Fructose-Conditioned Flavor-Flavor Preferences in the Rat: Role of Dopaminergic Receptor Subtypes in the Nucleus Accumbens, Amygdala, and Medial Prefrontal Cortex

by

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Abstract

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Danielle C. Malkusz

Advisor: Professor Richard J. Bodnar, Ph.D.

Systemic administration of dopamine (DA) D1 (SCH23390) and D2 (raclopride) antagonists blocked both acquisition and expression of fructose-conditioned flavor preferences (CFP). It is unclear what brain circuits are involved in mediating these effects. The present study investigated DA signaling within the nucleus accumbens shell (NAC), amygdala (AMY) and medial prefrontal cortex (mPFC) in the acquisition and expression of fructose-CFP. In Experiment 1, separate groups of rats were injected daily in the NAC or AMY with saline, SCH23390 (24 nmol) or raclopride (24 nmol) prior to training sessions with a flavor (CS+) mixed with 8% fructose and 0.2% saccharin (CS+/F) and a different flavor (CS-) mixed with only 0.2% saccharin. In two-bottle choice tests with the CS+ or CS- flavor presented in a 0.2% saccharin solution, only rats injected with raclopride in the AMY failed to acquire a CS+ preference (45-54%). In Experiment 2, new rats were identically trained, but saline, SCH23390 and raclopride were injected in the mPFC. In subsequent two-bottle choice tests, SCH23390 -and raclopride -treated rats failed to exhibit a CS+ preference (50-56%). In Experiment 3, new rats were trained with CS+/F and CS-without injections. Subsequent two-bottle choice tests were then conducted following bilateral injections of SCH23390 or raclopride in the mPFC at total doses of 12, 24 and 48 nmol. Expression of the CS+ preference failed to be affected by either

antagonist, indicating that the mPFC is not involved in the maintenance of this preference. These data indicate that the acquisition of fructose-CFP is dependent on DA signaling in the mPFC and AMY.

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This dissertation is dedicated to my children, to serve as a reminder that they can achieve anything if they find passion, work hard, and never give up.

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Glossary of Abbreviations

AMY = Amygdala
CB= Cannabinoid Receptor
CFP = Conditioned Flavor preference
CS = Conditioned Stimulus
CTA = Conditioned Taste Aversion
DA = Dopamine
DAMGO = D-Ala2, N-Met-Phe4, Gly-ol5-enkephalin, mu agonist
F-F= Flavor-Flavor
F-F= Flavor-Nutrient
GABA = gamma-aminobutyric acid
IG = Intragastric
LH = Lateral Hypothalamus
mPFC = Medial Prefrontal Cortex
NAC= Nucleus Accumbens
NMDA = N-methyl-D-aspartate
NTX = Naltrexone
NST = Nucleus of the Solitary Track
OFC = Orbitofrontal Cortex
THC= Δ^9-tetrahydrocannabinol
US =Unconditioned Stimulus
VTA= Ventral Tegmental Area

CHAPTER 1: INTRODUCTION,

SIGNIFICANCE AND SPECIFIC AIMS

General Significance: The increased incidence of obesity along with its related medical complications is considered to be a major health crisis today (Abelson and Kennedy, 2004). Laboratory research has documented that diets high in sugar and fat stimulate overeating, weight gain, and over time, unhealthy levels of body adipose in both animals and humans (Ackroff et al, 2007; Rogers & Blundell 1984; Swinburn et al., 2004). Additionally, the World Health Organization indicated that a sugar- and fat-rich diet combined with a sedentary lifestyle is the foremost contributor to weight gain and obesity (World Health Organization, 1997). Despite significant advances in our understanding of the biological underpinnings of energy regulation over the past decade, the "epidemic of obesity rages on, oblivious to scientific advances" (Filer, 2004), and changes in the environment rather than our biology seem to be driving force (Hill et al., 2003). Statistical data from the Center for Disease Control (CDC) show that from 2009 to 2010, 35.7% or 72.5 million American adults ages 20 and over were classified as obese (Ogden et al., 2012), whereas in 1960 only 25% of American adults met criteria for this classification. Likewise, childhood obesity has grown rapidly during the late twentieth and early twenty-first century, such that between 1999 to 2003 there was a 6% increase in the incidence of overweight and obese school age children (Lobstein and Jackson-Leach, 2007). The escalating prevalence of obesity displayed in children and adults is not limited to only the American population, it is a worldwide epidemic that has overwhelmed developed and developing countries, where calorically dense sugar- and fat-rich foods are both inexpensive and readily available (Dragone, 2009; World Health Organization, 1997).

