of fructose-CFP by DA D1 and D2 antagonists in the NacS, the next experiment (Chapter 4) examined the role of DA in the AMY in these responses.

# CHAPTER FOUR: DOPAMINE RECEPTOR ANTAGONISM IN THE AMYGDALA AND FRUCTOSE-CONDITIONED FLAVOR PREFERENCES

## Introduction

In the previous chapter, the nucleus accumbens shell (NAcS) was examined as one of the potential central anatomical sites of action for dopaminergic modulation of a fructose-based CFP because this was a site in which sweet taste stimulated DA efflux (e.g., Cheng and Feenstra, 2006; Genn et al., 2004), and in which DA antagonists suppressed lithium chloride-conditioned saccharin aversions (Fenu et al., 2001).

The AMY is also a site implicated in flavor aversion learning (Bures et al., 1998), flavor preference learning (Gilbert et al., 2003; Touzani and Sclafani, 2005), and in Pavlovian and instrumental reward learning (Baxter and Murray, 2002; Cardinal et al., 2002). Moreover, feeding and gastric nutrient infusions increased AMY DA turnover or efflux (Hajnal and Lenard, 1997; Heffner et al., 1980), and a Pavlovian CS for food elicited AMY DA efflux (Harmer and Phillips, 1999). Further, AMY microinfusions of amphetamine facilitated learning to respond to a food-related CS (Hitchcott et al., 1997), whereas AMY inactivation modulated feeding-stimulated DA efflux in the NAc (Ahn and Phillips, 2002). Therefore, the next two experiments examined whether DA D1 (SCH23390) or D2 (raclopride) antagonists administered bilaterally into the AMY would alter the acquisition and/or expression of a flavor preference conditioned by the sweet taste of fructose. This work has been published in the journal, Behavioural Brain Research (Bernal et al., 2009) and presented at Society for Neuroscience meetings (Bernal et al., 2006, 2007).

**Experiment 2A: Expression Procedure**: The first experiment determined whether D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **AMY** dose-dependently altered the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the General Methods Section.

Training: Rats were given ten one-bottle training sessions (30 min/day) with 16 ml of the CS+/Fs solution presented on odd-numbered days, and 16 ml of the CS-/s solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests. Training intakes were limited to 16 ml/session to minimize the difference between CS+/Fs and CS-/s intakes. The left-right position of the CS and water sipper tubes was counterbalanced over the two days across animals within groups. Following training, the rats were given eight two-bottle choice test sessions (30 min/day) with unlimited (50 ml) access to the CS+/s and CS-/s solutions. Solution intakes during the training and testing were measured by weighing (0.1 g) the bottles before and after the 30-min sessions.

Testing: Ten min prior to the two-bottle test sessions, the rats were given bilateral AMY injections (0.5 μl/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 by using a Hamilton microsyringe that was connected by polyethylene tubing to the internal cannula. For the first two sessions of two-bottle tests, all rats were given a vehicle (0.9% saline) injection. Based on their CS+/s and CS-/s intakes in these tests, the

rats were divided into two matched groups. The D1 group was treated with the D1 antagonist, SCH23390 (Sigma Chemical Co.) at total doses of 12 (6 nmol/side), 24 (12 nmol/side) and 48 (24 nmol/side) nmol administered into the AMY. Half of the rats were tested with an ascending dose order, and the remaining rats were tested in a descending dose order. The D2 group was similarly tested, but with AMY microinfusions of the D2 antagonist, raclopride (Sigma Chemical Co.) at total doses of 12, 24, and 48 nmol. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups. Ten min following drug treatment, the 50 ml rations of the CS+/s and CS-/s solutions were presented, and intake measured after 30 min.

**Experiment 2B: Acquisition Procedure**: The second experiment determined whether D1 (SCH23390) or D2 (raclopride) DA receptor antagonists injected bilaterally into the **AMY** dose-dependently altered the **acquisition** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the General Methods Section.

Training and Testing: Four groups of rats were matched for their intakes of an unflavored 0.2% saccharin solution prior to training. The rats were given eight one-bottle training sessions (60 min/day) with the CS+/Fs solution presented on odd-numbered sessions, and the CS-/s 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 AMY microinjections. Rats in the D1 and D2 groups were given bilateral injections of the D1 antagonist, SCH23390 (12 nmol, 6 nmol/side) or the D2 antagonist,

raclopride (12 nmol, 6 nmol/side), respectively, into the AMY 10 min prior to each one-bottle training session. These doses were identical to those employed in acquisition testing following microinjections into the NAcS (see previous chapter). A third group (Yoked Control) received AMY vehicle injections throughout one-bottle training, and their exposure to the CS+/Fs and CS-/s solutions was limited to the mean 60-min intakes of the D1 and D2 groups. A fourth group of unoperated rats (Control) was trained as above except without injections and with their CS+/Fs and CS-/s intakes limited to 16 ml/session; the purpose of this group was to evaluate the effectiveness of the training procedure. Following training, all groups were given six daily two-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. The positions of the CS+/s and CS-/s solutions were counterbalanced across sessions.

**Results**

*Histological Verification*: Figure 4 is a schematic representation (Paxinos and Watson, 1998) and contains a detailed description of the bilateral cannula placements (n=126) of all 63 animals in experiments 1 and 2. Cannulae were distributed in the rostral (n= 25 animals), middle (n= 25 animals) and caudal (n=13) levels of the AMY. Multiple animals had highly similar cannula placements, and there was considerable overlap of placements for the animals included in the five different groups.

*Experiment 2A. Expression study.* During one-bottle training, the mean intake of the CS+/Fs solution significantly exceeded that of the CS-/s solution (12.3 vs. 8.4 g/30 min, $t(28)= 6.84$, $p<0.0001$). In the two-bottle preference tests conducted with the D1 group, overall, CS+/s intakes significantly exceeded CS-/s intakes ($F(1,55)= 180.54$, $p<0.0001$)

with intakes significantly varying as a function of drug dose ($F(3,55)= 4.92$, $p<0.004$) and the interaction between CS conditions and drug doses ($F(3,55)= 4.92$, $p<0.004$). CS+/s intake was significantly higher than CS-/s intake following the vehicle and all SCH23390 doses (Figure 5A). The 48 nmol total dose of SCH23390, but not the 12 and 24 mol total doses, significantly reduced CS+/s intake relative to vehicle (Figure 5A). Significant differences in the percent CS+ intakes were observed across doses ($F(3,42)= 10.46$, $p<0.0001$), and the preference (66%) following the 48 nmol SCH23390 dose was significantly lower than the preference (77%) following vehicle (Figure 5A). Preferences at the 12 (71%) and 24 (74%) nmol doses of SCH23390 were intermediate, and failed to differ significantly from the vehicle preference. Significant differences in total intake were observed across SCH23390 doses ($F(3,42)= 14.99$, $p<0.0001$), and total CS intakes were less following the 12 (14.8 g), 24 (14.8 g) and 48 (12.3 g) nmol total doses relative to vehicle (18.7 g).

In the two-bottle preference tests conducted with the D2 rats, overall, CS+/s intakes significantly exceeded CS-/s intakes ($F(1,52)= 233.07$, $p<0.0001$) with intakes significantly varying as a function of drug dose ($F(3,52)= 2.99$, $p<0.039$) and the interaction between CS conditions and drug doses ($F(3,52)= 4.48$, $p<0.007$). CS+/s intake was significantly higher than CS-/s intake following vehicle and all raclopride doses (Figure 5B). The 48 nmol total dose of raclopride, but not the 12 and 24 mol doses, significantly reduced CS+/s intake relative to vehicle (Figure 5B). Significant differences in the percent CS+ intakes were observed across doses ($F(3,39)= 5.75$, $p<0.002$), and the preference (68%) following the 48 nmol raclopride dose was significantly lower than the preference (77%) following vehicle (Figure 5B). Preferences following the 12 (76%) and

Figure 25

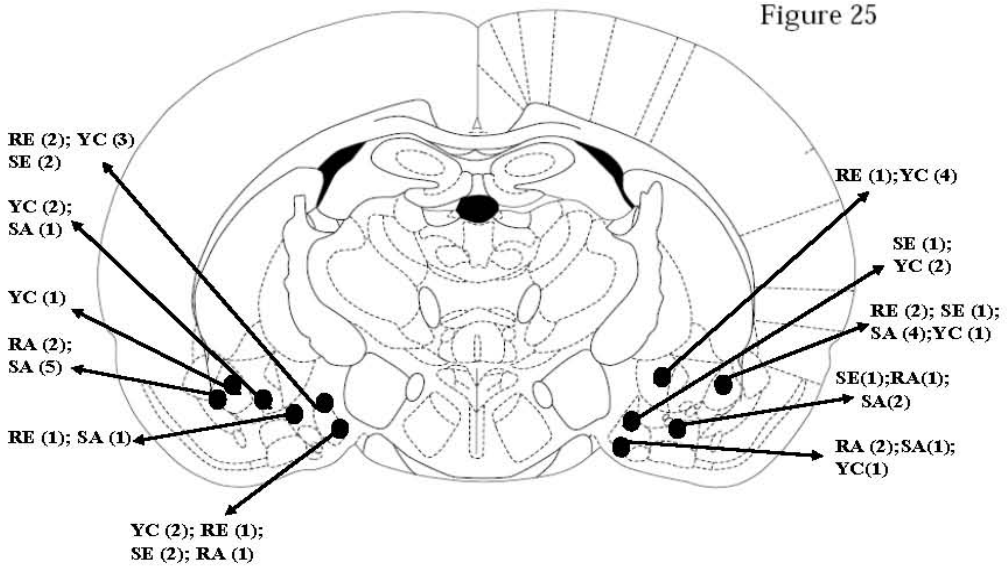



Figure 28

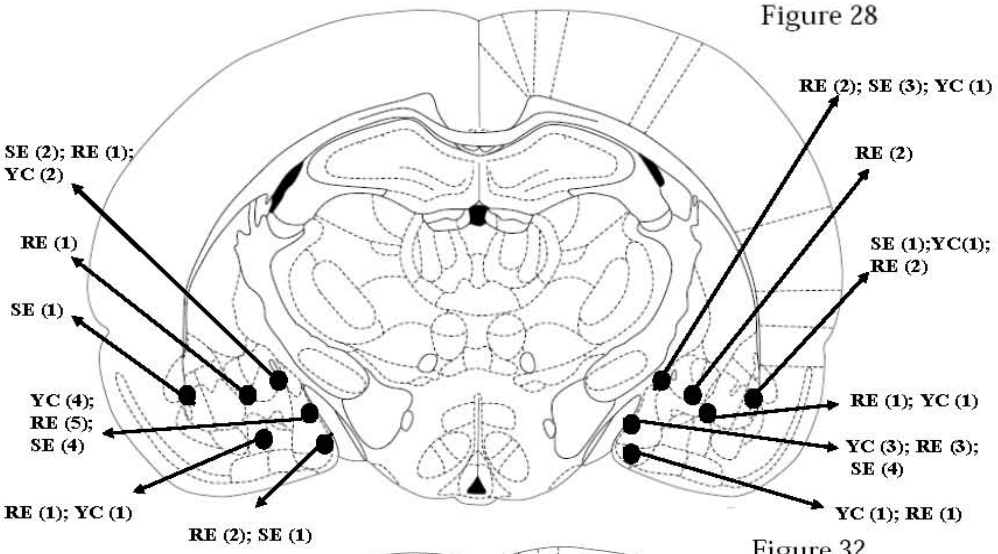



Figure 32

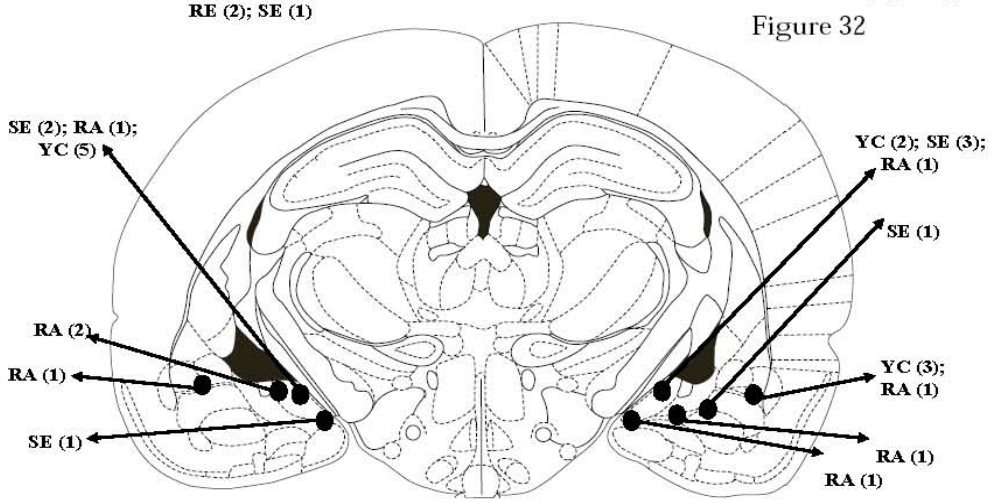


**Figure 4.** Bilateral representation of cannula sites (n = 126) aimed at the amygdala (AMY) of 63 animals in Experiment 1 (SCH23390 [SE, n=15]; raclopride [RE, n=14]) and Experiment 2 (SCH23390 [SA, n=7], raclopride [RA, n=7]; yoked [YA, n=20] using Figures 25 (Bregma -1.80 mm), 28 (Bregma -2.56 mm) and 32 (Bregma -3.60 mm) of the stereotaxic atlas of Paxinos and Watson (1997) based on a scale of 1.6 cm of the depicted structures equaling 1 mm of the actual distance between structures. Cannulae were localized in or near the medial AMY nuclei bilaterally (n= 20 animals), centro-medial AMY nuclei bilaterally (n= 5 animals), baso-lateral AMY nuclei bilaterally (n= 4 animals), unilateral medial and centro-medial AMY nuclei (n= 14 animals), unilateral medial and baso-lateral AMY nuclei (n= 18 animals), and unilateral centro-medial and baso-lateral AMY nuclei (n= 2 animals). Cannulae were distributed in the rostral (n= 25 animals), middle (n= 25 animals) and caudal (n=13) levels of the AMY. Multiple animals had highly similar cannula placements, and there was considerable overlap of placements for the animals included in the five different groups.

## Figure 5

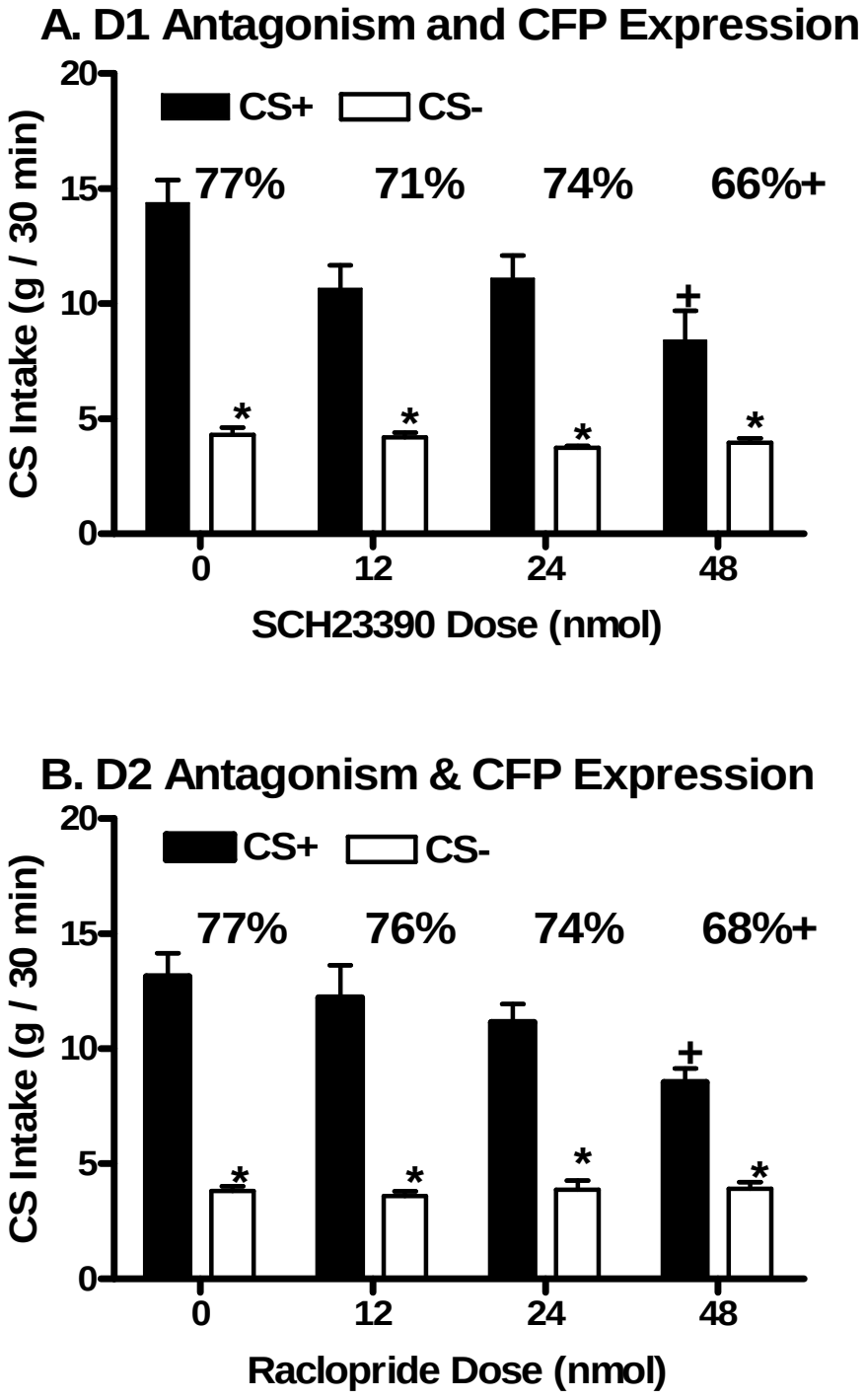

A. D1 Antagonism and CFP Expression
CS+
CS-
77%
71%
74%
66%+
CS Intake (g / 30 min)
SCH23390 Dose (nmol)
0
12
24
48
B. D2 Antagonism & CFP Expression
77%
76%
74%
68%+
Raclopride Dose (nmol)

**Figure 5. Experiment 2A (expression procedure**). Intakes (mean +SEM, g/30 min) of CS+/s and CS-/s solutions in two-bottle tests in animals receiving bilateral AMY microinjections of the D1-like dopamine antagonist, SCH23390 (upper panel) or the D2-like dopamine antagonist, raclopride (lower panel) at total doses of 0, 12, 24 or 48 nmol 10 min prior to testing. Significant differences are denoted between CS+/s and CS-/s intake within an injection condition (*) and between CS+/s intake following a drug dose relative to the vehicle treatment (+). The percentages of CS+/s intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (+) noted.

48 (74%) nmol raclopride doses failed to differ from the vehicle preference. Significant differences in total intake were observed across raclopride doses ($F(3,39)= 8.09$, $p<0.0003$) with total CS intakes following the 48 (12.3 g), but not the 12 (15.8 g) or 24 (15.0 g), nmol raclopride dose significantly reduced relative to that following vehicle (16.9 g).

*Experiment 2B. Acquisition study.* In the one-bottle training sessions, overall, CS+/Fs intake significantly exceeded CS-/s intake (12.9 vs. 9.7 g/1 h, $F(1,19)= 242.08$, $p<0.0001$), the four groups significantly differed from each other ($F(3,57)= 15.53$, $p<0.0001$), and there was a marginally significant interaction between groups and conditions ($F(3,57)= 2.653$, $p=0.057$). Overall mean CS intake for the Yoked Control group (9.2 g) was significantly less than the D1 (12.4 g), D2 (11.7 g) and the Control (12.0 g) groups. The yoked controls consumed less than the D1 and D2 groups apparently because they were provided with a lesser amount (12 ml per session) during training trials than the D1, D2 and untreated Control groups (16 ml per session). In particular, CS+/Fs intakes were significant greater than CS-/s intakes for the Control rats (13.4 vs. 10.6 g), Yoked Control rats (10.9 vs. 7.4 g) and D2 rats (13.9 vs. 9.5 g), but not for the D1 rats (13.7 vs. 11.2 g). Moreover, CS+/Fs intakes were significantly higher in the D1, D2 and Control groups relative to the Yoked Control group, and CS-/s intakes were significantly higher in the D1 and Control groups relative to the Yoked Control group.

In the two-bottle preference tests, there were significant differences in CS+/s and CS-/s intakes ($F(1,19)= 359.90$, $p<0.0001$), among the four training groups ($F(3,57)= 2.92$, $p<0.042$) and among the three tests ($F(2,38)= 17.63$, $p<0.0001$). In addition, there were significant interactions between groups and tests ($F(6,114)= 17.97$, $p<0.0004$),

between CS solutions and tests ($F(2,38)= 16.54$, $p<0.0001$), and among groups, CS solutions and tests ($F(6,114)= 9.35$, $p<0.0001$). Within-group comparisons revealed that significantly greater consumption of the CS+/s solution relative to the CS-/s solution occurred across all three tests in the Yoked Control (Figure 6C) and Control (Figure 6D) groups as well as the D2 group (Figure 6B). In contrast, although the D1 group consumed significantly more of the CS+/s relative to the CS-/s solution in Test 1, CS intakes failed to differ in Tests 2 and 3, indicating extinction of the CS+/s preference (Figure 6A). In particular, the D1 group displayed a selective and significant reduction in CS+/s intake from the first to the third test (Figure 6A). The CS+/s intakes of the D1 and D2 groups in Test 3 were also significantly lower than those of the Control and Yoked Control groups (Figures 6A, 6B), whereas CS-/s intakes failed to change over testing in any group.

Analysis of the percent CS+/s data failed to reveal any significant overall group difference ($F(3,57)= 1.96$, ns), but there were significant effects across tests ($F(2,38)= 33.36$, $p<0.0001$) and for the interaction between groups and tests ($F(6,114)= 8.73$, $p<0.0001$). Whereas percent CS+/s intakes remained stable across the three tests in the Control and Yoked Control groups (Figures 6C, 6D) as well as the D2 group (Figure 6B), the percent CS+ intakes of the D1 group were significantly lower in Tests 2 (59%) and 3 (57%) relative to Test 1 (70%) (Figure 6A). In addition, CS+/s preference of the D1 group in Test 3 (Figure 6A) was significantly below that of the Control group, again suggesting extinction of the CS+/s preference. Finally, significant differences in total CS solution intakes were observed among the four training groups ($F(3,57)= 2.92$, $p<0.042$), among the three tests ($F(2,38)= 17.63$, $p<0.0001$), and for the interaction between groups and tests ($F(6,114)= 17.97$, $p<0.0001$). The Yoked Control group significantly increased

total CS intake in Test 3 (17.8 g) relative to the first (14.6 g) and second (14.4 g) Tests, and the Control group significantly increased total CS intake in Test 3 (15.8 g) relative to Test 1 (13.3 g). Total CS intakes in Test 3 were significantly lower in D1 (12.9 g) and D2 (13.6 g) groups compared to the Yoked Control group.

# Figure 6

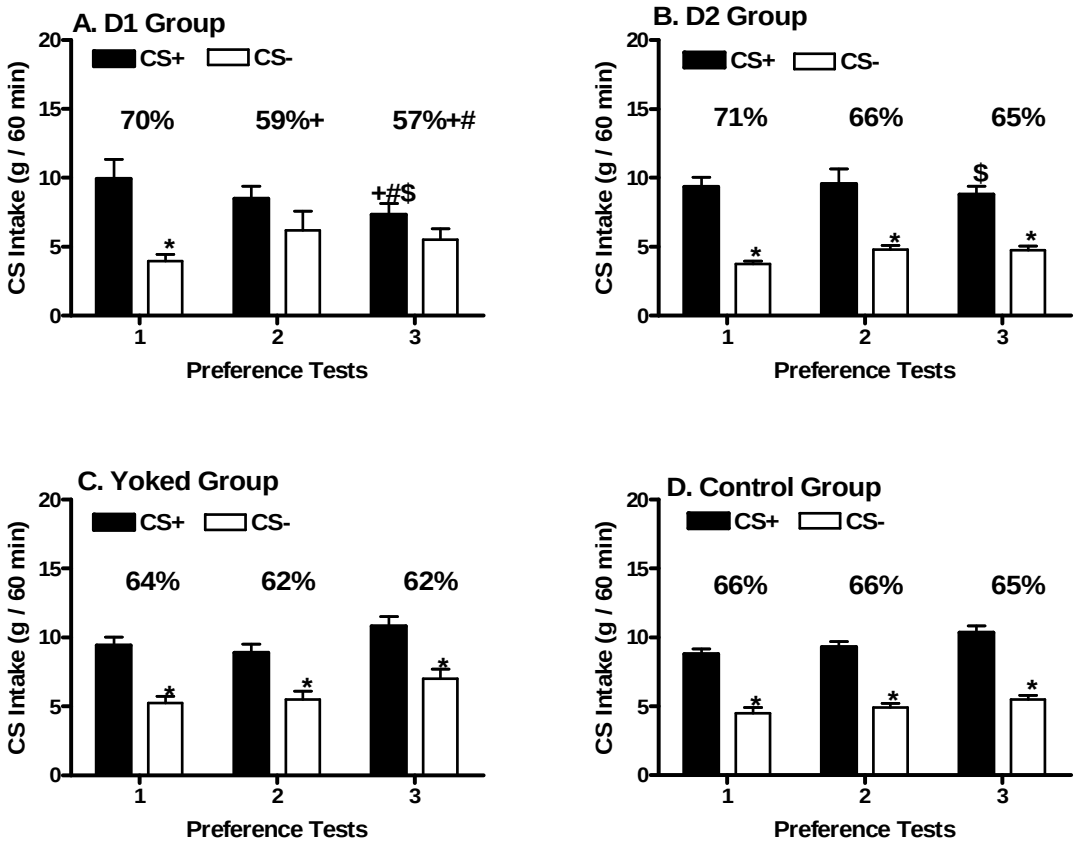

A. D1 Group
CS+
CS-
70%
59%+
57%+#
+#$
*
CS Intake (g / 60 min)
Preference Tests
B. D2 Group
71%
66%
65%
$
C. Yoked Group
64%
62%
62%
D. Control Group
66%
66%
65%

**Figure 6. Experiment 2B (acquisition procedure**). Intakes (mean +SEM, g/1 h) of CS+/s and CS-/s solutions during two-bottle Tests 1-3. During the training, the D1 group received bilateral AMY microinjections of SCH23390 (12 nmol total dose, Panel A) and the D2 group received bilateral AMY microinjections of raclopride (12 nmol total dose, Panel B); the yoked control group was limited to the CS intakes of the drug groups and received bilateral AMY vehicle microinjections (Panel C); the control group received no injections during training (Panel D). Numbers atop bars represent the mean percent intakes of CS+/s. Significant differences are denoted between CS+/s and CS-/s intake within each test (*). Significant differences in CS intake or percent CS+ intakes between Test 1 and subsequent tests are denoted (+) as are differences in the drug groups relative to the yoked control group ($) or control group (#).

## Discussion

In this study, we analyzed the role of dopamine transmission within the AMY in flavor preferences conditioned by the sweet taste of fructose. The results showed that dopamine D1-like and D2-like receptor antagonism at high (48 nmol), but not low (12-24 nmol), drug doses significantly attenuated the expression of a previously-learned fructose-CFP. D1-like antagonist treatment (12 nmol dose) during training had no effect on the initial establishment of fructose- CFP, but rather resulted in a rapid extinction of the learned fructose-CFP. These effects cannot be attributed to the spread of the drugs to structures outside the AMY. Large excitotoxic lesions of AMY with 0.7 □l of ibotenic acid never invaded structures outside of the AMY (Touzani and Sclafani, 2005). These results showed that dopamine transmission within the AMY plays an important role in the maintenance of learned fructose-CFP.

**Fructose-CFP Expression Effects.** The present study demonstrated that bilateral administration into the AMY of either the dopamine D1-like receptor antagonist SCH23390 or the D2-like receptor antagonist raclopride attenuated the expression of a fructose-CFP. These results are quite similar to the results obtained with the same drugs microinjected into the NAcS (Bernal et al., 2008). Thus, both the AMY and NAcS are implicated as central sites of action for the suppressive effects of systemic administration of dopamine D1-like and D2-like antagonists on the expression of flavor preferences conditioned by the sweet taste of sugars (Baker et al., 2003; Yu et al., 2000a, 2000b). However, the central and systemic injections of the D1-like antagonist differed in the degree to which they attenuated the fructose-CFP. SCH23390 (48 nmol) significantly reduced the expression of the fructose-CFP preference from 77% to 66% following AMY

administration, and from 76% to 62% following NAcS administration. This is in contrast to the elimination of the fructose-CFP preference from 77% to 39-55% across a 50-800 nmol/kg systemic dose range of SCH23390 (Baker et al., 2003). On the other hand, the central and systemic effects of the D2-like antagonist on the expression of the fructose-CFP were similar. Raclopride significantly reduced the expression of the fructose-CFP preference from 77% to 68% following AMY (48 nmol) administration, from 76% to 63% following NAcS (24 nmol) administration, and from 80% to 66% following systemic (200 nmol/kg) administration.

**Fructose-CFP Acquisition Effects:** In addition to attenuating the expression of a previously learned CS+ preference, injection of SCH23390 into the AMY during training influenced the acquisition of the fructose-conditioned CS+ preference. Although the D1 group displayed a significant preference for the CS+ flavor in Test 1 (70%), the preferences declined to 59% by Test 2, and to 57% by Test 3, and were no longer significant. In contrast, the D2 group treated with raclopride during training displayed a significant CS+ preference that persisted over the three pairs of tests. The CS+ preference of the D2 group declined somewhat by Test 3 (to 65%), but it did not differ from that of the Yoked and Control groups that displayed stable preferences of 62% and 66% over the three tests. In contrast to the present results, systemic treatment (200 nmol/kg) with SCH23390 or raclopride completely prevented the acquisition of a fructose-conditioned flavor preference in rats (Baker et al., 2003). Systemic SCH23390, but not raclopride also prevented the acquisition of a flavor preference conditioned by IG sucrose infusions (Azzara et al., 2001).

The results obtained with dopamine antagonism of the AMY showed both similarities and differences with fructose-CFP acquisition results obtained with drug injections into the NAcS (Bernal et al., 2008). First and foremost, injection of SCH23390 into either the AMY or NAcS during training produced similar effects with an initial CS+ preference in Test 1 (70%) giving way to no preference in Test 3 (57%). Both central D1-like antagonist treatments differ from systemic D1-like antagonist treatment, which as noted above, prevented the animals from showing any CS+ preference (Baker et al., 2003). It is possible that the single tested dose (12 nmol) of the D1-like antagonist administered into the AMY was not sufficient to induce effects upon acquisition of the fructose-CFP in the same manner that a systemic dose of 200 nmol/kg SCH23390 during training eliminated either sucrose-conditioned CFP in sham-feeding rats (Yu et al., 2000b) or fructose-conditioned CFP in real-feeding rats (Baker et al., 2003). However, the 12 nmol dose of SCH23390 in the AMY in the expression study reduced total CS intakes to the same degree as that produced by the systemic 200 nmol/kg SCH23390 dose in the systemic injection studies (Baker et al., 2003; Yu et al., 2000b).

Although SCH23390 administered during training did not block learning of the fructose-CFP, D1-like antagonism in the AMY, like that in the NAcS during training hastened the extinction of the fructose-CFP. The reason for this effect remains to be established. It is not readily explained by the drug treatment merely producing a weaker association between the CS+ flavor and fructose taste. If this were the case, the D1 AMY group would have been expected to show a weaker CS+ preference in Test 1 rather than showing a slightly greater preference than that displayed by the control groups (70% vs. 64-67%). However, AMY administration of the 12 nmol dose of SCH23390 during

training may have been producing a more “tenuous” association between the CS+ flavor and fructose taste. One potential mechanism of action involves the co-distribution of dopamine D-1 and NMDA/AMPA receptors within the AMY (Pickel et al., 2006). Thus, dopamine D1-like receptor antagonists in the cortico-lateral AMY block the enhancement of long-term potentiation elicited by low-frequency stimulation (e.g., Huang and Kandel, 2007). Further, dopamine receptor-mediated enhancement of hippocampal long-term potentiation requires the NR2B subunit of the NMDA receptor (Stramiello and Wagner, 2008). Thus, using this form of neuronal plasticity underlying learning and memory (see review: Bliss and Collingridge, 1993), a low dose of SCH23390 in the AMY can affect the stability of a NMDA-mediated neuroplasticity within the AMY underlying the acquisition of fructose-CFP learning. Such a mechanism has been proposed (see review: Sutton and Benninger, 1999) in that co-activation of D1-like receptors and NMDA/AMPA receptors contributes to consolidation of learned behavior, and that long-term memory (maintenance of the behavior) may depend upon the molecular cascade mediated by D1-like receptors that are interrupted by SCH23390. Indeed, a role for NMDA receptors in the acquisition of fructose-CFP has been established in that systemic administration of MK-801 and D-cycloserine respectively block and enhance acquisition, but not expression of a fructose-CFP (Golden and Houpt, 2007). Further studies are needed to address this important issue.

In contrast to the identical CS+ preferences observed with the D1 AMY and D1 NAcS groups, treatment with the D2-like antagonist during training produced a somewhat weaker decline in CS+ preference in the AMY rats of the present study (from 71% to 65%) than in the NAcS rats of our prior study (from 73% to 60%: Bernal et al.,

2008). Thus, fructose-CFP appears more dependent on D2 receptor activity in the NAcS than in the AMY. As in the case of the 12 nmol dose of SCH23390, the 12 nmol dose of raclopride administered into the AMY may not have been equivalent to the systemic 200 nmol/kg dose of raclopride that blocked flavor conditioning by fructose (Baker et al., 2003).

The dopamine drug effects on flavor conditioning by oral fructose also differ from those produced by IG glucose conditioning. Thus, SCH23390 injections into the AMY or NAcS during training did not block fructose conditioning, but they did block the acquisition of a flavor preference produced by glucose infusions (Touzani et al., 2008, 2009). On the other hand, the expression of an IG glucose-conditioned flavor preference was less affected by SCH23390 injections into the AMY or NAcS (Touzani et al., 2008, 2009) than was the expression of the fructose-CFP (present study, Bernal et al., 2008). It should be noted that the effect of AMY or NAcS injections of raclopride on IG glucose conditioning was not determined because systemic injections of the D2-like receptor antagonist did not block conditioning with IG sucrose (Azzara et al., 2001). Taken together, the central and systemic injection data indicate that there is differential involvement of D1 and D2 receptors in flavor-flavor and flavor-nutrient preference conditioning produced by oral fructose and IG glucose, respectively.

The AMY is a highly heterogeneous aggregate of nuclei. There is extensive evidence that the discretely sub-divided baso-lateral and central nuclei of the AMY (see review: Pitkanen, 2000) play highly distinct and specific roles in different forms of appetitive and aversive learning (see reviews: Davis, 2000; LeDoux, 2000; Gallagher, 2000; Balleine and Killcross, 2006). This would suggest that dopamine transmission

within these discrete subdivisions of the AMY plays differential role in fructose-conditioned flavor preferences. Given the relatively large microinfusion volume (0.5 µl) used in the present study, the dopamine antagonists presumably acted both in the baso-lateral and central nuclei of the AMY. Thus, it is not possible to determine whether the effect observed were due to the drugs' action within the baso-lateral or the central nuclei (or both). Recently Touzani et al. (2009) reported that flavor-nutrient conditioning by intragastric glucose infusions was eliminated by SCH23390 injections (12 nmol in 0.5 µl) that involved both the baso-lateral and central AMY nuclei. In contrast, flavor-nutrient conditioning was only attenuated by smaller volume (0.25 µl) infusions of the same drug dose into the baso-lateral or central AMY nuclei. These findings suggested that the baso-lateral and central nuclei are both involved in flavor conditioning, and that additive effects are produced by dopamine antagonism in both nuclei.

**Potential Central Mechanisms of Action:** As detailed above, D1-like and D2-like receptor antagonism in the AMY and NAcS resulted in similar reductions in, but not elimination of the expression of a fructose-CFP, and D1 receptor antagonism in the AMY and NAcS during training hastened the extinction of the fructose-CFP. These data suggest that dopamine-responsive neurons within the two sites are part of a regional network of brain sites that mediate flavor-flavor conditioning in a manner similar to proposed regional networks of interacting brain sites for other aspects of feeding behavior (e.g., Baldo and Kelley, 2007; Bodnar and Levine, 2008; Will et al., 2003). Several sources of evidence support the existence of an AMY–NAcS dopamine reward network. First, the source of dopamine into the AMY is derived from the mesolimbic dopamine pathway originating in the ventral tegmental area which also provides dopaminergic

innervation to the NAcS (e.g., Asan, 1997, 1998; Eliava et al., 2003; Lammel et al., 2008). Second, there is very strong evidence for AMY, particularly baso-lateral and lateral nuclei, projections to the NAc, particularly the shell region (Brog et al., 1993; Christie et al., 1987; Fudge et al., 2002) in an organized and highly compartmentalized fashion (Groenewegen et al., 1999; Phillipson and Griffiths, 1985; Wright and Groenewegen, 1995; Wright et al., 1996). Third, the NAcS sends projections to the AMY (Brog et al., 1993; Mello et al., 1992). Fourth, the NAcS is a site in which sweet taste stimulated dopamine efflux (e.g., Cheng and Feenstra, 2006; Genn et al., 2004), and in which dopamine antagonists suppressed lithium chloride-conditioned saccharin aversions (Fenu et al., 2001). Indeed and importantly, the NAcS is a site in which the motivational valence and novelty of hedonic food stimuli (e.g., Fonzies) is a critical component of dopamine release as compared to the core of the nucleus accumbens in which generic motivational values of food-related stimuli elicit dopamine release (Bassareo and DiChiara, 1997, 1999a, 1999b; Bassareo et al., 2002). Fifth, the AMY is another site in which Pavlovian and instrumental reward learning are supported (Baxter and Murray, 2002; Cardinal et al., 2002), in which feeding and gastric nutrient infusions increased DA turnover or efflux (Hajnal and Lenard, 1997; Heffner et al., 1980), and in which a Pavlovian CS for food elicited DA efflux (Harmer and Phillips, 1999). Further, AMY inactivation modulated feeding-stimulated DA efflux in the NAc (Ahn and Phillips, 2002). Finally, recent findings indicate that AMY and dopamine projections to the NAc interact to promote sugar seeking behavior, and that the AMY responses preceded and indeed excited the responses observed in the NAc (Ambroggi et al., 2008). The integrity of the AMY, and particularly the baso-lateral nucleus, is necessary for NAc responsivity.