Obesity is a condition largely associated with a myriad of medical complications resulting from co-morbid diseases such as Type II diabetes, coronary heart disease, hypertension, metabolic syndromes, gallbladder disease, and osteoarthritis (Must et al., 1999). Though current dietary guidelines recommend limiting sugar and fat intake, compliance with low-sugar and low-fat diets has been proven problematic, despite evidence implicating medical complications associated with obesity as the second leading cause of death in America (Mokdad et al., 2004). The widespread difficulty with compliance to recommended dietary guidelines may be due, in part, to an attraction to foods rich in fats and sugars. But why are high fat and high sugar diets so attractive? Diverse intermingled external and internal factors result in both preferences for specific food types, as well as control the amount of food consumed by an individual. Major internal factors include metabolic regulation, genetic and evolutionary predisposition, prenatal and postnatal experiences, age, mood, gender, hormone levels, as well as the oral, post-oral and neurochemical modulation of ingestion (McElroy et al., 2004; Nobel et al., 1993; Ogden et al, 2012; Smith et al., 2009). All of the above are in turn affected by external factors such as individual socioeconomic status, cultural influences, caloric expenditure versus intake, portion sizes, and proximity to available food and food cues (Cummings and Macintyre, 2005; Drewnowski and Specter, 2004; Rolls, 2003). Thus, orosensory attraction to a food is based on dynamic, complex interactions between the internal state of the individual, the given environment, and learning. Behavioral studies in humans and laboratory animals demonstrate that whereas there are some innate preferences (e.g., the sweet taste of sugar), most food preferences are learned through experience (Rozin and Zellner, 1985). Accordingly, the CDC and other public health organizations aim to combat obesity by promoting behavioral changes in target populations.

Significance of Food Preference: Extensive research performed within the scientific community has made clear that the learning of a food preference is due to both the oral and post-oral actions associated with that particular food. Food paired with positive oral and post-ingestive actions leads to a conditioned preference for the associated flavor (Sclafani, 2004). Conditioned flavor preferences (CFP) are strong, enduring, and are capable of increasing the value of food reward through modulation of both hedonic association and incentivizing value (Myers and Sclafani, 2001; Sclafani and Ackroff, 2006). Moreover, it has been suggested that such CFP's appear to be mediated by the same potent neurochemical systems involved in drug (eg: cocaine) addiction (Smith, 2004). CFP's can be induced through diverse models of learning. Our laboratories (see reviews: Touzani et al., 2010; Sclafani et al., 2011) have utilized a simple model of classical or Pavlovian conditioning, in which a novel flavor (the conditioned stimulus, CS) comes to be associated with the oral and/or post-oral properties of sugars (the unconditioned stimulus, US) through repeated exposure to CS/US pairings. Under normal consummatory conditions, both oral and post-oral mechanisms relay information about a food (eg: sweet taste, texture, associated fullness) to target neural locations, which in turn promotes learning about the flavor cue. However, this combined oral and post-oral association can be isolated in the laboratory setting into component parts, allowing for the oral and post-oral actions involved in the mediation of food related behavior to be studied independently. Flavor-Flavor (F-F) reward learning involves developing preferences for the taste, odor and/or texture of a substance based solely on associations between a previously-neutral oral stimulus (e.g., unflavored cherry Kool-Aid) and the orosensory properties of that nutrient (e.g., sweet taste) in the relative absence of post-ingestive factors. Flavor-Nutrient (F-N) learning entails preference development based on associations made between a previously-neutral oral stimulus (e.g., grape Kool-Aid) and positive

post-ingestive nutrient actions activated by intragastric infusion (Sclafani, 2004). Once acquired, CFP's induced through both types of flavor learning are enduring, and can lead to high rates of persistent over-consumption of the conditioned flavor, even in the absence of nutrient content.

Significance of Neuropharmacological Substrates: The neuropharmacology of food reward has been subject to extensive investigation, with a number of neurotransmitters implicated as prospective candidates in the mediation of food reward and preference learning. In systemic studies, dopamine (DA) has been found to be a major contributor to the mediation of F-F and F-N preference acquisition and F-F preference expression (Azzara et al., 2001; Baker et al., 2003; Hsiao and Smith, 1995; Yu et al., 2000a, 2000b). In F-N studies utilizing intragastric (IG) sucrose solutions to promote CFP, it was found that acquisition of this flavor preference was entirely eliminated by systemic administration of the DA D1 receptor antagonist SCH23390 during training. However, systemic administration of the DA D2 antagonist, raclopride during training failed to display significant alterations in the acquisition of F-N CFP. Expression of F-N CFP failed to be appreciably altered by either systemic DA D1 (SCH23390) or D2 (raclopride) receptor antagonism, except when profound reductions in CS intake was observed following high antagonist doses (Azzara et al., 2001). F-F CFP studies evaluated the effects of systemic DA D1 and D2 antagonism on the acquisition and expression of animals in both "real feeding" fructose (Baker et al., 2003) and "sham feeding" sucrose (Yu et al, 2000a, 2000b) paradigms. Both DA D1 and D2 antagonists systemically administered during training significantly reduced the acquisition of both sucrose-CFP in sham-feeding rats, and fructose-CFP in real-feeding rats, with the DA D1 effect more robust than the D2 effect. Both antagonists also significantly reduced the expression of sucrose-CFP in sham-feeding rats and fructose-CFP in real-feeding rats that already acquired this preference. These data collectively suggest that DA D1, but not D2

receptors are critically involved in the acquisition, but play a more limited role in the expression, of F-N CPF, whereas both DA D1 and D2 receptor subtypes are vital for the acquisition and expression of F-F induced CFP.