These findings are consistent with a network model of D1 and/or D2 receptor modulation of flavor preference learning, with differential involvement in the acquisition and expression of flavor-flavor and flavor-nutrient associations. Further systematic microinjection studies in the AMY and NAc, as well as other sites (e.g., medial prefrontal cortex, lateral hypothalamus) sites are necessary to identify the underlying system(s) mediating these forms of food-related learning.

# CHAPTER FIVE: OPIATE RECEPTOR ANTAGONISM IN THE NUCLEUS ACCUMBENS AND FLAVOR PREFERENCES CONDITIONED BY FRUCTOSE

## Introduction

There is extensive evidence that central opioid systems are importantly involved in the ingestive response to palatable foods and fluids (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006). In a variety of rodent studies, opioid receptor agonists selectively increased, whereas antagonists selectively reduced, the intake of preferred foods and fluids when administered systemically or directly into the brain. The opioid modulation of the hedonic, or more broadly the reward evaluation of sweet substances has been studied in particular detail. For example, general opioid receptor antagonism is reported to (a) suppress intake of sweet solutions more than plain water (Cooper, 1983; Le Magnen et al., 1980; Sclafani et al., 1982); (b) block the portion of feeding that appears driven by sweet taste in food-restricted animals (Levine et al., 1995); (c) reduce sucrose's positive hedonic qualities in a taste reactivity (TR) paradigm (Levine et al., 1995; Parker et al., 1992); (d) reduce sucrose intake in sham-feeding tests which minimize post-oral effects (Kirkham and Cooper, 1988; Rockwood and Reid, 1982), but (e) does not reduce sweet taste discrimination (O'Hare et al., 1994). Some human studies also report that opioid receptor antagonism reduces sweet taste pleasantness without reducing sweet taste discrimination (Arbisi et al., 1999; Fantino et al., 1986).

An early study also implicated the opioid system in flavor preference conditioning by sugar (Mehiel, 1996). Subsequent work in our laboratories, however, failed to support this view. In separate studies, we investigated the effects of systemic administration of the opioid receptor antagonist naltrexone (NTX) on flavor preferences conditioned by

either the sweet taste (flavor-taste conditioning) or the post-oral nutritive effects (flavor-nutrient conditioning) of sugars (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). Our studies revealed that NTX treatment during training trials failed to block preference conditioning, and that drug treatment during post-training choice tests failed to block the expression of previously learned flavor preferences. These findings would appear to conflict with the general idea that central opioid signaling modulates the avidity for preferred foods and fluids (see review: Cooper, 2007). It may be that opioid antagonism influences primarily unlearned rather than learned preferences. It is also possible, however, that the systemic drug treatments used in our conditioning experiments obscured an important contribution of specific central opioid circuits on conditioned food preferences. Systemically administered drugs will act on opioid circuits in various brain regions that may have different functions, such as energy homeostasis as compared to food hedonics (Glass et al., 1999; Gosnell and Levine, 2009).

A recent study by Wooley et al. (2006) indicates that the route of drug administration can influence the effect of NTX on food preference. Rats were given choice tests with two differently-flavored (chocolate and banana) but nutritionally-identical food pellets. Most animals displayed a mild preference (presumably unlearned) for the chocolate-flavored food. Systemic NTX treatment did not alter this preference but rather reduced the intakes of both flavored foods. However, NTX microinfusions into the nucleus accumbens (NAc) selectively reduced the intake of the preferred chocolate-flavored food. The NAc is recognized as a critical site of opioid action on food intake and reward (see reviews: Kelley et al., 2002; Peciña, 2008). Thus, it is not surprising that

NAc and systemic administration of opioid drugs might have different effects on food preferences.

In light of the food preference findings of Wooley et al. (2006), the following study determined if NAc microinfusions of NTX would substantially attenuate or block the expression of sugar-conditioned flavor preferences. This experiment has been published in Pharmacology Biochemistry and Behavior (Bernal et al., 2010), and has been presented at the Society for Neuroscience (Bernal et al., 2008b).

**Experiment 1: Expression Procedure**: This experiment determined whether the opioid receptor antagonist, NTX, injected bilaterally into the **NAc** dose-dependently altered the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the General Methods Section.

Training and Testing: The rats were given ten one-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. This familiarized them with the presence of two sipper tubes used during the choice tests. The position of the CS and water sipper tubes varied across days using a left-right-right-left pattern. Following training, the rats were given eight two-bottle choice test sessions (30 min/day) with unlimited access to the CS+ and CS- solutions. All injections occurred 10 min prior to the two-bottle tests. For the first two test sessions, the rats were given a vehicle (0.9% saline) microinfusion. Over the next six sessions the rats were given microinfusions of NTX at total doses of 1, 25, and 50 μg (0.5, 12.5, 25 μg/side) into the NAc shell or core. Half of the rats were tested

with an ascending dose order, and the remaining rats were tested in a descending dose order. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups.

**Results**

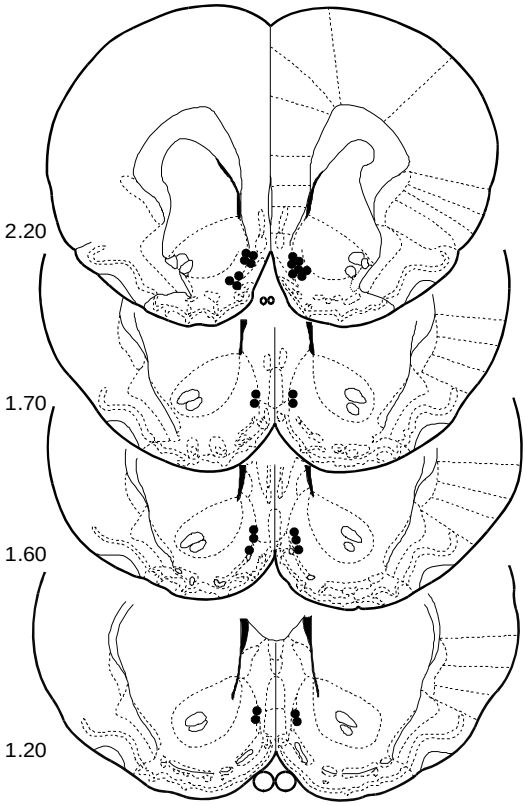
*Histological Verification:* Cannula tip placements for all rats used in these experiments are shown in Figure 7. Placements were deemed appropriate for fourteen rats in the NAc shell and twelve rats in the NAc core and were primarily restricted to the rostral portion between the Frontal Planes 2.20 and 1.20 mm of the Paxinos and Watson (1998) atlas. The remaining two rats with misplaced injection sites were discarded.

*Experiment 1A: NAc Shell:* During one-bottle training, the rats consumed more CS+ than CS- (12.9 vs. 10.5 g/30 min, $t(13) = 5.0$, $p < 0.001$). In the two-bottle preference tests (Fig. 8A), overall, the rats consumed significantly more CS+ than CS- [$F(1,13) = 41.4$, $p < 0.001$] while the drug dose main effect was not significant. The interaction of CS x dose was not significant although the rats tended to consume less CS+ and more CS- as the NTX dose increased; individual tests indicated that the rats consumed significantly more ( $p<0.05$) CS+ than CS- at all NTX doses. Percent CS+ intakes (calculated as percent of total CS intake) ranged from 80% in the vehicle test to 72%, 69%, and 64% following the 1, 25 and 50 μg NTX doses respectively; these differences were not significant (Fig. 8A).

*Experiment 1B: NAc Core:* The rats consumed more CS+ than CS- (13.2 vs. 9.3 g/30 min, $t(11) = 7.8$, $p < 0.001$) during one-bottle training. In the two-bottle preference tests (Fig. 8B), overall CS+ intakes exceeded CS- intakes [$F(1,11) = 168.3$, $p < 0.001$] while

the drug dose main effect was not significant. There was a significant CS x dose [$F(3,33) = 4.01$, $p < 0.05$] although individual tests indicated that the rats consumed significantly more ($p < 0.05$) CS+ than CS- at all NTX doses. CS+ intakes declined and CS- intakes increased somewhat as drug dose increased but these changes were not significant. Significant differences in the percent CS+ preference occurred across doses [$F(3,33) = 4.09$, $p < 0.014$] (Figure 8B) with the CS+ preference at the 50 μg NTX dose significantly lower ($p<0.05$) than that following vehicle (72% vs. 80%, $p<0.05$). It should be noted that the 50 μg dose in the NAc core produced a smaller decline in CS+ preference (8%) than that observed in the NAc shell (16%) yet the later difference was not significant because of the greater variability in the percent CS+ preferences obtained in the Shell experiment.

NAc Shell




NAc Core

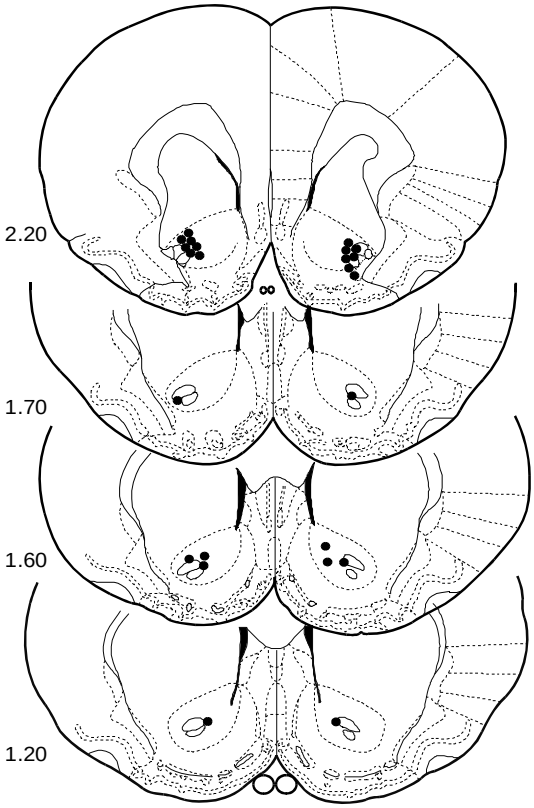


**Figure 7**. Bilateral representation of cannula sites of fourteen rats in the NAc shell and twelve rats in the NAc core which were primarily restricted to the rostral portion between the Frontal Planes 2.20 and 1.20 mm of the Paxinos and Watson (1998) atlas tested in the Fructose-CFP paradigm. The remaining two rats with misplaced injection sites were discarded. The atlas is based on a scale of 1.6 cm of the depicted structures equaling 1 mm of the actual distance between structures.

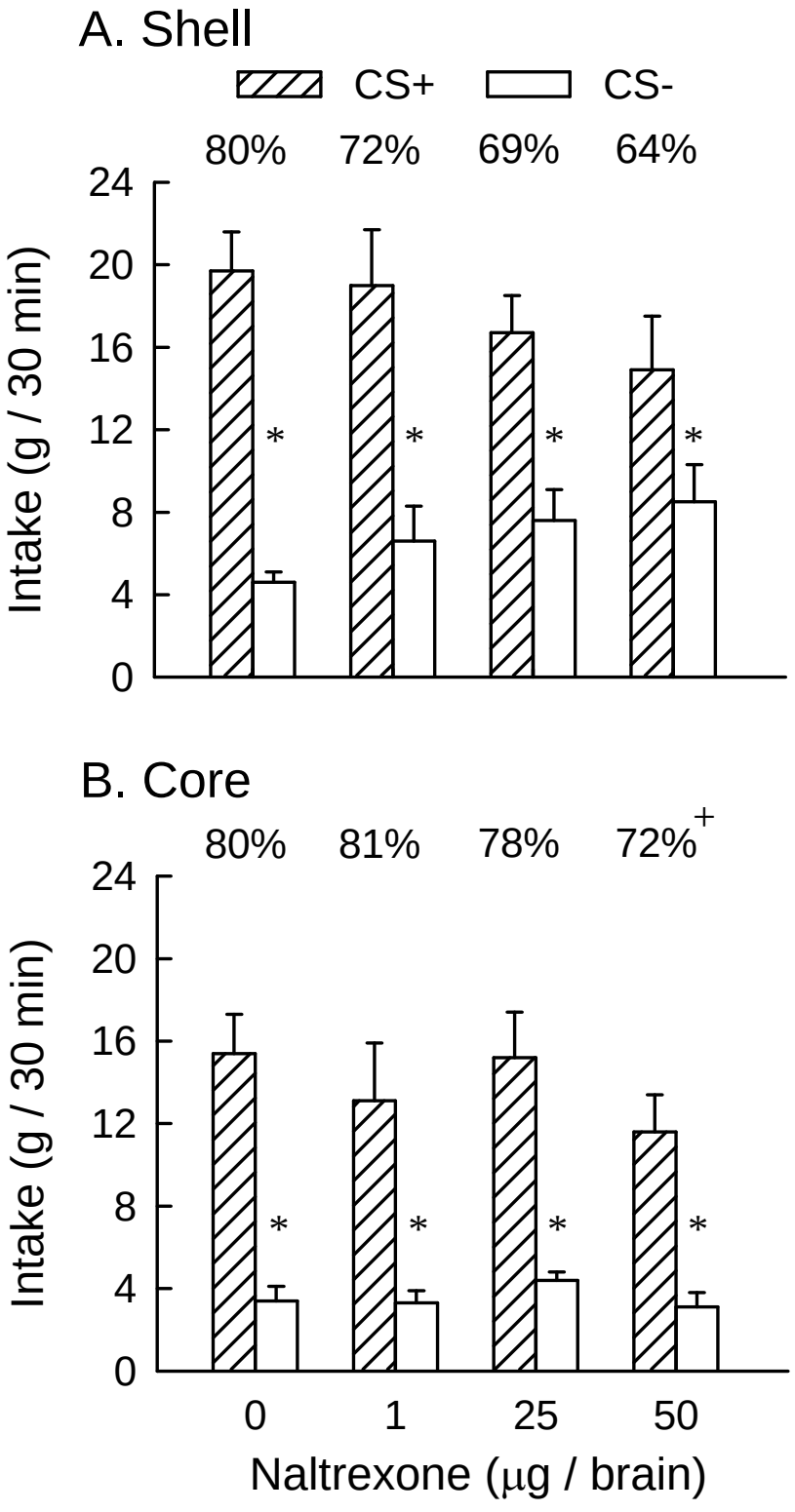

A. Shell
CS+
CS-
80%
72%
69%
64%
Intake (g / 30 min)
24
20
16
12
8
4
0
*
B. Core
80%
81%
78%
72%+
Intake (g / 30 min)
0
1
25
50
Naltrexone (μg / brain)

**Figure 8**. Oral fructose-conditioned flavor preferences. Intakes (+SEM, g/30 min) of CS+ and CS- solutions during two-bottle tests in animals receiving bilateral NAc shell (n=14, Panel A) or NAc core (n=12, Panel B) microinjections of naltrexone at total doses of 0, 1, 25 and 50 μg. A significant difference between CS+ and CS- intakes at a given dose is indicated by an asterisk (* $P < 0.05$). A significant difference in percent CS+ intakes at the dose of 50 □g as compared to the 0 dose is indicated by a plus (+ $P < 0.05$).The percentages of CS+ intake over total intake are indicated by the value above each CS+ bar.

**Discussion**

The present study evaluated the effect of opioid receptor antagonism in the NAc on the expression of sugar-conditioned flavor preferences. Confirming prior work, rats developed strong preferences (80-91%) for flavors paired with the sweet taste of fructose or the post-oral nutrient effects of glucose (Baker et al., 2004; Bernal et al., 2008). The expression of these fructose-ibnduced conditioned preferences was not substantially attenuated by NTX microinfusions into the NAc shell or core, regions where opioid agonists stimulate feeding (Zhang and Kelley, 2000). The CS+ flavor preferences were reduced by the highest NTX dose in the NAc shell (from 80% to 64%) and core (from 80% to 72%), although only the core effect was significant.

A parallel study (Bernal et al., 2010) was conducted in a collaborating laboratory (A Saclafani and K Touzani) that examined NTX effects in the NAC shell and core on flavor preferences conditioned by IG glucose. In this study, all cannula tip placements were in the same areas of the NAC shell and core as the fructose study. In the NacS, CS+, but not CS-, intakes declined as NTX increased from 0 to 50 μg (18 to 10 g/30 min). Nevertheless, the rats consumed significantly more CS+ than CS- at all NTX doses with percent CS+ intakes failing to change (91%: Veh; 89%-93%: NTX). In the NAC core, NTX failed to affect CS+ or CS- intakes, and percent CS+ intakes failed to change (81%: Veh; 76%-78%: NTX). Overall, the relatively limited effects of NAc NTX on CS+ flavor preferences and intakes are comparable to the results obtained in our prior sugar-conditioning studies using systemic NTX treatment (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). Systemic NTX selectively reduced CS+ intake but not percent preference in our fructose conditioning study (Baker et al., 2004) and did not

significantly alter CS+ intake or preference in our IG sugar conditioning study conducted with food-restricted animals (Azzara et al., 2000). Furthermore, at all NTX doses administered in the NAc or systemically, the rats consumed more CS+ than CS- in the two-bottle tests.

The impetus for the present study was the report that NAc NTX microinfusions selectively reduced the intake of a preferred flavored food (chocolate), whereas systemic naltrexone injection decreased the intake of both the preferred (chocolate) and less-preferred (banana) foods (Woolley et al., 2006). Based on these results, we predicted that NAc NTX administration would have a much greater effect on the expression of sugar-conditioned flavor preferences than we previously observed with systemic administration. However, this prediction was not confirmed: the reductions in the sugar-conditioned flavor preferences following NAc drug treatment were not much different from those observed in our prior studies with systemic NTX treatment. Our findings do not contradict the Woolley et al. results given the many differences between the two studies. Whereas our present and prior studies investigated relatively strong conditioned flavor preferences, the food preferences in the Wooley et al. study were relatively weak, and based presumably on unlearned palatability differences between the flavored foods. In addition, the choice tests in the Wooley et al. study were conducted with non-deprived rats fed nutritionally-identical diets, whereas the choice tests of the present experiment involved food-restricted rats given non-nutritive fluids. The type of test diet or fluid and deprivation state can influence the response to opioid drugs (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006).

Relevant to the issue of the selectivity of systemic versus NAc naltrexone treatments on flavor preferences is a recent study by Taha and co-workers (2006) which used an anticipatory contrast design. In this study, non-deprived rats were trained to drink a 4% sucrose solution only (4-0 group), or a 4% sucrose solution followed by a 20% sucrose solution (4-20 group). The 4-20 rats consumed less 4% sucrose than did the 4-0 group, indicating the reward value of 4% sucrose was reduced in those rats expecting the more preferred 20% sucrose solution. Systemic NTX injections (1 mg/kg) suppressed 4% sucrose intake in the 4-0 rats, but not in the 4-20 rats; the drug suppressed 20% sucrose intake in the latter group, however. In contrast to these findings, NTX microinfusions failed to suppress 4% and/or 20% sucrose intakes in the 4-0 and 4-20 groups. Note that other studies not involving a contrast design reported that NAc NTX infusions minimally reduced 10% or 20% sucrose intake in rats (Bodnar et al., 1995; Kelley et al., 1996). Thus, the effectiveness of NAc NTX infusions to alter flavor preferences or sweet solution intakes varies as a function of the specific test paradigm used.