Specific Aims: It is believed that feeding-related behavior is modulated by a neural network comprised of multiple brain sites and several chemical modulators. Systemic studies on the effects of DA D1 and D2 receptor involvement have yielded information indicating that DA is an important component in the neurochemical modulation of CFP. The meso-limbic and meso-cortical DA projections originating in the ventral tegmental area (VTA) and projects to sites such as the amygdala (AMY), nucleus accumbens (NAC) and medial prefrontal cortex (mPFC) (Swanson, 1982) emerged as primary candidates whereby DA D1 (and D2) antagonists would act to mediate F-F and F-N CFP. Similar to the ability for DA D1 antagonism to block the acquisition of a F-N CFP induced by intragastric sucrose infusions (Azzara et al., 2001), a series of studies demonstrated that the acquisition of F-N CFP induced by intragastric glucose infusions was eliminated by administration of the DA D1 antagonist, SCH23390, during training at a dose of 12 nmol into the NAC (Touzani et al., 2008), the AMY (Touzani and Sclafani., 2009), or the mPFC (Touzani et al., 2010). Parallel studies examining DA D1 and D2 antagonist effects in the NAC and AMY upon the expression and acquisition of F-F fructose-induced CFP (Bernal et al., 2008, 2009) did not produce comparable results. Thus, whereas both systemic DA D1 and D2 antagonists eliminated the expression of fructose-CFP (Baker et al., 2003), DA D1 or D2 antagonist administration into either the NAC or AMY produced significant reductions, but not elimination of fructose-CFP (Bernal et al., 2008, 2009). Moreover, using an identical (12 nmol) dose of the DA D1 and D2 antagonists, thel acquisition of fructose-CFP was unaffected by administration into the NAC or AMY. However, the persistence of the learned fructose-CFP

was subject to hastened extinction following DA D1 antagonism in the NAC or AMY, and following DA D2 antagonism in the NAC (Bernal et al., 2008, 2009). Thus, the elimination of the acquisition of fructose-CFP following systemic DA D1 or D2 antagonism was not observed following direct NAC or AMY injection (Baker et al., 2003). This pattern of results could have occurred for at least two reasons. First, the dose of the DA D1 and D2 antagonists administered into either the NAC or the AMY necessary to reduce the acquisition of fructose-CFP might be higher for F-F-mediated fructose-CFP relative to F-N-mediated intragastric glucose-CFP. Second, the site of action mediating the ability of DA D1 and D2 antagonists to block the acquisition of F-F-mediated fructose-CFP may not be within the NAC and AMY, but rather, in the third major projection site of the mesocorticolimbic DA system, the mPFC. These questions form the basis of this dissertation, which will focus on the role of DA D1 and D2 receptor antagonist actions in the AMY and NAC on the acquisition of F-F-mediated fructose-induced CFP, and the role of mPFC DA D1 and D2 receptor antagonist actions on both the acquisition and expression of this type of learning. The following four Specific Aims were designed to explore DA functions upon F-F-mediated fructose-CFP:

The **First Specific Aim** examines whether administration of a 24 nmol dose of either the DA D1 receptor antagonist SCH23390 or the DA D2 receptor antagonist raclopride into the NAC will influence the acquisition of fructose-CFP. It is hypothesized that DA D1 or D2 antagonists administered into the NAC at this higher dose will significantly reduce the acquisition of fructose-CFP without appreciably affecting CS intake during training.

The **Second Specific Aim** examines whether administration of a 24 nmol dose of either the DA D1 receptor antagonist SCH23390 or the D2 receptor antagonist raclopride into the AMY will influence the acquisition of fructose-CFP. It is hypothesized that DA D1 or D2

antagonists administered into the AMY at this higher dose will significantly reduce the acquisition of fructose-CFP without appreciably affecting CS intake during training.

The **Third Specific Aim** examines whether administration of a 24 nmol dose of either the DA D1 receptor antagonist SCH23390 or the D2 receptor antagonist raclopride into the mPFC will influence the acquisition of fructose-CFP. It is hypothesized that DA D1 or D2 antagonists administered into the mPFC will significantly reduce the acquisition of fructose-CFP without appreciably affecting CS intake during training.