The present study investigated the effects of NTX on the expression of previously learned sugar-conditioned flavor preferences. It is possible that infusing NTX into the NAc during initial training trials might have greater effects on the acquisition of sugar-conditioned preferences. This seems unlikely, however, in view of the failure of systemic NTX injections to block flavor-sugar conditioning (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). On the other hand, systemic or NAc treatment with dopamine receptor antagonists attenuate, at least to some degree, flavor preference conditioning by the sweet taste and post-oral nutrient effects of sugars (Azzara et al., 2001; Baker et al., 2003; Bernal et al., 2008; Touzani et al., 2008; Yu et al. 2000). Other systemic studies

also implicate endocannabinoid and glutamate receptor signaling in preference conditioning by the sweet taste of fructose (Golden and Houpt, 2007; Miner et al., 2008). Our studies do not argue against the involvement of opioid systems in food palatability and reward processing, but rather indicate that these systems have a limited role in sugar-conditioned changes in flavor preferences (Cooper, 2007). Other types of food-related learning, such as place preference conditioning, appear to be more dependent upon opioid signaling (Ågmo et al., 1993; Delamater et al., 2000; Jarosz et al., 2006).

The following chapter describes an experiment evaluating NTX effects in the AMY upon fructose-CFP using the rationale and reasoning employed for the NAC.

# CHAPTER SIX: OPIATE RECEPTOR ANTAGONISM IN THE AMYGDALA AND FLAVOR PREFERENCES CONDITIONED BY FRUCTOSE

As explained in the previous chapter, there is extensive evidence that central opioid systems are importantly involved in the ingestive response to palatable foods and fluids (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006). Using the reasoning stated in the previous chapter, it is reasonable to assume a role for NTX in the reward evaluation of sweet substances (Cooper, 1983; Kirkham and Cooper, 1988; Le Magnen et al., 1980; Levine et al., 1995; Parker et al., 1992; Rockwood and Reid, 1982; Sclafani et al., 1982) and flavor preference conditioning by sugar (Mehiel, 1996). Subsequent work in our laboratories (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999) failed to observe systemic NTX effects on flavor preferences conditioned by either the sweet taste (flavor-taste conditioning) or the post-oral nutritive effects (flavor-nutrient conditioning) of sugars. A recent study by Wooley et al. (2006) indicates that the route of drug administration can influence the effect of NTX on food preference measured by choice tests with two differently-flavored (chocolate and banana) but nutritionally-identical food pellets. Whereas systemic NTX treatment did not alter this preference but rather reduced the intakes of both flavored foods, NTX microinfusions into the nucleus accumbens (NAc) selectively reduced the intake of the preferred chocolate-flavored food. The previous chapter however showed that NAc  NTX, like systemic NTX failed to alter fructose-CFP.

The AMY has been intricately implicated in the opioid mediation of food intake with feeding elicited by mu and delta opioid agonists administered into the AMY (Gosnell, 1988; Gosnell et al., 1986; Stanley et al., 1989). DAMGO-induced feeding

elicited from the central nucleus of the AMY was blocked by naltrexone pretreatment in the PVN, but DAMGO-induced feeding elicited from the PVN was not blocked by naltrexone pretreatment in the central nucleus of the AMY (Giraudo et al., 1998a). Importantly, a bidirectional opioid–opioid signaling pathway between the NTS and the central nucleus of the AMY given that DAMGO-induced feeding elicited from the central nucleus of the AMY was blocked by naltrexone pretreatment in the NTS, and that DAMGO-induced feeding elicited from the NTS was blocked by naltrexone pretreatment in the central nucleus of the AMY (Giraudo et al., 1998b). NTX in the central nucleus of the AMY selectively reduced preferred diet intake (Glass et al., 2000). Both OFQ/N and butorphanol, mixed mu and kappa opioid agonist stimulated c-fos activity in the AMY (Kim et al., 2001; Olszewski et al., 2000). Finally, chronic food restriction decreased mu opioid receptor binding in the AMY while increasing dynorphin A(1-17) in the AMY (Berman et al., 1994, 1997; Wolinsky et al., 1994). Given the important role of opioid activation in the AMY, and in light of the food preference findings of Wooley et al. (2006), the present study determined if AMY microinfusions of NTX would substantially attenuate or block the expression of sugar-conditioned flavor preferences. This experiment has been presented at the Society for Neuroscience (Bernal et al., 2008b).

**Experiment: Expression Procedure**: This experiment will determine whether the opioid receptor antagonist, naltrexone (NTX), injected bilaterally into the **AMY** dose-dependently alters the **expression** of fructose-conditioned flavor-flavor preferences in food restricted rats.

The subjects, surgery, histology, training solutions and statistics are described in the General Methods Section.

Training and Testing: The rats were given ten one-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. This familiarized them with the presence of two sipper tubes used during the choice tests. The position of the CS and water sipper tubes varied across days using a left-right-right-left pattern. Following training, the rats were given eight two-bottle choice test sessions (30 min/day) with unlimited access to the CS+ and CS- solutions. All injections occurred 10 min prior to the two-bottle tests. For the first two test sessions, the rats were given a vehicle (0.9% saline) microinfusion. Over the next six sessions the rats were given microinfusions of NTX at total doses of 1, 25, and 50 μg (0.5, 12.5, 25 μg/side) into the AMY. Half of the rats were tested with an ascending dose order, and the remaining rats were tested in a descending dose order. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups.

**Results**

*Histological Verification:* Figure 9 is a schematic representation (Paxinos and Watson, 1998) and contains a detailed description of the bilateral cannula placements deemed appropriate for all rats (n= 11) in the AMY and were primarily restricted to the rostral portion between the Frontal Planes 2.20 and 1.20 mm of the Paxinos and Watson (1998) atlas.

During one-bottle training, the rats consumed more CS+ than CS- (13.1 vs. 10.7 g/30 min, $t(10) = 6.69$, $p < 0.001$). In the two-bottle preference tests (Fig. 10), overall, the rats

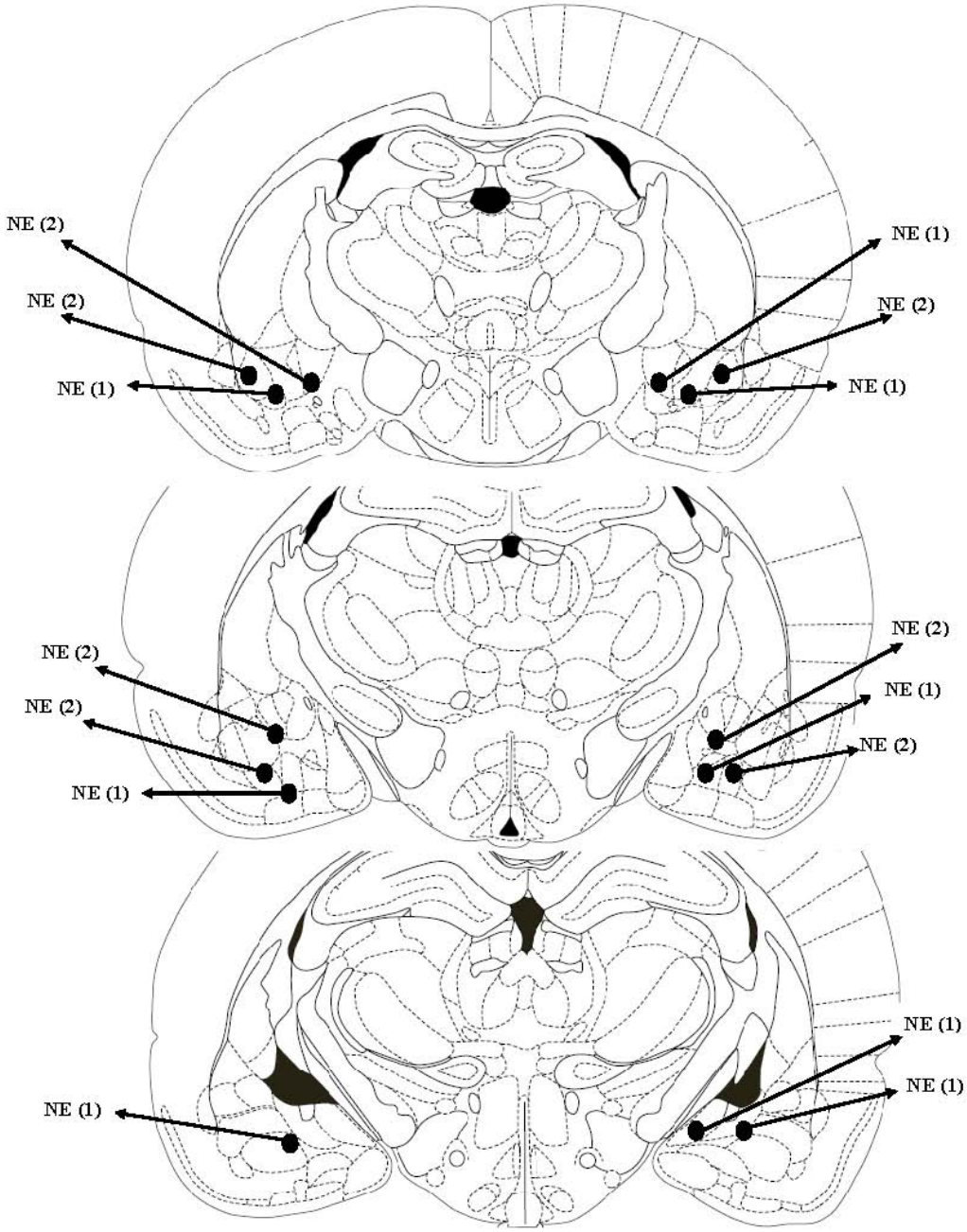

NE (2)
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**Figure 9**. Bilateral representation of cannula sites aimed at the AMY of eleven rats using figures 25 (Bregma -1.80 mm), 28 (Bregma -2.56 mm), and 32 (Bregma -3.60 mm) of the stereotaxic atlas of Paxinos and Watson (1998) based on a scale of 1.6 cm of the depicted structures equaling 1 mm of the actual distance between structures.

consumed significantly more CS+ than CS- [$F(1,10) = 40.6$, $p < 0.001$] with intakes significantly varying as a function of drug dose [$F(3,30) = 7.7$, $p < 0.001$]. The interaction of CS x dose was also significant ($F(3,30)= 3.08$, $p< 0.04$). The rats consumed less CS+ solution as the NTX dose increased; CS- intakes failed to be affected. Individual tests indicated that the rats consumed significantly more ( $p<0.05$) CS+ than CS- at all NTX doses. Percent CS+ intakes (calculated as percent of total CS intake) ranged from 80% in the vehicle test to 75%, 61%, and 64% following the 1, 25 and 50 μg NTX doses respectively; these differences failed to achieve significance ($F(3,30)= 2.18$, ns) (Fig. 10).

**Discussion**

The AMY has been intricately implicated in the opioid mediation of food intake with feeding elicited by mu and delta opioid agonists administered into the AMY (Gosnell, 1988; Gosnell et al., 1986; Stanley et al., 1989). DAMGO-induced feeding elicited from the central nucleus of the AMY was blocked by naltrexone pretreatment in the PVN, but DAMGO-induced feeding elicited from the PVN was not blocked by naltrexone pretreatment in the central nucleus of the AMY (Giraudo et al., 1998a). Importantly, a bidirectional opioid–opioid signaling pathway between the NTS and the central nucleus of the AMY given that DAMGO-induced feeding elicited from the central nucleus of the AMY was blocked by naltrexone pretreatment in the NTS, and that DAMGO-induced feeding elicited from the NTS was blocked by naltrexone pretreatment in the central nucleus of the AMY (Giraudo et al., 1998b). NTX in the central nucleus of the AMY selectively reduced preferred diet intake (Glass et al., 2000). Both OFQ/N and butorphanol, mixed mu and kappa opioid agonist stimulated c-fos activity in the AMY

(Kim et al., 2001; Olszewski et al., 2000). Finally, chronic food restriction decreased mu opioid receptor binding in the AMY while increasing dynorphin A(1-17) in the AMY (Berman et al., 1994, 1997; Wolinsky et al., 1994). Therefore, it is reasonable to assume that the AMY is a site at which general opioid antagonism might interfere with the expression of fructose-CFP. However, the present data clearly indicate that NTX infusions into the AMY dose-dependently reduced total intake of the two flavored saccharin solutions, but failed to significantly reduce the expression of fructose-CFP despite selectively reducing CS+ intake.

The impetus for the present study was the report that NAc NTX microinfusions selectively reduced the intake of a preferred flavored food (chocolate), whereas systemic naltrexone injection decreased the intake of both the preferred (chocolate) and less-preferred (banana) foods (Woolley et al., 2006). Based on these results, we predicted that NAc NTX administration would have a much greater effect on the expression of sugar-conditioned flavor preferences than we previously observed with systemic administration. However, this prediction was not confirmed in the previous study (Bernal et al., 2010). Given the important role of opioid activation in the AMY, and in light of the food preference findings of Wooley et al. (2006), the present study determined if AMY microinfusions of NTX would substantially attenuate or block the expression of sugar-conditioned flavor preferences. Again, this hyopothesis was not confirmed as the reductions in the sugar-conditioned flavor preferences following NAc drug treatment were not much different from those observed in our prior studies with systemic NTX treatment. Both of these findings do not contradict the Woolley et al. results given the many differences between the two studies. Whereas our present and prior studies

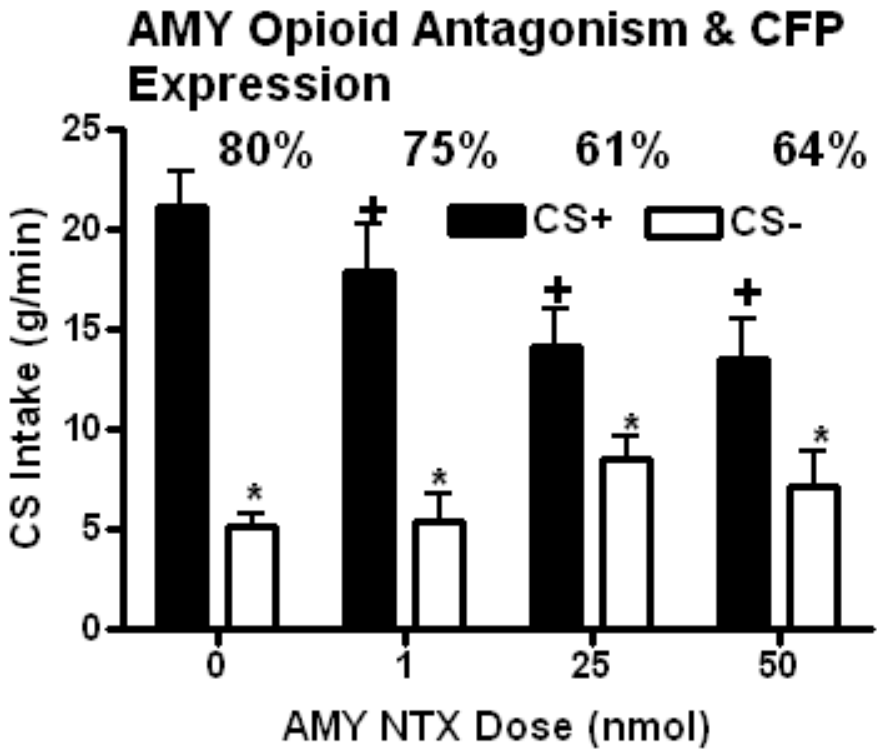
AMY Opioid Antagonism & CFP Expression
80%
75%
61%
64%
CS+
CS-
CS Intake (g/min)
25
20
15
10
5
0
0
1
25
50
AMY NTX Dose (nmol)

**Figure 10. Oral fructose-conditioned flavor preferences**. Intakes (+SEM, g/30 min) of CS+ and CS- solutions during two-bottle tests in animals receiving bilateral AMY (n=11) microinjections of naltrexone at total doses of 0, 1, 25 and 50 μg. A significant difference between CS+ and CS- intakes at a given dose is indicated by an asterisk (* $P < 0.05$), and between CS+ intakes relative to vehicle is indicated by a cross (+). The percentages of CS+ intake over total intake are indicated by the value above each CS+ bar.

investigated relatively strong conditioned flavor preferences, the food preferences in the Wooley et al. study were relatively weak, and based presumably on unlearned palatability differences between the flavored foods. In addition, the choice tests in the Wooley et al. study were conducted with non-deprived rats fed nutritionally-identical diets, whereas the choice tests of the present experiment involved food-restricted rats given non-nutritive fluids. The type of test diet or fluid and deprivation state can influence the response to opioid drugs (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006).

The present study investigated the effects of NTX on the expression of previously learned sugar-conditioned flavor preferences. It is possible that infusing NTX into the AMY during initial training trials might have greater effects on the acquisition of sugar-conditioned preferences. This seems unlikely, however, in view of the failure of systemic NTX injections to block flavor-sugar conditioning (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). On the other hand, systemic or AMY treatment with dopamine receptor antagonists attenuate, at least to some degree, flavor preference conditioning by the sweet taste and post-oral nutrient effects of sugars (Azzara et al., 2001; Baker et al., 2003; Bernal et al., 2009; Touzani et al., 2009b; Yu et al. 2000). Other systemic studies also implicate endocannabinoid and glutamate receptor signaling in preference conditioning by the sweet taste of fructose (Golden and Houpt, 2007; Miner et al., 2008). Our studies do not argue against the involvement of opioid systems in food palatability and reward processing, but rather indicate that these systems have a limited role in sugar-conditioned changes in flavor preferences (Cooper, 2007). Other types of food-related learning, such as place preference conditioning, appear to be more dependent upon opioid signaling (Ågmo et al., 1993; Delamater et al., 2000; Jarosz et al., 2006).

# CHAPTER SEVEN: GENERAL DISCUSSION

This final chapter will initially review the findings of DA D1 and D2 antagonist effects in the NAc and AMY upon expression and acquisition of fructose-CFP relative to the hypotheses posited for Specific Aims 1 and 2 followed by the findings of opioid antagonist effects in the NAc and AMY upon expression of fructose-CFP relative to the hypotheses posited for Specific Aims 3 and 4. This will be followed by a general discussion of the following topics: A) Role of the opioid system in spontaneous relative to learned food intake; B) Differential roles of DA D1 and D2 antagonists in distinct flavor/flavor and flavor-nutrient processes in CFP; C) Proposal of a distributed brain network mediating DA signaling and CFP; D) Future directions of research; and E) Implications of CFP pharmacology in human ingestive function and dysfunction.

**I A. DA D1 and D2 antagonism in the NAc and AMY alter expression and acquisition of fructose-CFP (Specific Aims 1 and 2).**

The complex relationship of neural DA circuits in food reward processes (Ikemoto & Panksepp, 1999; Smith, 2004; Wise, 2004; Barbano et al., 2009; Berridge, 2009; Salamone et al., 2009) was extended in the present dissertation to its relationship to CFP. Early studies (Hsiao & Smith, 1995; Mark *et al.*, 1994) revealed that intake of a bitter solution stimulated NAc DA efflux in rats previously trained to prefer this solution by pairing it with IG carbohydrate infusions, and that systemic D2 antagonism reduced the preference for a sucrose-paired flavor. Systemic D1 antagonist treatment blocked the acquisition of flavor-nutrient conditioning by IG sugar infusions, whereas systemic D1 and D2 antagonists blocked the acquisition and expression of flavor-flavor conditioning by oral sugar solutions using sham-feeding of sucrose and real-feeding of fructose

(Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). The NAcS and AMY were proposed as primary central sites of action at which these pharmacological effects would be mediated. Thus, the goal of **First Specific Aim** was to examine whether D1 or D2 DA receptor antagonists injected bilaterally into the NAcS would dose-dependently decrease the expression and/or acquisition of fructose-CFP. The corresponding goal of the **Second Specific Aim** was to investigate these same DA receptor antagonists injected bilaterally into the AMY would dose-dependently reduce the expression and/or acquisition of fructose-CFP.