The **Fourth Specific Aim** examines whether the same dose range (12, 24 or 48 nmol) of either the DA D1 receptor antagonist SCH23390 or the D2 receptor antagonist raclopride used previously in the NAC or AMY (Bernal et al., 2008, 2009) will influence the expression of fructose-CFP when administered into the mPFC. It is hypothesized that DA D1 or D2 antagonists administered into the mPFC will significantly and dose-dependently reduce the expression of fructose-CFP.

The following Background section is organized in the following manner to present the underlying evidence leading to the development of these Specific Aims: 1. Food Preference, 2. Specific-Nutrient Conditioned Flavor Preferences, 3. Roles of Orosensory and Post-Ingestive Factors in Preference Conditioning, 4. Neurochemical Candidates in the Mediation of CFP, 5. Dopamine effects upon F-F and F-N CFP, 6. Putative Sites of Action of action in the Dopaminergic Mediation of CFP, and 7. A Rationale for the Proposed Specific Aims.

The full extent of experimental finding discussed in this dissertation was published in Behavioral Brain Research in 2012.

B. Background

1. Food Preference

This section will review evidence for food preference as functions of: a. Evolution, Genetics and Environment, b. Hunger, Wanting, Liking, and Learning, c. Human Food Preference Studies, and d. Conditioned Flavor Preferences.

Evolution, Genetics, and Environment: A principal goal of food consumption is to maintain homeostasis. But, unlike other homeostatic regulatory functions, such as body temperature and blood pressure, energy intake can vary dramatically from what is needed for maintenance. There are a multitude of factors that have an impact upon the eating behavior of humans and other animals, including physiological needs, hedonic value, and learning (Berthound, 2007; Kringelbach 2004; Waynforth, 2009). As previously stated, obesity has become a major worldwide health crisis; a crisis due to the overconsumption of calorically-dense foods, combined with a sedentary lifestyle. But the question remains: Why do humans overeat when aware of the detrimental physiological effects caused by high body adiposity? At a fundamental level, evolutionary factors may be partly to blame.

The scientific community generally believes that evolution works to sustain life through the simple rules Darwin proposed in "The Origin of Species" (1859); essentially the passing on of genetic material that has allowed an organism to thrive in its unique environment thereby ensuring the long-term survival of the species. In his book, Darwin described that animals with poor diets exhibited impaired reproductive ability. If the goal of evolution is the production of genetically fit offspring, then acquisition of nutrient-rich foods has been a paramount force in shaping the current genetic landscape (Hosken and Balloux, 2002; Warren and Dominguez, 2004). As Darwin's Galapagos Island finches' were equipped with differently shaped beaks to

acquire foods specific to their environments, so too have human beings adapted various behavioral, cognitive, and metabolic strategies to ensure appropriate nutrient intake. The evolutionary mechanisms involved in the procurement of food have a large neural presence, with distributed networks of interacting nuclei involved in both the physiological components of intake (such as how much and what types of foods to consume), and higher-order functions (such as the creation of multimodal memories about food experiences that guide future behavior) (Berthound, 2007).

According to Neel (1962), human beings are equipped with a "thrifty gene". Neel first hypothesized the existence of a “thrifty gene” to pose an explanation for the growing incidences of diabetes in developed countries. Since then, this “thrifty gene” hypothesis has become a staple in the evolutionary theory of human feeding behavior. The thrifty gene hypothesis arose from the harsh, unpredictable lifestyle of our hunter-gatherer ancestors. During times of plentiful nutrient availability, ancient humans needed to consume large amounts to rapidly increase subcutaneous fat stores in preparation to survive periods of food scarcity (Neel, 1962). Humans who consumed beyond their immediate homeostatic need were able to survive off of stored energy in times of deprivation, and therefore were more likely to successfully reach sexual maturity and pass on the genetic traits that made their procreation possible. In the laboratory setting, it is observed that neuroadaptive brain processes occur in food restrictied animals, particularly in reward neurocircuitry, which has been shown to induce increased foraging behavior as well as increased reward sensitivity. Moreover food restricted animals are more likely to take part in binging behaviors, in relation to food as well as drugs of reward (Carr, 2012). Thus in times of scarcity the food restricted animal is neutrally “primed” to seek out and intake food substances, and is likely to exhibit binging behavior (intake of more food than

needed to maintain homeostasis) during times of plenty. This neural adaptation is likely an evolutionary mechanism geared to facilitate individual survival in times of food scarcity.

Given that adequate feeding behavior is a necessity in both the survival of the individual and the prevalence of that individual's genetic code, it is reasonable to assert that evolution has played a role not only in the amount of food consumed, but has also guided selection or preference for foods and flavors that are associated with positive post-ingestive actions (e.g.: sugar and sweet taste). For example, it is believed that at a genetic level, humans and many mammals prefer sweet foods and have distaste for bitter foods, as evident in studies that have demonstrated that human infants and rats readily accept sweet solutions and reject bitter solutions (Steiner, 1979; Steiner et al, 2001). This inborn tendency is believed to be due to the association between sweet tastes and foods high in vitamins and energy (e.g.: fruit), and bitter taste associations with substances likely to cause gastro-intestinal malaise, or even poison-related fatality (Glendinning et al., 2008; Menella, 2008). This phenomenon is believed to be an example of evolutionary processes guiding food/ flavor preferences.