The hypothesis (**Specific Aim 1A**) that direct bilateral administration into the NAcS of D1 or D2 receptor antagonists would block the *expression* of fructose-CFP was only *partially confirmed*. The respective effects of systemic and NAcS administration of the DA antagonists differed in magnitude. Whereas systemic SCH23390 eliminated the expression of the CS+ preference (39-55%) at all doses tested (50–800 nmol/kg) compared to the vehicle (77%) preference (Baker et al., 2003), SCH23390 administered into the NAcS significantly reduced, but did not eliminate, fructose-CFP from 76% (vehicle) to 62% (48 nmol). Whereas systemic raclopride also dose-dependently eliminated the expression of fructose-CFP, raclopride in the NAcS reduced, but did not eliminate the expression of fructose-CFP (75%: vehicle, 63%: 24 nmol). These data suggest that the NAcS is one, but not the only, site that participates in DA mediation of the expression of fructose-CFP.

The hypothesis (**Specific Aim 1B**) that direct bilateral administration into the NAcS of D1 or D2 receptor antagonists would block the *acquisition* of fructose-CFP was *not confirmed.* Systemic treatment with SCH23390 or raclopride (200 nmol/kg)

completely prevented the acquisition of a fructose-conditioned CS+ flavor-flavor preference (Baker et al., 2003) in that systemic injection of the D1 and D2 antagonists produced indifference (46-56%) in fructose-CFP relative to Yoked Control rats (66%). In contrast, SCH23390 or raclopride administration into the NAcS failed to alter the initial acquisition of fructose-CFP. However, whereas Yoked and untreated Control groups displayed stable preferences (~65%) through all three pairs of two-bottle tests, the NAcS D1 and D2 groups displayed preferences that declined from 70–73% in the first pair of tests to 57–60% in the third pair of tests, suggesting a process of hastened extinction. Analyses of methodological issues do not readily explain why the D1 and D2 rats, unlike the control rats, lost their CS+ preference with repeated two-bottle testing. It may be, however, that the salience of the CS+/Fs solution was reduced by the SCH23390 and raclopride treatment during training such that the D1 and D2 rats behaved as if they consumed less CS+/Fs than did the control rats during training. This explanation is supported by a proposed role for incentive salience in DA control of food intake and reward (e.g., Berridge, 2007; Berridge et al., 1998). According to this analysis, increasing the salience of the CS+/Fs solution during training (e.g., by increasing the sugar concentration) may prevent the extinction of CS+ preference observed in rats treated with DA receptor antagonists in the NAcS. Again, these data strongly suggest that the NAcS is not the critical site for the *acquisition* of fructose-CFP.

The hypothesis (**Specific Aim 2A**) that direct bilateral administration into the AMY of D1 or D2 receptor antagonists would block the *expression* of fructose-CFP was only *partially confirmed*. Again, the respective effects of systemic and AMY administration of the DA antagonists differed in magnitude. Whereas systemic

SCH23390 eliminated the expression of fructose-CFP, only the highest (48 nmol) SCH23390 dose administered into the AMY significantly reduced, but did not eliminate, the expression of fructose-CFP. Further, whereas systemic raclopride also dose-dependently eliminated the expression of fructose-CFP, only the highest (48 nmol) raclopride dose in the AMY significantly reduced, but did not eliminate fructose-CFP. These data strongly suggest that the AMY, like the NAcS, is one, but not the only, site that participates in DA mediation of the *expression* of fructose-CFP.

The hypothesis (**Specific Aim 2B**) that direct bilateral administration into the AMY of D1 or D2 receptor antagonists would block the *acquisition* of fructose-CFP was *not confirmed.* As described above, systemic treatment with SCH23390 or raclopride completely prevented the acquisition of a fructose-conditioned CS+ flavor-flavor preference (Baker et al., 2003). D1 antagonist treatment (12 nmol) in the AMY during training had no effect on the initial establishment of fructose-CFP, but rather resulted in a rapid extinction of the learned fructose-CFP. This pattern parallelled effects observed in the NAcS. In contrast, D2 antagonist treatment (12 nmol) in the AMY during training failed to affect the initial acquisition and subsequent maintenance of fructose-CFP across the 6-day paradigm. This lack of effect is in marked contrast to the initial systemic effects as well as the effectivess of D2 antagonists in the NAcS and D1 antagonists in the NAcS and AMY to hasten extinction. Again, these data strongly suggest that the AMY is not the critical site for the *acquisition* of fructose-CFP. The general similarities of the NAcS and AMY in DA mediation of fructose-CFP suggest that they may be part of a distributed brain network mediating this responses; this idea is discussed in further detail in Section C.

**I B. Opioid antagonism in the NAc and AMY alter expression and acquisition of fructose-CFP (Specific Aims 3 and 4).**

Brain opioid systems mediate the intake of palatable foods and fluids (Kelley et al., 2002; Yamamoto, 2003; Levine & Billington, 2004; Bodnar, 2004) in a model which proposes that opioid receptive neurons encode the positive hedonic affect aroused by tasty foods and drinks, and promote behavior related to food palatability (Kelley *et al.*, 2002; Levine & Billington, 2004), particularly by maintaining rather than initiating the consumption of palatable foods (Kirkham & Cooper, 1989; Frisina & Sclafani, 2002). This model predicts that opioid systems should play a critical role in flavor preference learning, particularly with those conditioned by the palatable flavors of sugar and fat. Yet systemic NTX failed to affect the acquisition or expression of either sucrose-CFP in sham-feeding rats (Yu et al., 1999) or fructose-CFP in real-feeding rats (Baker et al., 2004). However, the route of drug administration can influence the effect of NTX on food preference (Woolley et al., 2006) in a paradigm in which rats were given choice tests with two differently-flavored (chocolate and banana) but nutritionally-identical food pellets. Most animals displayed a mild preference (presumably unlearned) for the chocolate-flavored food. Systemic NTX treatment did not alter this preference, but rather reduced the intakes of both flavored foods. However, NTX microinfusions into the NAc selectively reduced the intake of the preferred chocolate-flavored food. The NAc is recognized as a critical site of opioid action on food intake and reward (see reviews: Kelley et al., 2002; Peciña, 2008). Thus, it is not surprising that NAc and systemic administration of opioid drugs might have different effects on food preferences based on the role of opioid signaling in the NAc on food intake and reward (Woolley et al., 2006).

Thus, **Specific Aim 3** examined whether NTX injected bilaterally into the NAc would dose-dependently alter the *expression* of fructose-CFP. Given the substantial evidence (Giraudo et al., 1998a, 1998b; Glass et al., 2000; Gosnell, 1988; Gosnell et al., 1986; Kim et al., 2001; Olszewski et al., 2000; Stanley et al., 1989) implicating the AMY in opioids control of food intake, **Specific Aim 4** investigated whether NTX injected bilaterally into the AMY would dose-dependently alter the *expression* of fructose-CFP. Based on the results of these two experiments, the hypotheses that NAc and/or AMY NTX administration would significantly reduce the *expression* of fructose-CFP were *not confirmed*. In fact, the small reductions in fructose-CFP following NAc or AMY NTX drug treatment were not much different from those observed in our prior studies with systemic NTX treatment.

However, the present findings do not negate the previous findings (Woolley et al., 2006) given the many methodological and conceptual differences between the two studies. Whereas our present and prior studies investigated relatively strong conditioned flavor preferences, the food preferences in Wooley's study were relatively weak, and based presumably on unlearned palatability differences between the flavored foods. In addition, the choice tests in Wooley's study were conducted with non-deprived rats fed nutritionally-identical diets, whereas the choice tests of the present experiment involved food-restricted rats given non-nutritive fluids. The type of test diet or fluid and deprivation state can influence the response to opioid drugs (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006). The following section will detail the involvement of opioid systems in unlearned relative to learned intake situations.

**II A. Role of the opioid system in spontaneous relative to learned food intake.**

General opioid antagonism with NTX failed to alter the acquisition or expression of either flavor-flavor or flavor-nutrient CFPs using sucrose, glucose, or fructose as the unconditioned stimulus (US) (Yu et al., 1999; Azzara et al., 2000; Baker et al., 2004). Because this finding appeared inconsistent with the central role of opioids in the response to palatable foods, three possible explanations are plausible as to why systemic NTX injections did not alter sugar-CFP. First, systemic NTX treatment failed to alter sugar-CFP because it acts on multiple brain opioid circuits that have differing functions in feeding and other behaviors. Supporting this view is a report that systemic NTX failed to alter the rat's inherent preference for one flavored food over another (i.e., chocolate vs. banana chow) whereas NTX microinfusions into the NAc selectively reduced the intake of the preferred chocolate food (Woolley *et al.*, 2006).Yet, NTX microinfusions into the NAc shell or core failed to alter the expression of flavor preferences produced by either Fructose-CFP or Glucose-CFP procedures (Bernal et al., 2010). This lack of effect was similar to another recent study (Taha et al., 2006) showing that NAc NTX was less effective than systemic NTX in reducing sucrose intake using an incentive devaluation conditioning paradigm. The second possibility (Sclafani et al., in preparation) is that the specific dependent variable used in our conditioning experiments, (i.e., CS solution intakes in 2-bottle tests) may be insensitive to subtle effects of NTX on the conditioned hedonic response to the CS+ flavor. Sugar conditioning produces hedonic as well as non-hedonic changes in the reward evaluation of CS flavors, not all of which are evident by simple intake measures (Myers & Sclafani, 2001; Sclafani & Ackroff, 2006). Therefore, lick microstructure (lick cluster size, number) was analyzed using oral 8% polycose, a

glucose polymer, which produced stronger and more persistent flavor preferences than did glucose or fructose. A flavor associated with 8% Polycose produced a CFP relative to a flavor associated with 2% Polycose, and this preference was unaffected by NTX. Microstructure analysis revealed that the size, but not the number of the lick clusters was greater with the CS+ than CS- (91.1 vs. 63.6 licks/cluster), which was unaffected by NTX. Moreover, in the previous studies, both training solutions were sweet, and perhaps their attractiveness was reduced by NTX. This led to the third possibility (Bonacchi et al., 2010) that compared NTX effects on preferences for flavors paired with sugar or starch drinks that have distinctive tastes to rats. Food-restricted rats displayed a significant 8% sucrose preference relative to 8% starch which increased following systemic NTX even though total intake of both solutions declined. Rats, trained to drink flavored (cherry or grape) starch and sucrose solutions in separate one-bottle sessions displayed a preference for the flavor paired with starch in two-bottle choice tests with flavors presented in a sucrose-starch mixture; NTX blocked the expression of this starch-conditioned preference. Rats treated with saline or NTX throughout one-bottle training with flavored sucrose and starch solutions displayed similar significant preferences for the starch-paired flavor, indicating that opioid antagonism failed to alter the acquisition of this conditioned preference. These findings suggest that the reward value of starch in liquid form is more dependent upon opioid signaling than that of sugar.

If the above findings indicate a lack of and opioid role in CFP, it is abundantly clear that brain opioid systems are deeply involved in the unlearned aspects of intake of palatable foods and fluids: (a) general opioid antagonists reduce and selective opioid agonists increase the intake of palatable foods and drinks (Sclafani et al., 1982; Ruegg et

al., 1997; Kelley et al., 2002); (b) β-endorphin- and enkephalin-deficient mice show reduced motivation to work for sugar- or fat-rich foods (Hayward *et al.*, 2002); (c) intake of palatable sugar solutions and liquid diets increase brain endorphin levels and alter enkephalin gene expression (Yamamoto et al., 2000; Kelley et al., 2003); and (d) opioid agonists and antagonists increase and decrease, respectively, "hedonic" taste reactivity (TR) responses elicited by intraoral sugar infusions (Parker *et al.*, 1992; Peciña & Berridge, 2000). As stated previously, these data support an opioid model of food palatability which posits that opioid receptive neurons encode the positive hedonic affect aroused by tasty foods and drinks and promote behavior related to food palatability (Kelley *et al.*, 2002; Levine & Billington, 2004), particularly by maintaining rather than initiating the consumption of palatable foods (Kirkham & Cooper, 1989; Frisina & Sclafani, 2002). Opioid systems are also involved in another aspect of sugar-induced learning. NTX dose-dependently reduced the expression, but not the acquisition of a sucrose-conditioned place preference (Delamater et al., 2000). Further, sucrose-preferring rats that have sucrose restricted display reductions in the total amount of energy generated by sucrose relative to starch following naltrexone infusions. In contrast, sucrose-preferring rats without subsequent sucrose restrictions failed to show reductions in sucrose preference (Levine et al., 2002). Thus, it may be that opioid antagonism influences primarily unlearned rather than learned preferences.

**II B. Differential roles of DA D1 and D2 antagonists in distinct flavor-flavor and flavor-nutrient processes in CFP.**

As indicated throughout, two interrelated types of conditioning operate in learned preferences: flavor-flavor conditioning and flavor-nutrient conditioning. Flavor-flavor

CFP refers 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 to the process by which a preference develops for a target flavor that is paired with the postingestive actions of a nutrient (e.g., sucrose) (Sclafani, 2004). Flavor-flavor and flavor-nutrient CFPs can be separated in the laboratory, and both warrant study because they appear to be mediated to some extent by separate neural mechanisms and exert different effects on behavior. Flavor-nutrient CFP can be quite strong (>90%: e.g., Azzara et al., 2000, 2001; Touzani et al., 2008, 2009) as compared to lesser (~75%), but significant sucrose- and fructose- flavor-flavor CFP (Baker et al., 2003, 2004; Yu et al., 1999, 2000a, 2000b). Robust flavor-nutrient preferences can be conditioned for flavored solutions that rats usually avoid (e.g., "bitter" sucrose octaacetate (SOA) and "sour" citric acid solutions) (Drucker *et al.*, 1994), situations that are obviously difficult to condition in flavor-flavor processes. Moreover, flavor-nutrient CFP can occur in a single trial and are very resistant to extinction and forgetting (Drucker *et al.*, 1994; Myers, 2007). In contrast, flavor-flavor CFP are evaluated in a choice test with both the CS+ and CS- flavors presented in water or saccharin solutions of the same concentration, and therefore, the CS+ and US flavors must be presented simultaneously or sequentially with delays no longer than a few seconds (Elizalde & Sclafani, 1990; Lyn & Capaldi, 1994). In addition, IG nutrient conditioning can increase the absolute intake of the CS+ flavored solution when presented alone, which is known as conditioned acceptance (Sclafani, 2004).

The above data thereby suggest that, to some degree, different neurobehavioral processes mediate the two types of flavor preference learning (Capaldi *et al.*, 1987;

Touzani & Sclafani, 2002b). Collaboration between the laboratories of Drs. Richard Bodnar and Anthony Sclafani has systematically compared the systemic and central DA and opioid effects on flavor-flavor and flavor-nutrient CFP. As described earlier, systemic administration of DA D1 and D2 receptor antagonists attenuated at lower doses and completely blocked at higher doses both the acquisition and expression of flavor-flavor learning (Baker et al., 2003; Yu et al., 2000a, 2000b). However, systemic DA D1, but not D2, receptor antagonism blocked the acquisition, but not the expression, of flavor-nutrient CFP (Azzara et al., 2001), indicating that the D2 receptor is involved in one (flavor-flavor), but not the other (flavor-nutrient) conditioning process. Subsequent central studies on the NAc and AMY determined the role of DA antagonism relative to flavor-flavor and flavor-nutrient types of learning. Bilateral administration of the D1 receptor antagonist, SCH23390, into the NAc significantly reduced the expression of flavor-flavor CFP (Bernal et al., 2008), but not of flavor-nutrient CFP, except at higher doses that severely affected intake (Touzani et al., 2008). Further, an identical dose (12 nmol) of a D1 antagonist in the NAc eliminated the acquisition of flavor-nutrient CFP (Touzani et al., 2008), but failed to affect the initial acquisition of flavor-flavor CFP. Rather, NAc D1 antagonism hastened the subsequent extinction of flavor-flavor learning (Bernal et al., 2008). Further, bilateral microinfusions of the DA D2 receptor antagonist, raclopride, into the NAc attenuated the expression, but not the acquisition, of flavor-flavor CFP, and hastened the extinction of these flavor-flavor processes (Bernal et al., 2008). A similar pattern of results was obtained in DA antagonism studies in the AMY. Bilateral administration of DA D1 receptor antagonist into the AMY significantly reduced the expression of flavor-flavor CFP (Bernal et al., 2009), but not of flavor-

nutrient CFP, except at higher doses that severely affected total intake (Touzani et al., 2009a). In contrast, DA D1 antagonism in the AMY eliminated the acquisition of flavor-nutrient CFP (Touzani et al., 2009a), but failed to affect the initial acquisition of flavor-flavor CFP (Bernal et al., 2009). Furthermore, blockade of the whole AMY was needed to completely eliminate the acquisition of flavor-nutrient CFP as opposed to just reducing this initial acquisition by selective D1 antagonist injections into the central or the baso-lateral nuclei of the AMY (Touzani et al., 2009a). Like the results obtained from the NAc, bilateral D2 administration into the AMY reduced the expression of flavor-flavor, but not flavor-nutrient, learning, but it did not hasten extinction of CFP (Bernal et al., 2009). Once again, given the separate methodological procedures that allow the evaluation of flavor-flavor and flavor-nutrient CFPs, as well as the differential results from systemic and central studies, it seems logical to conclude that separate, although related, neural mechanisms underlie these distinct learning processes. The underlying neural system mediating both of these effects is described in the next section.

### II C. Proposal of a distributed brain network mediating DA signaling and CFP

Given some of the striking similarities observed in DA antagonist modulation of both flavor-flavor and flavor-nutrient processes, and given the fact that DA antagonism only partially reduces the expression of flavor-flavor CFP following antagonist treatment in the NAcS and AMY, it appears quite plausible that a single site does not mediate all of the effects observed following systemic DA antagonism. This raises the possibility that a distributed brain network is responsible for these effects, and this section will discuss

inter-relationships between the two sites of interest, the NAcS and AMY, as well as a third site, the medial prefrontal cortex (mPFC).

The NAc is a site in which palatable foods and sweet solutions, in particular, stimulate DA efflux (Bassareo & Di Chiara, 1997; Bassareo & Di Chiara, 1999; Genn et al., 2004; Cheng & Feenstra, 2006). Recent findings further indicate that the postingestive effects of glucose promote NAc DA release (De Araujo *et al.*, 2008; Ren *et al.*, 2009). In addition, intake of a CS+ solution previously paired with IG carbohydrate, but not a CS- solution, increased NAc DA efflux (Mark *et al.*, 1991). DA receptor antagonism in the NAc impairs learning in several paradigms such as Pavlovian approach conditioning to a CS paired with sugar (Parkinson *et al.*, 1999), operant responding for sugar (Parkinson *et al.*, 1999; Smith-Roe & Kelley, 2000), and conditioned avoidance of sweet taste (Fenu *et al.*, 2001).