Although we as a species may be predisposed via evolution and genetics to have certain consummatory behaviors, it is important to recognize that individual adaptation to the environment also plays a significant role. The ability to learn; acquiring information, forming memories based on experience, and utilizing that information to dictate current and future behavior is an adaptive technique (Arbilly et al., 2010). Learning about individual food experiences such as where to find food, what to consume, and what to reject, is important for individual survival. Consequently, learning brought about by food intake is so potent that it can turn an innately positive response to orally rewarding stimuli (e.g., sweet taste) into an aversion when associated with negative intragastric consequences (e.g., Glendinning et al., 2008).

For ancient humans this consumption strategy was useful and allowed for development to sexual maturity. However, the dawn of the agricultural revolution brought about a new food environment, one in which the danger of food scarcity has been markedly reduced. As modern agriculture advanced with science and technology, the food environment itself has evolved, allowing for food to be readily available at any time to most people (Mariani-Constantini, 2000). Although substantially reduced risk of famine is beneficial to the survival of the human species, a conflict has occurred because the evolution of our modern food environment clashes with the genetic evolution of human eating behavior. The current food environment has been described as "toxic" and "obesogenic", primarily due to the evolutionary-driven aspects of feeding behavior (i.e., thrifty gene, evolution of learned feeding behavior) meeting an endless supply of plentiful food resources.

Hunger, Wanting, Liking, and Learning: The ever-increasing number of obese and overweight individuals worldwide suggests that the consumption of food does not occur merely in response to energy deregulation, but instead is also heavily mediated by the hedonic aspects of foods. Consumption occurring in response to the physiological need for increased caloric intake due to an impending or existing energy deficit has been defined as "homeostatic hunger". Homeostatic hunger sensations (eg: stomach pain, etc.) motivate an individual to seek out and intake food, a process similarly accomplished by hedonic hunger sensations, such as thoughts and urges pertaining to food consumption. Hedonic hunger, like homeostatic hunger, is motivational, and occurs when "people experience frequent thoughts, feelings, and urges about food in absents of energy deprivation" (Lowe and Butryn, 2007). Although perceived food palatability is a feature of both types of hunger, it seems that for hedonic hunger, palatability is imperative (Lowe and Butryn, 2007). For example, adult rats allowed standard chow ad libitum

presented alongside highly palatable "supermarket foods" quickly gained substantial amounts of adipose tissue. Control animals exposed to the same standard chow but not the "supermarket foods" failed to gain weight at the same rate as the experimental animals, with significant differences in weight observed after only 10 days of experimentation (Sclafani and Springer, 1976). These data suggest that the difference in weight gain between the control and experimental subjects was due to frequent exposure to palatable food, and that consumption did not indicate a physical need for increased caloric intake, but instead was representative of associated hedonic experience. The lack of physiological need instigating consummatory behavior is highly intriguing, and has sparked much discussion about the dissociable biological and affective processes mediating food intake.

Compartmentalization of eating behavior has yielded three interrelated, yet neutrally discernible processes associated with intake; liking, wanting, and learning (Berridge, 1996, Berridge et al., 2009). "Liking" is the passive subjective hedonic experience associated with the consumption of flavors (foods) associated with the pleasurable experience. "Liking" is usually measured by taste reactivity tests that rate facial expression as a motoric manifestation of derived pleasure (Finlayson et al., 2007). "Wanting", or incentive salience, is the motivational component that, through associative Pavlovian type conditioning, simultaneously causes us to desire a substance and energize us to act upon the desire to consume a substance associated with oral and post oral pleasure. "Wanting", usually measured by timed forced choice tests (Finlayson and Dalton, 2012), does not necessitate a cognitive or affective component and can occur at an entirely “unconscious” level. However, both wanting and liking must occur together for the full reward experience to occur (Berridge et al., 2009). Learning is a process very much fused with incentive salience, it is simply the association of a neutral stimulus with a reward (the

pleasure of consuming the food) or reward-related cues (positive post-ingestive actions, or in a preceding event a light or tone that signals the presentation of the reward), that can arouse the energizing condition of incentive salience, causing the stimulus to become an attention grabbing "motivational magnet" (Berridge, 2012).

Under normal eating conditions, all three of these components occur together. However in the laboratory setting, each can be more independently studied. The neural actions that mediate sensory aspects of taste, food reward, and learning require activation of pathways which include both sub-cortical (i.e., brainstem, nucleus accumbens (NAC), amygdala (AMY), ventral tegmental area (VTA)), and more complex cortical (i.e., medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC)) structures (Berridge et al, 2009). The limbic and thalamo-cortico pathways are believed to be involved in feeding behavior, with both emanating from the nucleus of the solitary tract (NTS) of the brainstem and bifurcating to individual neural paths(Berridge, 2007; Woosley et al., 2003). Taste sensations are coded by the thalamo-cortical path. Information originating from receptors on the surface of the tongue, palate and pharynx reach the Nucleus of the Solitary Tract (NST) in the medulla via the facial, glossopharyngeal, and vagus nerves, from the NTS information is passed to the to the gustatory cortex (GC), and ascends through the parabrachial nucleus (PBN) of the pons and the ventro-postero-medial nucleus (VPM) of the thalamus onto cortical structures, including prefrontal structures like the OFC (Berridge, 2007; Woosley et al, 2003). A second pathway also emerges from the NTS which is believed to encode food reward. In this path information is sent to limbic forebrain structures including the hypothalamus, substantia innominata, and AMY (Berridge, 2007). At a rudimentary level, the involvement of these pathways in eating behavior is well established,

however the exact interactions (structural and neurochemical) between both the nuclei within individual paths and the greater neural network mediating food related actions remains vague.

Investigation into the neuroanatomical and neurochemical components of consumption, food- related reward, and related learning has yielded detailed information critical to a more complete understanding. Recently several neural hedonic "hotspots" have been identified. Hotspots in the rostrodorsal medial shell of the NAC and the posterior ventral pallidum have been discovered due to a twofold amplification of the "liking" facial response to sweet taste when these areas are centrally infused with the mu-opioid agonist, DAMGO or the CB-1 receptor agonist, Anandamide (Berridge et al., 2009). Opioid agonisim of the ventral pallidum and NAC "hotspots" not only increased hedonic reaction to sucrose reward, but also induced increased sucrose consumption, a response indicative of increased "wanting" (Pecina and Berridge, 2005; Smith and Berridge, 2005). Anandamide centrally infused into the rostrodorsal medial shell of the NAC hotspot has been shown to increase the wanting response in animals, in a manner similar to that observed with opioid manipulations, (Berridge et al, 2009; Smith and Berridge, 2005). Moreover, facial reactions indicating "disliking" to bitter quinine were suppressed by DAMGO or Anandamide injections in and around the NAC hotspot in the absents of an enhanced "liking" response (Peciña, 2008). Due to the observed similarities between opioid and endocannidinioid receptor stimulation, it has been posited that these neurochemicals may work cooperatively within the NAC hotspot to enhance the "liking" experience (Berridge et al., 2009; Mahler et al., 2007). The described "hotspots" are extremely centralized (1 cubic mm) and infusions of DAMGO at a location even minutely outside the "hotspot" can produce a very different outcome. For example, DAMGO administered into the NAC, but not within the centralized hotspot, fails to increase the muscular facial responses indicative of "liking", but

succeeds in escalating the "wanting" response for foods (Berridge et al., 2009). Additionally, site-specific injections of DAMGO into the anterior ventral palladium decreases "liking" and is associated with decreased feeding behavior (wanting) (Berridge et al., 2009). Collectively, these data indicate that "liking" is mediated by both opioid and endocannabinoid receptor activity within very specific neural areas, but that the wanting reaction may be induced by more widespread neural activity.

Learning is an integral aspect of "wanting" as well as the modulation of reward experience and eating behaviors. Incentive salience is composed of two equally important collaborative components, Pavolovian type learning and the neurobiological state of the animal (eg: hungry v. sated), which together mediate motivational intensity experienced by the animal to gain access to a particular reward (Berridge, 2012). Animals naturally gravitate towards consuming food products that are found to be both palatable and associated with positive ingestive consequences, and conversely endeavor to avoid foods that have a foul taste and/or cause gastro-intestinal malaise. For example, mammals tend to find diets high in carbohydrates and fats appealing due to pleasurable orosensory experiences and the nutritional value of these substances (Myers and Sclafani, 2006). Associative learning equips an animal with information about a food substance and can cause an animal to "want" a substance previously associated with positive value when the CS is encountered. Learning associations between a flavor and nutritive value plays an important role in food choice from an early age. Thus, young preweaning rats given intraoral infusions of a flavor paired with either a 20% glucose solution or a 0.05% saccharin solution display a stronger preference for the flavor associated with the more nutritive glucose solution then the less nutritive saccharin solution when tested subsequently as young adults (Myers et al., 2005). Associations formed through experience between a food and the oral/

post-oral consequences gives rise to the establishment of either CTP or Conditioned Taste Aversions (CTA). For instance, if food is associated with a gastrointesional distress, nausea, vomiting, the animal will associate the taste, smell, texture of the food with the distressing experience and avoid future consumption resulting in CTA (Touzani and Sclafani, 2009). Likewise, food associated with pleasant flavor, and positive post-ingestive consequences (as demonstrated above by Myers and Sclafani, 2005) will result in a CTP. Development of a CTP can produce changes in the hedonic and motivational value (incentive salience) of the preferred substance, as illustrated by rats exhibiting increased hedonic facial responses to flavors associated with positive postoral actions (Myers and Scalfani, 2001) and higher rates of licking for flavors associated with positive post oral actions (e.g.: glucose) over a flavor associated with neutral intragastric consequences (e.g.: water) (Sclafani and Ackroff, 2006).