The AMY has also been implicated in learning related to flavor aversion (Bures *et al.*1998), flavor preference (Gilbert *et al.*, 2003; Touzani & Sclafani, 2005), and in Pavlovian and instrumental reward (Cardinal et al., 2002; Baxter & Murray, 2002). Feeding and gastric nutrient infusions increased AMY DA turnover or efflux (Heffner et al., 1980; Hajnal & Lenard, 1997), and a Pavlovian CS for food elicited AMY DA efflux (Harmer & Phillips, 1999). The mPFC is a component of the mesocorticolimbic DA network and plays a crucial role in reward-related learning (Kelley, 2004). It has intimate connections with the NAc and AMY and presents a moderate density of D1-like receptors (Mansour *et al.*, 1990). Resuls from central studies of DA D1 and D2 receptor antagonists suggest that DA-responsive neurons within the NAc and the AMY are part of a regional network of brain sites that mediate flavor-flavor conditioning in a manner

similar to proposed regional networks of interacting brain sites for other aspects of feeding behavior (e.g., Baldo and Kelley, 2007; Bodnar and Levine, 2008; Will et al., 2003).

Several sources of evidence support the existence of an AMY–NAcS DA reward network. First, the source of DA into the AMY is derived from the mesolimbic DA pathway originating in the VTA which also provides DA innervation to the NAcS (e.g., Asan, 1997, 1998; Eliava et al., 2003; Lammel et al., 2008). Second, there is very strong evidence for AMY, particularly baso-lateral and lateral nuclei, projections to the NAc, particularly the shell region (Brog et al., 1993; Christie et al., 1987; Fudge et al., 2002) in an organized and highly compartmentalized fashion (Groenewegen et al., 1999; Phillipson and Griffiths, 1985; Wright and Groenewegen, 1995; Wright et al., 1996). Third, in turn, the NAcS sends projections to the AMY (Brog et al., 1993; Mello et al., 1992). Fourth, the NAcS is a site in which sweet taste stimulated DA efflux (e.g., Cheng and Feenstra, 2006; Genn et al., 2004), and in which DA antagonists suppressed lithium chloride-conditioned saccharin aversions (Fenu et al., 2001). Indeed and importantly, the NAcS is a site in which the motivational valence and novelty of hedonic food stimuli (e.g., Fonzies) is a critical component of DA release as compared to the core of the NAc in which generic motivational values of food-related stimuli elicit DA release (Bassareo and DiChiara, 1997, 1999a, 1999b; Bassareo et al., 2002). Fifth, the AMY is another site in which Pavlovian and instrumental reward learning are supported (Baxter and Murray, 2002; Cardinal et al., 2002), in which feeding and gastric nutrient infusions increased DA turnover or efflux (Hajnal and Lenard, 1997; Heffner et al., 1980), and in which a Pavlovian CS for food elicited DA efflux (Harmer and Phillips, 1999). Further, AMY

inactivation modulated feeding-stimulated DA efflux in the NAc (Ahn and Phillips, 2002). Finally, recent findings indicate that AMY and DA projections to the NAc interact to promote sugar seeking behavior, and that the AMY responses preceded and indeed excited the responses observed in the NAc (Ambroggi et al., 2008). The integrity of the AMY, and particularly, the baso-lateral nucleus is necessary for NAc responsivity. These findings are consistent with a network model of D1 and/or D2 receptor modulation of flavor preference learning, with differential involvement in the acquisition and expression of flavor-flavor and flavor-nutrient associations. Further systematic microinjection studies in the AMY and NAc, as well as other sites (e.g., mPFC, lateral hypothalamus) sites are necessary to identify the underlying system(s) mediating these forms of food-related learning.

Among other brain sites mediating DA involvement in sugar-conditioned flavor preferences is the mPFC. The mPFC is a component of the mesocorticolimbic DA network and plays a crucial role in reward-related learning (Kelley, 2004) through intimate connections with the NAc and AMY. Most relevant to CFP are reports of increased DA efflux in the mPFC induced by feeding and food-related cues in both Pavlovian and instrumental learning tasks (D'Angio & Scatton, 1989; Hernandez & Hoebel, 1990; Izaki et al., 1999; Bassareo et al., 2002). Touzani (Touzani et al., 2010) recently found that the acquisition of flavor-nutrient conditioining was eliminated by mPFC D1 receptor antagonism (54%) relative to vehicle (71%); again, the expression of flavor-nutrient CFP was largely unaffected by mPFC D1 receptor antagonism. These effects were very similar to D1 antaghonist-mediated flavor-nutrient effects in the NAcS (Touzani et al., 2008) and AMY (Touzani et al., 2009a). Moreover, the acquisition of

fructose-CFP observed in yoked control rats (67%) was eliminated by a 24 nmol dose of SCH23390 (53%) or raclopride (54%) (Malkusz et al., 2010). Expression of fructose-CFP was significantly reduced by mPFC D2, but not D1 receptor antagonism (Malkusz et al., 2008, 2009).

When considering all of the theories related to DA control of food intake as well as any DA role in learning related to food intake, the ideas of hedonics, incentive salience, stimulus-outcome and stimulus-action mechanisms have come into play. As reviewed above, these concepts have been largely attributed to function of the meso-limbic and meso-cortical DA systems. These systems were the subject of study because the major terminal sites, the NAc, AMY and mPFC, all display strong DA efflux responses to oral sugar stimulation, and even to intragastric glucose stimulation. A second major DA system, the nigro-striatal tract, has been implicated in different forms of DA-mediated learning responses including the strengthening of S->R relationships and habit-related motor learning. Although these processes are certainly important, the striatal terminal sites have not been as strongly implicated in DA efflux responses to sugars, and the effects of DA antagonists in such terminals have stronger non-specific motor impairments that could affect the proper determination of preferences.

**II D. Future Directions.**

DA signaling in the NAc, AMY, and mPFC appears critical to flavor preference conditioning by the postingestive actions of glucose, and these areas may form a distributed network. One area of interest for future studies involves potential functional interactions among these network regions in supporting glucose conditioning in selective disconnection experiments. These network regions may also act together to support flavor

conditioning by the sweet taste of fructose, given that selective DA antagonism in selected areas (NAc, AMY) fails to block such conditioning. Thus, the effect of simultaneous DA antagonism in multiple regions (NAc, AMY) on fructose conditioning should be determined using a disconnection procedure in which a mixed solution of the GABA-A and GABA-B agonists baclofen and muscimol (B/M) will be administered into one site (e.g., NAcS) and the DA antagonists are administered into a second site (e.g., the AMY or mPFC). Systematic analyses of where the inactivation takes place would then be paired with DA antagonist effects for flavor-flavor conditioning.

A second series of studies should evaluate the potential interaction of glutamate with the observed DA effects. Golden and Houpt (2007) demonstrated that systemic administration of NMDA antagonists blocked the acquisition, but not expression of fructose-CFP. An interesting series of studies would determine whether the acquisition, but not thexpression of flavor-flavor conditioning would be correspondingly blocked following NMDA and AMPA receptor antagonists in the NacS, AMY and mPFC as well as the source of the DA afferents, the VTA. Such studies would not only expand our knowledge about the nature of the distributed brain network mediating these conditioning effects, but expand the list of neurochemical “players” involved in these responses.

**II E. Implications.**

As indicated in the outset of this dissertation, obesity is recognized as a major public health problem in the United States and elsewhere (Caballero, 2007). The etiology of obesity is complex but there is general agreement that the overabundance of palatable, energy dense foods is an important contributing factor. Considerable progress has been made during the past years in identifying and characterizing the central neural systems

that regulate energy balance. These homeostatic systems appear more effective in protecting against undernutrition and weight loss than overeating and weight gain (Saper et al., 2002; Schwartz et al., 2003; Halford et al., 2004). The sight, smell, taste, and texture of palatable foods activate potent brain reward systems that can override the homeostatic energy balance system. These brain reward systems have been the subject of extensive investigations from the perspectives of natural rewards (e.g., food) and drugs of abuse. In the past two decades there have also been advances in our understanding of the role of learning in determining food preferences and appetite. The studies compiled in this dissertation help to elucidate the central neurotransmitter systems and brain circuits involved in food preference learning, particularly for sugar. The understanding of the basic mechanisms involved in the development of food preferences in animals may be directly translatable and thereby provide insights into the clinical treatment of overeating and obesity.

## REFERENCES

Accolla, R., Bathellier, B., Petersen, C.C.H., & Carleton, A. (2007). Differential spatial representation of taste modalities in the rat gustatory cortex. The Journal of Neuroscioence, 27(6), 1396-1404.

Ackroff, K. (2008). Learned flavor preferences: The variable potency of post-oral nutrient reinforces. Appetite, 51, 743-746.

Ackroff, K., & Sclafani, A. (1999). Palatability and Foraging Cost Interact to Control Caloric Intake. Journal of Experimental Psychology, 25(1), 28-36.

Ackroff, K., & Sclafani, A. (2004). Fructose-conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration. Appetite, 42, 287-297.

Ackroff, K., & Sclafani, A. (2006). Energy density and macronutrient composition determine flavor preference conditioned by intragastric infusions of mixed diets. Physiology and Behavior, 89, 250-260.

Ackroff, K., Bonacchi, K., Magee, M., Yiin, Y.M., Graves, J.V., & Sclafani, A. (2007). Obesity by choice revisited: Effects of food availability, flavor variety and nutrient composition on energy intake. Physiology and Behavior, 92, 468-478.

Ackroff, K., Dym, C., Yiin, Y.M., & Sclafani, A. (2009). Rapid acquisition of conditioned flavor preferences in rats. Physiology & Behavior, 97, 406-413.

Ackroff, K., Lucas, F., & Sclafani, A. (2005). Flavor preference conditioning as a function of fat source. Physiology and Behavior, 85, 448-460.

Ackroff, K., Rozental, D., & Sclafani, A. (2004). Ethanol-conditioned flavor preferences compared with sugar- and fat-conditioned preferences in rats. Physiology and Behavior, 81, 699-713.

Ackroff, K., Touzani, K., Peets, T.K., & Sclafani, A. (2001). Flavor preferences conditioned by intragastric fructose and glucose Differences in reinforcement potency. Physiology and Behavior, 72, 691-703.

Adan, R.A., Vanderschuren, L.J., & La Fleur, S. (2008). Anti-obesity drugs and neural circuits of feeding. Trends in Pharmacological Sciences, 29(4), 208-217.

Ågmo A, Galvan A, & Talamantes B. (1995). Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. Pharmacol Biochem Behav. 52(2), 403-14.

Ahn S, Phillips AG. (2002). Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. J Neurosci., 22(24), 10958-65.

Albertella, L., & Boakes, R.A. (2006). Persistence of Conditioned Flavor Preferences Is Not Due to Inadvertent Food Reinforcement. Journal of Experimental Psychology, 32(4), 386-395.

Alcaro, A., Huber, R., & Panksepp, J. (2007). Behavioral functions of the mesolimbic dopaminergic system: An effective neuroethological perspective. Brain Research Reviews, 56, 283-321.

Ambroggi, F., Ishikawa, A., Fields, HL., & Nicola, SM. (2008). Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron, 59, 648-61.

Andrzejewski, M.E., Spencer, R.C., & Kelley, A.E. (2005). Instrumental learning, but not performance, requires dopamine D1-receptor activation in the amygdala. Neuroscience, 135, 335-345.

Asan, E. (1997). Ultrastructural features of tyrosine-hydroxylase-immunoreactive afferents and their targets in the rat amygdala. Cell Tissue Res, 288, 449-69.

Asan, E. (1998). The catecholaminergic innervation of the rat amygdala. Adv Anat Embryol Cell Biol, 142, 1-118.

Azzara, A.V., Bodnar, R.J., Delamater, A.R., & Sclafani, A. (2000). Naltrexone fails to block the acquisition or expression of a flavor preference conditioned by intragastric carbohydrate infusions. Pharmacology Biochemistry and Behavior, 67, 545-557.

Azzara, A.V., Bodnar, R.J., Delamater, A.R., & Sclafani, A. (2001). D1 but not D2 dopamine receptor antagonism blocks the acquisition of a flavor-preference conditioned by intragastric carbohydrate infusions. Pharmacology Biochemistry and Behavior, 68, 709-720.

Baker, R.M., Shah, M.J., Sclafani, A., & Bodnar, R.J. (2003). Dopamine D1 and D2 antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats. Pharmacology Biochemistry and Behavior, 75, 55-65.

Baker, R.W., Li, Y., Lee, M.G., Sclafani, A., & Bodnar, R.J. (2004). Naltrexone does not prevent acquisition or expression of flavor preferences conditioned by fructose in rats. Pharmacology Biochemistry and Behavior, 78, 239-246.

Baker, R.W., Osman, J., & Bodnar, R.J. (2001). Differential actions of dopamine receptor antagonism in rats upon food intake elicited by either mercaptoacetate or exposure to a palatable high-fat diet. Pharmacology Biochemistry and Behavior, 69, 201-208.

Baldo, B.A., & Kelley, A.E. (2007). Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. Psychopharm, 191, 439-459.

Balleine, BW., & Killcross, S. (2006). Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci., 29, 272-9.

Barbano, M.F., & Cador, M. (2007). Opioids for hedonic experience and dopamine to get ready for it. Psychopharmacology, 191, 497-506.

Bassareo V, & Di Chiara G. (1997). Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci., 17(2), 851-61.

Bassareo V, & Di Chiara G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. Eur J Neurosci., 11(12), 4389-97.

Bassareo V, De Luca MA, & Di Chiara G. (2002). Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. J Neurosci. 22(11), 4709-19.

Baxter MG, Murray EA. (2002). The amygdala and reward. Nat Rev Neurosci., 3(7), 563-73.

Beczkowska, I.W., Bowen, W.D., and Bodnar, R.J. (1992). Central opioid receptor subtype antagonists differentially alter sucrose and deprivation-induced water intake in rats. Brain Research, 589, 291-301.

Berman, Y., Devi, L., & Carr, KD. (1994). Effects of chronic food restriction on prodynorphin-derived peptides in rat brain regions. Brain Res., 664, 49–53.

Berman, Y., Devi, L., Spangler, R., Kreek, MJ., & Carr, KD. (2006). Chronic food restriction and streptozotocin-induced diabetes differentially alter prodynorphin mRNA levels in rat brain regions. Mol Brain Res., 46, 25–30.

Bernal, S.Y., Dostova, I., Kest, A., Abayev, Y., Kandova, E., Touzani, K., Sclafani, A., & Bodnar, R.J. (2008a). Role of dopamine D1 and D2 receptors in the nucleus accumbens shell on the acquisition and expression of fructose-conditioned flavor-flavor preferences in rats. Behavioural Brain Research, 190, 59-66.

Bernal, S.Y., Miner, P., Abayev, Y., Kandova, E., Gerges, M., Touzani, K., Sclafani, A., & Bodnar, R.J. (2009). Role of amygdala dopamine D1 and D2 receptors in the acquisition and expression of fructose-conditioned flavor-flavor preferences in rats. Behavioural Brain Research, 205, 183-190.

Bernal, S.Y., Touzani, K., Gerges, M., Abayev, Y., Sclafani, A., & Bodnar, R.J. (2010). Opioid receptor antagonism in the nucleus accumbens fails to block the expression of sugar-conditioned flavor preferences in rats. Pharmacology, Biochemistry and Behavior, 95, 56-62.

Bernal, SY, Abayev, Y., Miner, P., Kandova, E., Pinhas, A., Touzani, K., Sclafani, A., & Bodnar, RJ. (2006). Expression of fructose-conditioned flavor preferences: role of D1 and D2 dopamine receptors in the amygdala. Soc. Neurosci. Abstr. 32: [SFN Abstract Viewer].

Bernal, SY, Miner, P., Abayev, Y., Kandova, E., Pulatov, P., Touzani, K., Sclafani, A., & Bodnar, RJ. (2007). Acquisition of fructose-conditioned flavor preferences in rats: role of D1 and D2 dopamine receptors in the nucleus accumbens and amygdala. Soc Neurosci Abstr. 33: [SFN Abstract Viewer].

Bernal, SY., Miner, P., Abayev, Y., Kandova, E., Burstein, A., Rogawski, E., Wapniak, R., Dimopoulos, S., Touzani, K., Sclafani, A., & Bodnar, RJ. (2008b). Expression of

fructose-conditioned flavor preferences in rats: role of opioid receptors in the amygdala and nucleus accumbens shell and core. Soc Neurosci Abstr. 34: [SFN Abstract Viewer].

Berridge, K.C. (1996). Food Reward: Brain Substrates of Wanting and Liking. Neuroscience and Behavioral Reviews, 20(1), 1-25.

Berridge, K.C. (2007). The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology, 191, 391-431.

Berridge, K.C. (2009). 'Liking' and 'wanting' food rewards: Brain substrates and role in eating disorders. Physiology and Behavior, 97, 537-550.

Berridge, K.C., & Kringelbach, M.L. (2008). Affective neuroscience of pleasure: Reward in humans and animals. Psychopharmacology, 199, 457-480.

Berridge, K.C., & Robinson T.E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev, 28, 309-69.

Berridge, K.C., Robinson, T.E., & Aldridge, J.W. (2009). Dissecting components of reward: 'liking,' 'wanting,' learning. Current Opinion in Pharmacology, 9, 65-73.

Berridge, K.C., Venier, I.L., & Robinson, T.E. (1989). Taste Reactivity Analysis of 6-Hydroxydopamine-Indiced Aphagia: Implications for Arousal and Anhedonia Hypothesis of Dopamine Function. Behavioral Neuroscience, 103(1), 36-45

Bliss, TV., & Collingridge, GL. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature, 361, 31-9.

Boakes, R.A., Albertella, L., & Harris, J.A. (2007). Expression of Flavor Preference Depends on Type of Test and on Recent Drinking History. Journal of Experimental Psychology, 33(3), 327-338.

Bodnar, R.J. (2004). Endogenous opioids and feeding behavior: a 30-year historical perspective. Peptides, 25, 697-725.

Bodnar, R.J., & Levine, A.S. (2008). Role of opioid peptides in regulating energy balance. In: Harvey J, Withers DJ, editors. Neurobiology and Obesity. United Kingdom: Cambridge University Press, 232-265

Bodnar, R.J., Glass, M.J., Ragnauth, A., & Cooper, M.L. (1995). General, μ and κ opioid antagonists in the nucleus accumbens alter food intake under deprivation, glucoprivation and palatable conditions. Brain Research, 700, 205-212.

Bonacchi, K.B., Ackroff, K., & Sclafani, A. (2008). Sucrose taste but not polycose taste conditions flavor preferences in rats. Physiology and Behavior, 95, 235-244.

Broberger, C., & Hökfelt, T. (2001). Hypothalamic and vagal neuropeptide circuitries regulating food intake. Physiology & Behavior, 74, 669-682.

Brog, JS., Salyapongse, A., Deutch, AY., & Zahm, DS. (1993). The patterns of afferent innervation of the core and shell in the “accumbens” part of the ventral striatum: immuno-histochemical detection of retrogradely transported fluoro-gold. J Comp Neurol, 338, 255-78.

Brunstrom, J.M., & Fletcher, H.Z. (2008). Flavour-flavour learning occurs automatically and only in hungry participants. Physiology & Behavior, 93, 13-19.

Capaldi, E.D., & Privitera, G.J. (2008a). Decreasing dislike for sour and bitter in children and adults. Appetite, 50, 139-145.

Capaldi, E.D., & Privitera, G.J. (2008b). Potentiation of taste and extract stimuli in conditioned flavor preference learning. Learning & Behavior, 36(1), 62-66.

Cardinal RN, Parkinson JA, Lachenal G, Halkerston KM, Rudarakanchana N, Hall J, Morrison CH, Howes SR, Robbins TW, & Everitt BJ. (2002). Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. Behav Neurosci., 116(4), 553-67.

Caulliez R, Meile MJ, & Nicolaidis S. (1996). A lateral hypothalamic D1 dopaminergic mechanism in conditioned taste aversion. Brain Res. 729(2), 234-45.

Chambers, E.S., Bridge, M.W., & Jones, D.A. (2009). Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. Journal of Physiology, 587(8), 1779-1794.

Cheer, J.F., Aragona, B.J., Heien L.A.V., & Seipel, A.T. (2007). Coordinated Accumbal Dopamine Release and Neural Activity Drive Goal-Directed Behavior. Neuron, 54, 237-244.

Cheng J., & Feenstra, M.G. (2006). Individual differences in dopamine efflux in nucleus accumbens shell and core during instrumental conditioning. Learn Mem, 13, 168-177.

Christie, MJ., Summers, RJ., Stephenson, JA., Cook, CJ., Beart, PM. (1987). Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retro-grade transport study utilizing D[3H]aspartate and {3H]GABA. Neuroscience, 22, 425-39.

Cleary, J., Weldon, D.T., O'Hare, E., Billington, C., & Levine, A.S. (1996). Naloxone effects on sucrose-motivated behavior. Psychopharmacology, 126(2), 110-114.

Cohen, D.A. (2008). Neurophysiological pathways to obesity: below awareness and beyond individual control. Diabetes, 57(7), 1768-1773.

Colantuoni, C., Rada, P., McCarthy, J., Patten, C., Avena, N.M., Chadeayne, A., & Hoebel, B.G. (2002). Evidence That Intermittent, Excessive Sugar Intake Causes Endogenous Opioid Dependence. Obesity Research, 10(6), 478-488.

Cooper, S.J. (1983). Benzodiazepine-opiate antagonist interactions in relation to feeding and drinking behavior. Life Sci., 32(10), 1043-1051.

Cooper, S.J. (2004). Endocannabinoids and food consumption: comparisons with benzodiazepine and opioid palatability-dependent appetite. European Journal of Pharmacology, 500, 37-49.

Cooper, S.J. (2005). Palatability-dependent appetite and benzodiazepines: new directions from the pharmacology of GABA(A) receptor subtypes. Appetite, 44(2), 133-50.

Cota D, Tschöp MH, Horvath TL, & Levine AS. (2006). Cannabinoids, opioids and eating behavior: the molecular face of hedonism? Brain Res Rev. 51(1), 85-107.

Curtis, K.S., Stratford, J.M., & Contreras, R.J. (2005). Estrogen increases the taste threshold for sucrose in rats. Physiology & Behavior, 86, 281-286.

Danaei, G., Ding, E.L., Mozaffarian, D., Taylor, B., Rehm, J., Murray, C.J.L., & Ezzati, M. (2009). The preventable causes of death in the United States: Comparative risk assessment of dietary, lifestyle, and metabolic risk factors. Plos Medicine, 6(4), e1000058.

De Araujo, I.E., Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Garon, M.G., Nicoleis, M.A.L., & Simon, S.A. (2008). Food reward in the absence of taste receptor signaling. Neuron, 57, 930-941.

Delamater, A.R. (2007). Extinction of Conditioned Flavor Preferences. Journal of Experimental Psychology, 33(2), 160-171.

Delamater, A.R., Campese, V., LoLordo, V.M., & Sclafani, A. (2006). Unconditioned Stimulus Devaluation Effects in Nutrient-Conditioned Flavor Preferences. Journal of Experimental Psychology, 32(3), 295-306.

Delamater, A.R., Sclafani, A., & Bodnar, R.J. (2001). Pharmacology of Sucrose-Reinforced Place-Preference Conditioning: Effects of Naltrexone. Pharmacology Biochemistry and Behavior, 65(4), 697-704.

Di Chiara, G., & Bassareo, V. (2007). Reward system and addiction: what dopamine dies and doesn't do. Current Opinion in Pharmacology, 7, 1-8.

Dostova, I., Kest, A., Bernal, SY., Abayev, Y., Kandova, E., Touzani, K., Sclafani, A., & Bodnar, RJ. (2006). Expression of fructose-conditioned flavor preferences: role of D1 and D2 dopamine receptors in the nucleus accumbens shell. Soc. Neurosci. Abstr. 32: [SFN Abstract Viewer].

Dragone, D. (2009). A rational eating model of binges, diets and obesity. Journal of Health Economics, 28(4), 799-804.

Dwyer, D.M. (1999). Retrospective revaluation or mediated conditioning? The effect of different reinforcers. The Quarterly J. of Experimental Psychology, 52B(4), 289-306.

Dwyer, D.M. (2005). Reinforcer devaluation palatability-based learned flavor preferences. Journal of Experimental Psychology, 3(4), 487-492.

Dwyer, D.M. (2009). The effects of midazolam on the acquisition and expression of fructose- and maltodextrin-based flavour preferences. Pharmacology, Biochemistry and Behavior, 91, 503-510.

Dwyer, D.M., & Quirk, R.H. (2008). Context Conditional Flavor Preferences in the Rat Based on Fructose and Maltodextrin Reinforcers. Journal of Experimental Psychology, 34(2), 294-302.

Dwyer, D.M., Bennett, C.H., & Mackintosh, N.J. (2001). Evidence for inhibitory associations between the unique elements of two compound flavours. The Quarterly J. of Experimental Psychology, 54B(2), 97-107.

Dwyer, D.M., Boakes, R.A., & Hayward, A.J. (2008). Reduced palatability in lithium- and activity-based, but not in amphetamine-based, taste aversion learning. Behavioral Neuroscience, 122(5), 1051-1050.

Eliava, M., Yilmazer-Hanke, D., & Asan, E. (2003). Interrelationships between monoaminergic afferents and corticotrophin-releasing factor-immunoreactive neurons in the rat central amygdaloid nucleus: ultrastructural evidence for dopaminergic control of amygdaloid stress systems. Histochem Cell Biol, 120, 183-97.

Elizalde, G., & Sclafani, A. (1990). Flavor preference conditioned by intragastric polycose infusions: A detailed analysis using an electronic esophagus preparation. Physiology and Behavior, 47, 63-77.

Epstein, L.H., & Leddy, J.J. (2006). Food reinforcement. Appetite, 46, 22-25.

Fanselow, M.S. & Birk, J. (1982). Flavor-flavor associations induce hedonic shifts in taste preferences. Anim Learn Behav, 10, 223-228.

Fenu, S., Bassareo, V., & Di Chiara, G. (2001). A Role for Dopamine D1 Receptors of the Nucleus Accumbens Shell in Conditioned Taste Aversion Learning. The journal of Neuroscience, 21(17), 6897-6904.

Finlayson G, King N, Blundell JE. (2007). Liking vs. wanting food: importance for human appetite control and weight regulation. Neurosci Biobehav Rev. 31(7), 987-1002.

Frisina, P.G., & Sclafani, A. (2002). Naltrexone suppresses the late but not early licking response to a palatable sweet solution: opioid hedonic hypothesis reconsidered. Pharmacology Biochemistry and Behavior, 74, 163-172.

Fudge, JL., Kunishio, K., Walsh, P., Richard, C., & Haber, SN. (2002). Amygdaloid projections to ventromedial striatal subterritories in the primate. Neuroscience, 110, 257-75.

Furudono, Y., Ando, C., Yamamoto, C., Kobashi, M., & Yamamoto, T. (2006). Involvement of specific orexigenic neuropeptides in sweetner-induced overconsumption in rats. Behavioural Brain Research, 175, 241-248.

Gallagher, M. (2000). The amygdala and associative learning. In: Aggleton JP, editor. The amygdala: a functional analysis. 2nd ed. Oxford, UK: Oxford University Press; p. 311-29.

Gallo M, & Bures J. (1991). Acquisition of conditioned taste aversion in rats is mediated by ipsilateral interaction of cortical and mesencephalic mechanisms. Neurosci Lett., 133(2), 187-90.

Garcia J, Lasiter PS, Bermudez-Rattoni F, Deems DA. (1985). A general theory of aversion learning. Ann N Y Acad Sci., 443, 8-21.

Geary, N. & Smith, GP. (1985). Pimozide decreases the positive reinforcing effect of sham fed sucrose in the rat. Pharmacol Biochem Behav. 22(5), 787-90.

Geary, N. (2001). Estradiol, CCK and satiation. Peptides, 22, 1251-1263.

Genn, R.F., Ahn, S., & Phillips, A.G. (2004). Attenuated dopamine efflux in the rat nucleus accumbens during successive negative contrast. Behavioral Neuroscience, 118, 869-873.

Gilbert, P.E., & Kesner, R. (2002). The Amygdala but Not the Hippocampus Is Involved in Pattern Sepatation Based on Reward Value. Neurobiology of Learning and Memory, 77, 338-353.

Gilbert, P.E., Campbell, A., & Kesner, R.P. (2003). The role of the amygdala in conditioned flavor preference. Neurobiology of Learning and Memory, 79, 118-121.

Giraudo SQ, Billington CJ, Levine AS. (1998). Effects of the opioids antagonist naltrexone on feeding induced by DAMGO in the central nucleus of the amygdala and in the paraventricular nucleus in the rat. Brain Res., 782, 18–23.

Giraudo SQ, Kotz CM, Billington CJ, Levine AS. (1998). Associationbetween the amygdala and the nucleus of the solitary tract in mu-opioid induced feeding in the rat. Brain Res., 802, 184–8.

Glass MJ, Billington CJ, Levine AS. (2000). Naltrexone administered to central nucleus of amygdala or PVN: neural dissociation of diet and energy. Am J Physiol., 279, 86–92.

Glendinning, J.I., Yiin, Y.M., Ackroff, K., & Sclafani, A. (2008). Intragastric infusion of denatonium conditions flavor aversions and delays gastric emptying in rodents. Physiology and Behavion, 93, 757-765.

Golden, G.J., & Houpt, Thomas, A. (2007). NMDA receptor in conditioned flavor-taste preference learning: Blockade by MK-801 and enhancement by D-cycloserine. Pharmacology Biochemistry and Behavior, 86, 587-596.

Gondek-Brown, M., Sclafani, A., McNally, S., Gordon, R., Farkas, J., Torres, M., & Kissileff, H. (2007). Measuring the Reward Value of Aspartame-Sweetened Yogurt Shake via Continuous and Progressive Ratio Schedules in Humans. Columbia Undergraduate Science Journal, 2(1), 52-64.

Goodwin, F.L.W., Campisi, M., Babinska, I., & Amit, Zalman (2001). Effects of Naltrexone on the intake of ethanol and flavores solutions in rats. Alcohol, 25, 9-19.

Gosnell BA, Morley JE, & Levine AS. (1986). Opioid-induced feeding: localization of sensitive brain sites. Brain Res., 369, 177–84.

Gosnell BA. (1988). Involvement of mu opioid receptors in the amygdala in the control of feeding. Neuropharmacology, 27, 319–26.

Greenberg, D., & Smith, G.P. (1996). The controls of fat intake. Psychosom. Med., 58, 559-569.

Groenewegen, HJ., Wright, CI., Beijer, AV., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. Ann NY Acad Sci, 877, 49-63.

Grossman, S.E., Fontanini, A., Wieskopf, J.S., & Katz, D.B. (2008). Learning-Related Plasticity of Temporal Coding in Simultaneously Recorded Amygdala-Cortical Ensembles. The Journal of Neuroscience, 28(11), 2864-2873.

Hajnal A, & Norgren R. (2004). Sucrose sham feeding decreases accumbens norepinephrine in the rat. Physiol Behav.82(1), 43-7.

Hajnal A, Lénárd L. (1997). Feeding-related dopamine in the amygdala of freely moving rats. Neuroreport., 8(12), 2817-20.

Hajnal A, Smith, GP., & Norgren R. (2003). Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol., 286R, 31-7.

Halpern, B.P. (2000). Glutamate and the Flavor of Foods. The Journal of Nutrition, 130(4S), 910S-914S.

Harmer CJ, Phillips GD. (1999). Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated D-amphetamine. Neuroscience., 90(1), 119-30.

Hayward, M.D., Schaich-Borg, A., Pintar, J.E., & Low, M.J. (2006). Differential involvement of endogenous opioids in sucrose consumption and food reinforcement. Pharmacology Biochemistry and Behavior, 85, 601-611.

Heffner TG, Hartman JA, Seiden LS. (1980). Feeding increases dopamine metabolism in the rat brain. Science, 208(4448), 1168-70.

Hernandez, P.J., Andrzejewski, M.E., Sadeghian, K., Panksepp, J.B., & Kelley, A.E. (2005). AMPA/Kainate, NMDA, and dopamine D1 receptor function in the nucleus accumbens core: A context-limited role in the encoding and consolidation of instrumental memory. Learning and Memory, 12, 285-295.

Hitchcott PK, Harmer CJ, Phillips GD. (1997). Enhanced acquisition of discriminative approach following intra-amygdala d-amphetamine. Psychopharmacology (Berl)., 132(3), 237-46.

Huang, YY., & Kandel, ER. (2007). Low-frequency stimulation induces a pathwathway-specific late phase of LTP in the amygdala that is mediated by PKA and dependent on protein synthesis. Learn Mem., 14, 497-503.

Hsiao, S., & Smith, G.P. (1995). Raclopride reduces sucrose preference in rats. Pharmacol Biochem Behav, 50, 121-125.

Inui, T., Shimura, T., & Yamamoto, T. (2007). The role of the ventral pallidum GABAergic system in conditioned taste aversion: Effects of microinjections of a $GABA_A$ receptor antagonist on taste palatability of a conditioned stimulus. Brain Reseach, 1164, 117-124.

Israel, Y., Kandov, Y., Khaimova, E., Kest, A., Lewis, S.R., Pasternak, G.W., Pan, Y.X., Rossi, G.C., & Bodnar, R.J. (2005). NPY-induced feeding: pharmacological characterization using selective opioid antagonists and antisense probes in rats. Peptides, 26(7), 1167-1175.

Jarret, M.M., Limebeer, L., & Parker, Linda.A. (2005). Effect of $\Delta^9$-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. Physiology & Behavior, 86, 475-479.

Jarret, M.M., Scantlebury, J., & Parker, Linda.A. (2007). Effect of $\Delta^9$-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. Physiology & Behavior, 90, 425-430.

Kelley, A.E. (2004). Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neuroscience and Behavioral Reviews, 27, 765-776.

Kelley, A.E., Bakshi, V.P., Fleming, S., & Holahan, M.R. (2000). A Pharmacological Analysis of the Substrates Underlying Conditioned Feeding Induced by Repeated Opioid Stimulation of the Nucleus Accumbens. Neuropsychocopharmacology, 23(4), 455-467.

Khaimova, E., Kandov, Y., Israel, Y., Cataldo, G., Hadjimarkou, M.M., & Bodnar, R.J. (2004). Opioid receptor subtype antagonists differentially alter GABA agonist-induced feeding elicited from either the nucleus accumbens shell or ventral tegmental area regions in rats. Brain Research, 1026, 284-294.

Kim EM, Shi Q, Olszewski PK, Grace MK, O'Hare E, & Billington CJ. (2001). Identification of central sites involved in butorphanol-induced feeding in rats. Brain Res., 907, 125–9.

Kirkham TC, & Cooper SJ. (1988). Attenuation of sham feeding by naloxone is stereospecific: evidence for opioid mediation of orosensory reward. Physiol Behav. 43(6), 845-7.

Klein, D.A., Schebendach, J.S., Devlin, M.J., Smith, G.P., & Walsh, B.T. (2006). Intake, sweetness and liking during modified sham feeding of sucrose solutions. Physiology & Behavior, 87, 602-606.

Lammel, S., Hetzel, A., Hackel, O., Jones, I., Liss, B., & Roeper, J. (2008). Unique properties of meso-prefrontal neurons with a dual mesocorticolimbic dopamine system. Neuron, 57; 760-73.

LeDoux, J. (2000). The amygdala and emotion: a view through fear. In: Aggleton JP, editor. The amygdala: a functional analysis. 2nd ed. Oxford, UK: Oxford University Press; p. 289-310.

Leventhal, L., Mathis, J.P., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1998). Orphan opioid receptor antisense probes block orphanin FQ-induced hyperphagia. European Journal of Pharmacology, 349, R1-R3.

Liang NC, Hajnal A, & Norgren R. (2006). Sham feeding corn oil increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol. 291(5), R1236-9.

Lucas, F., & Sclafani, A. (1999). Differential Reinforcing and Satiating Effects of Intragastric Fat and Carbohydrate Infusions in Rats. Physiology and Behavior, 66(3), 381-388.

Lucas, F., & Sclafani, A. (1999). Flavor Preferences Conditioned by High-Fat versus High-Carbohydrate Diets Vary as a Function of Session Length. Physiology and Behavior, 66, 389-395.

Lucas, F., Azzara, A.V., & Sclafani, A. (1998). Flavor Preferences Conditioned by Intragastric Polycose in Rats: More Concentrated Polycose Is Not Always More Reinforcing. Physiology and Behavior, 63(1), 7-14.

Mahler, S.V., Smith, K.S., & Berridge, K.C. (2007). Endocannabinoid hedonic hotspot for sensory pleasure: Anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology, 32(11), 2267-2278.

Malkusz, D.C., Banakos, T.. Bohn, T., Aviel, M., Malkusz, G., Touzani, K., Sclafani, A. & Bodnar, R.J. Differential dopamine D1 and D2 receptor modulation in the medial prefrontal prelimbic and orbitofrontal cortex of expression of fructose-conditioned flavor preferences in rats. Soc. Neurosci. Abstr. 35: 2009. [SFN Abstract Viewer]

Malkusz, D.C., Banakos, T., Vongwattanakit, T., Mohammed, A., Bohn, T., Mahmud, F., Martinez, S., Saeed, S., Orr, J., Touzani, K., Sclafani, A. & Bodnar, R.J.. Acquisition of fructose-conditioned flavor preference is blocked by dopamine D1 and D2 receptor antagonists in the medial prefrontal cortex in rats. Soc. Neurosci. Abstr. 36: 2010. [SFN Abstract Viewer]

Malkusz, D.C., Malkusz, G., Banakos, T., Mehl, E., Touzani, K., Sclafani, A. & Bodnar, R.J. Expression of fructose-conditioned flavor preferences in rats: role of D1 and D2 dopamine receptors in the medial prefrontal cortex. Soc Neurosci Abstr. 34: 2008 [SFN Abstract Viewer]

Mark, G.P., Smith, S.E., Rada, P.V., & Hoebel, B.G. (1994). An appetitively conditioned taste elicits a preferential increase in mesolimbic dopamine release. Pharmacol Biochem Behav, 48(3), 651-660.

Marschner, A., Mell, T., Watenburger, I., Villringer, A., Reischies, F.M., & Heekeren, H.R. (2005). Reward-based decision making and aging. Brain Research Bull., 67, 382-390.

McCaughey, S.A. (2008). The taste of sugars. Neuroscience and Biobehavioral Reviews, 32, 1024-1043.

McCormack, D.N., Clyburn, V.L., & Pittman, D.W. (2006). Detection of free fatty acids following a conditioned taste aversion in rats. Physiology and Behavior, 87, 582-594.

Mello, LE., Tan, AM, & Finch, DM. (1992). Convergence of projections from the rat hippocampal formation, medial geniculate and basal forebrain onto single amygdaloid neurons: an in vivo extra- and intracellular electrophysiological. Brain Res, 587, 24-40.