Human Food Preference Studies: Studies investigating various aspects of feeding behaviors often utilize rats due to the remarkable similarities between the rat and human model with respect to this phenomenon. Due to the invasive and highly controlled nature of most feeding paradigms, humans are rarely utilized in such studies for ethical and logistical reasons. Nevertheless, there is a limited amount of relevant data collected from human subjects that supply the scientific community with important information on purely human F-F and F-N preferences. A particularly interesting study performed on 44 non-sated human subjects showed that the amount of energy supplied (calories) by two similarly flavored yogurt drinks (distinguished by a blue or pink marker) was the main factor in conditioning a preference, with subjects choosing the high energy drink over lower energy drink at a significantly higher frequency. These data suggest that human preference can be conditioned by delayed energy reward, regardless of flavor association (Zandstra and El-Derey, 2011). Likewise, there are

several studies that report conditioned preferences to flavors associated with higher caloric value. Subjects reported higher rates of liking for more energy dense dessert flavors then for flavors associated with lower energy the dessert (Brunstrom and Mitchell, 2007) and cream cheese flavors (orange or banana) containing higher fat contents were preferred to flavors associated with lower fat content when conditioned subjects were exposed to choice testing (Capaldi and Privitera, 2007). In energy-deprived, or hungry, states humans tend to increase liking of flavors associated with higher caloric intake. Rated liking for teas mixed with sucrose were significantly greater than those mixed with the non-nutritive sweetener aspartame when subjects were hungry, but when in a sated state subjects demonstrated increases in liking for the aspartame tea solution (Mobini et al., 2007). Similarly, children tested with novel flavored drinks that were either higher in caloric value or lower caloric value due to either a high or low amount of glucose maltodextrin exhibited increased preference for the higher calorie drink. Furthermore, children consumed more foods available ad libitum during snack time following exposure to the low calorie drink, but not in response to the more calorically dense drink (Birch et al., 1990). These data show both that humans tend to prefer foods higher in nutritive and energy values, and that preference for these particular foods are more pronounced when the individual is in an energy-deprived state.

Although numerous human studies demonstrate development of F-N pairings there are very few that test preference based on the orosensory properties of a flavor alone. Sham feeding techniques used with human participants include the spit out technique, where subjects are required to sip a solution or chew a food substance and then spit it out of the oral cavity to reduce post-ingestive actions. In one study (Klein et al., 2006), subjects were required to sip as much of either a unsweetened or sucrose-sweetened (2.5%, 5%, 10% and 20% concentration) cherry-

flavored Kool-Aid solution as they desired in a two minute test period and spit out the contents after each sip. Both intake and rated liking were significantly affected by sucrose concentration, such that the sucrose solutions were significantly preferred to the unsweetened solution as measured by rated liking and intake. Liking and intake rates were highest for the sucrose solutions with higher (10% and 20% sucrose) concentrations (Klein et al., 2006). These data suggest that the flavor of the sucrose alone (minus any post oral consequences) is uniquely responsible for the amount of intake and rated liking.

Flavor preference in human populations can vary widely due to many factors, including, but not limited to, gender, age, and level of education. For instance, women are more likely than men to comply with nutritional guidelines, and tend to rate "healthy" foods, such as fruit and vegetables as more pleasurable than men (Turrell, 1997), and education level in adults is correlated to reduced food neophobia and therefore fewer food aversions are observed for themselves and their offspring (Mustonen et al., 2012). Moreover, studies demonstrate that age and gender are major determinants in preference. In a study that examine rated liking of a sucrose concentration, it was found that children had higher preferences for foods containing higher sucrose levels then did college age or elderly adults, and that males expressed a greater liking for sweeter sucrose concentrations than females, even though perceived sweetness was identical for the male and female groups (Enns et al., 1979). Additionally, males consume more food than woman when it is presented ad libitum, and rate the pleasantness of sweetened yogurt as significantly higher than women when presented in a continuous, but not a progressive schedule of reinforcement (Kissileff et al., 2007).