Miner, P., Abayev, Y., Kandova, E., Gerges, M., Styler, E., Wapniak, R., Touzani, K., Sclafani, A., & Bodnar, R.J. (2008). Role of systemic endocannabinoid CB-! Receptor antagonism in the acquisition and expression of fructose-conditioned flavor-flavor preferences in rats. Pharmacology Biochemistry and Behavior, 90, 318-324.

Mizushige, T., Inoue, K., & Fushiki, T. (2007). Why Is Fat so Tasty? Chemical Reception of Fatty Acids on the Tongue. Journal Nut Sci Vitaminol, 53, 1-4.

Mobini, S., Chambers, L.C., & Yeomans, M.R. (2007). Effects of Hunger state on flavour pleasantness conditioning at home: Flavour-nutrient learning vs. flavour-flavour learning. Appetite, 48, 20-28.

Mokdad, A.H., Marks, J.S., Stroup, D.F., & Gerberding, J.L. (2004). Actual causes of death in the United States, 2000. JAMA, 291, 1238-1245.

Montmayeur JP, Liberles SD, Matsunami H, & Buck LB. (2001). A candidate taste receptor gene near a sweet taste locus. Nat Neurosci. 4(5), 492-8.

Myers, K.P. (2007). Robust preference for a flavor paired with intragastric glucose acquired in a single trial. Appetite, 48, 123-127.

Myers, K.P., & Sclafani, A. (2001). Conditioned enhancement of flavor evaluation reinforced by intragastric glucose. Physiology & Behavior, 74, 481-493.

Myers, K.P., & Sclafani, A. (2006). Development of Learned Flavor Preferences. Developmental Psychobiology, 42(5), 380-388.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, & Zuker CS. (2001). Mammalian sweet taste receptors. Cell. 106(3), 381-90.

Nishijo, H., Ono, T., Uwano, T., Kondoh, T., & Torii, K. (2000). Hypothalamic and Amygdalar Neuronal Responses to Various Tastant Solutions during Ingestive Behavior in Rats. The Journal of Nutrition, 130(4S), 954S-959S.

Nolan, L.J., McCaughey, S.A., Giza, B.K., Rhinehart-Doty, J.A., Smith, J.C., & Scott, T.R. (1997). Extinction of a conditioned taste aversion in rats: I. Behavioral effects. Physiology & Behavior, 62(2), 319-323.

Nunes, I., Tovmasian, L.T., Silva, R.M., Burke, R.E., & Goff, S.P. (2003). Pitx3 is required for development of substantia nigra dopaminergic neurons. PNAS, 100(7), 4245-4250.

Olszewski PK, Billington CJ, & Levine AS. (2000). Fos expression in feeding-related brain areas following intracerebroventricular administration of orphanin FQ in rats. Brain Res., 855, 171–5.

Parada, M.A., De Parada, M.P., & Hoebel, B.G. (1995). Rats Self-Inject a Dopamine Antagonist in the Lateral Hypothalamus Where it Acts to Increase Extracellular Dopamine in the Nucleus Accumbens. Pharmacology Biochemistry and behavior, 52(1), 179-187.

Parker, L.A. (2003). Taste avoidance and taste aversion: Evidence for two different processes. Learning & Behavior, 31(2), 165-172.

Parker, L.A., Rana, S.A., & Limebeer, C.L. (2008). Conditioned nausea in rats: Assessment by conditioned disgust reactions, rather than conditioned taste avoidance. Can J. Exp Psychology, 62(3), 198-209.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, & Everitt BJ. (1999). Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian

approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci., 19(6), 2401-11.

Paxinos G, Watson C. (1998). The Rat Brain in Stereotaxic Coordinates. Fourth Ed. Knoxville, TN: Neuroscience Associates.

Peciña, S. & Berridge, KC. (2005). Hedonic hot spot in the nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? Neurosci, 25, 11777-86.

Peciña, S. (2008). Opioid reward 'liking' and 'wanting' in the nucleus accumbens. Physiology and Behavior, 94, 675-680.

Perez, C., Lucas, F., & Sclafani, A. (1998). Increased Flavor Acceptance and Preference Conditioned by the Postingestive Actions of Glucose. Physiology and Behavior, 64 (4), 483-492.

Phillipson, OT., & Griffiths, AC. (1985). The topographic order of inputs to nucleus accumbens in the rat. Neuroscience, 16, 275-96.

Pickel, VM., Colago, EE., Mania, I., Molosh, AI., & Rainnie, DG. (2006). Dopamine D1 receptors co-distribute with N-methyl-D-aspartic acid type-1 subunits and modulate synaptically-evoked N-methyl-D-aspartic currents in rat baso-lateral amygdala. Neuroscience, 142, 671-90.

Pitkanen, A. (2000). Connectivity of the rat amygdaloid complex. In: Aggleton JP, editor. The amygdala: a functional analysis. 2nd ed. Oxford, UK: Oxford University Press; p. 31-115.

Pratt, W.E., Spencer, R.C., & Kelley, A.E, (2007). Muscarinic Receptor Antagonism of the Nucleus Accumbens Core Causes Avoidance to Flavor and Spatial Cues. Behavioral Neuroscience, 121(6), 1215-1223.

Prescott, J. (2004). Effects of added glutamate on liking for novel food flavors. Appetite, 42, 143-150.

Ragnauth, A., Moroz, M., & Bodnar, R.J. (2000). Multiple oipoid receptors mediate feeding elicited by mu and delta opioid receptor subtype agonists in the nucleus accumbens shell in rats. Brain Research, 876, 76-87.

Ragnauth, A., Ruegg, H., & Bodnar, R.J. (1997). Evaluation of opioid receptor subtype antagonist effects in the ventral tegmental area upon food intake under deprivation, glucoprivic and palatable conditions. Brain Research, 767, 8-16.

Ragnauth, A., Znamensky, V., Moroz, M., & Bodnar, R.J. (2000). Analysis of dopamine receptor antagonism upon feeding elicited by mu and delta opioid agonists in the shell region of the nucleus accumbens. Brain Research, 877, 65-72.

Ramirez, I. (1996). Stimulus Specificity in Flavor Acceptance Learning. Physiology & Behavior, 60(2), 595-610.

Reid, L.D. (1996). Endogenous opioids and alcohol dependence: opioid alkaloids and the propensity to drink alcoholic beverages. Alcohol, 13(1), 5-11.

Ren J, Xu H, Choi JK, Jenkins BG, & Chen YI. (2009) Dopaminergic response to graded dopamine concentration elicited by four amphetamine doses. Synapse, 63(9), 764-72.

Renteria, A.F., Silbaugh, B.C., Tolentino, J.C., & Gilbert, P.E. (2008). Age-related changes in conditioned flavor preference in rats. Behavioural Brain Research, 188, 56-61.

Rockwood GA, & Reid LD. (1982). Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. Physiol Behav. 29(6), 1175-8.

Roitman, M.F., Stuber, G.D., Phillips, P.E.M., Wightman, R.M., & Carelli, R.M. (2004). Dopamine Operates as a Subsecond Modulator of Food Seeking. The Journal of Neuroscience, 24(6), 1265-1271.

Roman, C., & Reilly, S. (2007). Effects of insular cortex lesions on conditioned taste aversion and latent inhibition in the rat. European Journal of Neuroscience, 26, 2627-2632.

Salamone, J.D., Cousins, M.S., & Snyder, B.J. (1997). Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. Neurosci Behav Rev, 21, 341-359.

Sclafani, A. (2001). Post-ingestive positive controls of ingestive behavior. Appetite, 36, 79-83.

Sclafani, A. (2001). Psychobiology of food preferences. International Journal of Obesity, 25(5), S13-S16.

Sclafani, A. (2002). Flavor preferences conditioned by sucrose depend upon training and testing methods: Two-bottle tests revisited. Physiology and Behavior, 76, 633-644.

Sclafani, A. (2004). Oral and postoral determinants of food reward. Physiology & Behavior, 81, 773-779.

Sclafani, A. (2004). The sixth taste?. Appetite, 43, 1-3.

Sclafani, A. (2006). Oral, post-oral and genetic interactions in sweet appetite. Physiology & Behavior, 89, 525-530.

Sclafani, A. (2007). Sweet taste signaling in the gut. PNAS, 104(38), 14887-14888.

Sclafani, A., & Ackroff, K. (1994). Glucose- and fructose-conditioned flavor preferences in rats: taste versus post-ingestive conditioning. Physiology & Behavior, 56, 399-405.

Sclafani, A., & Ackroff, K. (2004). The relationship between food reward and satiation revisited. Physiology & Behavior, 82, 89-95.

Sclafani, A., & Ackroff, K. (2006). Nutrient-conditioned flavor preference and incentive value measured by progressive ratio licking in rats. Physiology and Behavior, 88, 88-94.

Sclafani, A., & Clare, R.A. (2004). Female Rats show a Bimodal Preference to the Artificial Sweetener Sucralose. Chemical Senses, 29(6), 523-528.

Sclafani, A., Bodnar, R.J., & Delamater, A.R. (1998). Pharmacology of Food Conditioned Preferences. Appetite, 31, 406.

Sclafani, A., Cardieri, C., Tucker, K., Blusk, D., & Ackroff, K. (1993). Intragastric glucose but not fructose conditions robust flavor preferences in rats. Am J Physiol, 265 (2 Pr 2), R320-5.

Scott, C.L., & Downey, R.G. (2007). Types of Food Aversions: Animal, Vegetable, and Texture. The Journal of Psychology, 141(2), 127-134.

Sewards, T.V. (2004). Dual separate pathways for sensory and hedonic aspects of taste. Brain Research Bulletin, 62, 271-283.

Shabir, S., and Kirkham, T.C. (1999). Diet-induced enhancement of naloxone sensitivity is independent of changes in body weight. Pharmacology Biochemistry and Behavior, 62, 601-605.

Smith, G.P. (2004). Accumbens dopamine mediates the rewarding effect of orosensory stimulation by sucrose. Appetite, 43 (1), 11-13.

Smith, G.P., & Dockray, G.J. (2006). Introduction to the reviews on appetite. Phil. Trans. R. Soc. B., 361, 1089-1096, doi: 10.1098/rstb.2006.1848

Smith, J.C., & Sclafani, A. (2002). Saccharin as a Sugar Surrogate Revisited. Appetite, 38, 155-160.

Smith, K.S., Tindell, A.J., Aldridge, J.W., & Berridge, K.C. (2009). Ventral pallidum roles in reward and motivation. Behavioural Brain Research, 196, 155-167.

Smith-Roe SL, & Kelley AE. (2000). Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci. 20(20), 7737-42.

Stanley BG, Lanthier D, & Leibowitz SF. (1989). Multiple brain sites sensitive to feeding stimulation by opioid agonists: a cannula-mapping study. Pharmacol Biochem Behav., 31, 825–32.

Stramiello, M., & Wagner, JJ. (2008). D1/5 receptor-mediated enhancement of LTP requires PKA, Src family kinases, and NR2B-containing NMDRs. Neuropharmacology, 55, 871-7.

Stratford, TR., & Kelley, AE. (1999). Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. J Neurosci, 19, 11040-8.

Stromberg, M.F., Meister, S., Volpicelli, J.R., & Ulm, R.R. (1997). Morphine Enhances Selection of Both Sucrose and Ethanol in a Two-Bottle Test. Alcohol, 14(1), 55-62.

Sutton, M.A., & Beninger, R.J. (1999). Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. Psychopharmacology, 144, 95-110.

Swithers, S.E., & Davidson, T.L. (2008). A Role for Sweet Taste: Calorie Predictive Relations in Energy Regulation by Rats. Behavioral Neuroscience, 122(1), 161-173.

Taha, S.A., & Fields, H.L. (2005). Encoding of Palatability and Appetitive Behaviors by Distinct Neuronal Populations in the Nucleus Accumbens. The Journal of Neuroscience, 25(5), 1193-1202.

Taha, S.A., Norsted, E., Lee, L.S., Lang, P.D., Lee, B.S., Woolley, J.D., & Fields, H.L. (2006). Endogenous opioids encode relative taste preference. European Journal of Neuroscience, 24, 1220-1226.

Tallet, A.J., Blundell, J.E., & Rodgers, R.J. (2008). Behaviourally-selective hypophagic effects of naloxone in non-deprived male rats presented with palatable food. Behavioural Brain Research, 187, 417-427.

Tordoff, M.G. (2002). Obesity by Choice: the powerful influence of nutrient availability on nutrient intake. AJP Regulatory Integrative Comp Physiol, 282, R1536-R1539.

Touzani, K., & Sclafani, A. (2001). Conditioned Flavor Preference and Aversion: Role of the lateral hypothalamus. Behavioral Neuroscience, 115(1), 84-93.

Touzani, K., & Sclafani, A. (2002). Lateral hypothalamic lesions impair flavor-nutrient and flavor-toxin trace learning in rats. European Journal of Neuroscience, 16, 2425-2433.

Touzani, K., & Sclafani, A. (2005). Critical role of amygdala in flavor but not taste preference learning in rats. European journal of Neuroscience, 22, 1767-1774.

Touzani, K., & Sclafani, A. (2007). Insular cortex lesions fail to block flavor and taste preference learning in rats. European Journal of Neuroscience, 26, 1692-1700.

Touzani, K., Bodnar, R.J., & Sclafani, A. (2006). Critical role of dopamine D1 receptors in the nucleus accumbens shell in flavor preferences conditioned by intragastric glucose infusion. Appetite: 388.

Touzani, K., Bodnar, R.J., & Sclafani, A. (2008). Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of nutrient-conditioned flavor preferences in rats. European Journal of Neuroscience, 27, 1525-1533.

Touzani, K., Bodnar, R.J., & Sclafani, A. (2009a). Lateral hypothalamus dopamine D1-like receptors and glucose-conditioned flavor preferences in rats. Neurobiology of Learning and Memory, 92(3), 464-467.

Touzani, K., Bodnar, RJ., & Sclafani, A. (2009b). Dopamine D1-like receptor antagonism in amygdala impairs the acquisition of glucose-conditioned flavor preferences in rats. Eur J Neurosci., 30, 289-298.

Uematsu, A., Tsurugizawa, T., Kondoh, T., & Torii, K. (2009). Conditioned flavor preference learning by intragastric administration of L-glutamate in rats. Neuroscience Letters, 451, 190-193.

Vargas-Perez, H., Ting-A-Kee, R., Heinmiller, A., Sturgess, J.E., & Van Der Kooy, D. (2007). A test of the opponent-process theory of motivation using lesions that selectively block morphine reward. European Journal of Neuroscience, 25, 3713-3718.

Ventura, R., Morrone, C., & Puglisi-Allegra, S. (2007). Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. PNAS, 104(12), 5181-5186.

Wang, Y., Fontanini, A., & Katz, D.B. (2006). Temporary basolateral amygdala lesions disrupt acquisition of socially transmitted food preferences in rats. Learning & Memory, 13, 794-800.

Weatherford SC, Greenberg D, Gibbs J, & Smith GP. (1990). The potency of D-1 and D-2 receptor antagonists is inversely related to the reward value of sham-fed corn oil and sucrose in rats. Pharmacol Biochem Behav. 37(2), 317-23.

Will, MJ., Franzblau, EB., & Kelley, AE. (2003). Nucleus accumbens mu-opioids regulate intake of a high-fat diet via activation of a distributed brain network. J Neurosci, 23, 2882-8.

Williams, G., Bing, C., Cai, X.J., Harrold, J.A., King, P.J., & Liu, X.H. (2001). The hypothalamus and the control of energy homeostasis Different circuits, different purposes. Physiology and Behavior, 74, 683-701.

Wise, R.A. (2006). Role of brain dopamine in food reward and reinforcement. Philos. Tans. R. Soc. Lond. B. Biol. Sci., 361(1471), 1149-1158.

Wise, R.A. (2008). Dopamine and reward: The anhedonia hypothesis 30 years on. Neurotoxixity Research, 14(2, 3), 169-183.

Wolinsky TD, Carr KD, Hiller JM, & Simon EJ. (1994). Effects of chronic food restriction on mu and kappa opioid binding in rat forebrain: a quantitative autoradiographic study. Brain Res., 656, 274–80.

Woolley, J.D., Lee, B.S., & Fields, H.L. (2006). Nucleus Accumbens opioids regulate flavor-based preferences in food consumption. Neuroscience, 143, 309-317.

Woolley, J.D., Lee, B.S., Kim, B., & Fields, H.L. (2007). Opposing effects of intra-nucleus Accumbens mu and kappa opioid agonists on sensory specific satiety. Neuroscience, 146, 1445-1452.

Woolley, J.D., Lee, B.S., Taha, S.A., & Fields, H.L. (2007). Nucleus accumbens opioid signaling conditions short-term flavor preferences. Neuroscience, 146, 19-30

Wright, CI. & Groenewegen, HJ. (1995). Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic and basal amygdaloid afferents. J Comp Neurol, 361, 383-403.

Wright, CI., Beijer, AV., & Groenewegen, HJ. (1996). Basal amygdaloid complex afferents to the rat nucleus accumbens are compartmentally organized. J Neurosci, 16, 1877-93.

Xenakis S, & Sclafani A. (1981). The effects of pimozide on the consumption of a palatable saccharin-glucose solution in the rat. Pharmacol Biochem Behav. 15(3), 435-42.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. The Journal of Nutrition, 130(4S), 921S-926S.

Yamamoto, T. (2006). Neural substrates for the processing of cognitive and affective aspects of taste in the brain. Arch Histol Cytol, 69(4), 243-255.

Yamamoto, T., Nagai, T., Shimura, T., & Yasoshima, Y. (1998). Roles of Chemical Mediators in the Taste System. Journal of Pharmacology, 76, 325-348.

Yeomans, M.R., Jacson, A., Lee, M.D., Steer, B., Tinley, E., Durlach, P., & Rogers, P.J. (2000). Acquisition and extinction of flavour preferences conditioned by caffeine in humans. Appetite,35, 131-141.

Yeomans, M.R., Leitch, M., Gould, N.J., & Mobini, S. (2008). Differential hedonic, sensory and behavioral changes associated with flavor-nutrient and flavor-flavor learning. Physiology & Behavior, 93, 798-806.

Yiin, Y.M., Ackroff, K., & Sclafani, A. (2004). Food deprivation enhances the expression but not acquisition of flavor acceptance conditioning in rats. Appetite, 45, 152-160.

Yiin, Y.M., Ackroff, K., & Sclafani, A. (2005). Flavor preferences conditioned by intragastric nutrient infusions in food restricted and free-feeding rats. Physiology and Behavior, 84, 217-231.

Yu, W.Z., Sclafani, A., Delamater, A.R., & Bodnar, R.J. (1999). Pharmacology of Flavor Preference Conditioning in Sham-Feeding Rats: Effects of Naltrexone. Pharmacology Biochemistry and Behavior, 64(3), 573-584.

Yu, W.Z., Silva, R.M., Sclafani, A., Delamater, A.R., & Bodnar, R.J. (2000a). Pharmacology of Flavor Preference Conditioning in Sham-Feeding Rats: Effects of Dopamine Receptor Antagonists. Pharmacology Biochemistry and Behavior, 65(4), 635-647.

Yu, W.Z., Silva, R.M., Sclafani, A., Delamater, A.R., & Bodnar, R.J. (2000b). Role of D1 and D2 dopamine receptors in the acquisition and expression of flavor-preference conditioning in sham-feeding rats. Pharmacology Biochemistry and Behavior, 67, 537-544.

Zhang, M., & Kelley, A.E. (2002). Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens. Psychopharmacology, 159, 415-423.119-1124.