FMRI data have also illuminated gender and age related differences in response to hunger and satiety. In energy-deprived states, men display greater activation in frontotemporal and

paralimbic neural locations than their female counterparts. In satiated states, women display greater activation of the occipital and parietal sensory association areas and in the dorsolateral prefrontal cortex, precentral gyrus, superior temporal gyrus, and putamen as well as decreased activation in the hypothalamus and amygdala in comparison to males. Males showed greater activation in the ventromedial prefrontal cortex, ventral striatum, insula, orbitofrontal and medial orbitofrontal cortex, which is accompanied by decreased activation in somatosensory locations (Del Parigi et al., 2002; Smeets et al., 2006). Hungry children and adolescents with BMI's within normal range exhibited increased activity in the amygdala, medial frontal/orbitofrontal cortex, and insula in response to food cues (Holsen et al., 2005). Furthermore, fMRI studies of adults show that there are neural differences in early adulthood (mean participant age 23.3) and older adulthood (mean participant age 72.2) during states of energy depletion. Elderly adults exhibit more activation of the gustatory and reward processing regions, posterior cingulate, parahippocampal gyrus, lingual gyrus, occipital gyrus, anterior cingulate, medial frontal gyrus, caudate nucleus, superior frontal gyrus, middle frontal gyrus, cuneus, middle and superior temporal gyri, putamen, lentiform nucleus, claustrum, hypothalamus, inferior frontal gyrus, precentral gyrus, amygdala, and uncus then younger adults, while younger adults displayed significantly greater activation of the thalamus, hippocampus, caudate tail, and postcentral gyrus (Jackobson et al., 2010). Hence, differences in preference and intake displayed across age and gender in human subjects have underlying neural correlates.

Individual food choice and preference are complex phenomena influenced by many "conscious" and "unconscious" determinants. Genetics (DeCastro, 1988), familial and cultural eating habits (Roos et al,. 2012), neural and homeostatic activities (Woods and Ramsay, 2011), hedonic/ nutritional value (Mobini et al., 2007), social environment, physical proximity (King et

al,. 2007), mood (Gibson, 2006), age, and gender (Ares and Gambaro, 2007) all play pivotal roles in the foods we are exposed to as well as the foods for which we develop preferences. Hence, food consumption is mediated by a complex internal and external milieu mediated by learning and associative neural activity that either motivates an individual to eat foods that have specific nutritional or hedonic values, or restrains global food consumption or the intake of foods associated with negative consequences.

Conditioned Flavor Preferences: Bitter, sweet, salty, sour, and glutamate (or umami) (Yamaguchi and Ninomiya, 2000) are the five known tastents that chemically stimulate receptors along the tongue, throat, and larynx leading to activation of the gustatory system and neural gustatory locations. These basic tastants only partially contribute to the experience of a flavor; textures, temperature, physiological states of hunger and flavorant concentration are also important contributing factors. For instance, "allesthesia" refers to increased perception of taste pleasantness when in a state of energy deprivation (Cabanac, 1971). In addition to increased palatability resulting from a general physiological state of hunger, allesthesia can also occur in response to specific nutrient deficits, causing increased hedonic evaluations for flavors associated with that nutrient. It is known that animals can develop preferences for a previously non-preferred or unpleasant flavor when that flavor is paired with positive post-ingestive actions (e.g.: bitter solution paired with IG sucrose) (Gonzalez et al., 2010). Likewise, animals develop preferences for normally unpleasant flavors if that flavor is associated with nutrients in which the animal is deprived. For instance, under normal homeostatic conditions salty flavors, above that which is intrinsic to typical bodily concentration, are considered unpleasant. Non-sodium deprived rats given oral infusions of a 10% NaCl solution display disliking facial responses, including gaping or the mouth and turning away from the stimuli, however, when under a state of

sodium deprivation rats readily display musculo-facial movements indicative of strong hedonic reward in response to the same solution (Berridge and Schulkin, 1989). Perception of taste pleasantness in non-hungry or nutrient deprived subjects is generally related to exposure to and concentration of the flavorant. For example, sensory specific satiety refers to a decline in the hedonic evaluation of a specific flavor generated by the consumption of that flavor. This phenomena occurs quickly, in some cases in as little as 2 minutes following the intake of a meal, resulting in lower "pleasantness" rating for the meal then the rating given before exposure (Rolls et al., 1981). Additionally, concentration of a flavor significantly impacts perceived palatability. For instance, ratings of glutamate palatability increases at higher concentrations (Prescott, 2004) with soups containing higher levels of monosodium L-glutamate (MSG) eliciting a stronger preference. Though post-oral consequences may be responsible for increased MSG liking, human sip-and-spit sham-feeding studies show similar results with higher intake levels and palatability ratings for sucrose solutions at more intense concentrations (Klein et al., 2006). Hence, conditioning of a flavor preference is very much related to the physiological state of the animal and perceived pleasantness of the tastant.

The conditioning of a flavor preference is based on a variety of multimodal factors as previously discussed. Although consumption along with associated physiological and mental activity that prompt intake, rejection, acceptance, and cessation are complex and dynamic the behavioral aspect of conditioning a flavor preference is based on the rather simple principles of associative learning. Associative learning is the process by which an association is made between a stimulus and a response. There are two main forms of associative learning; classical or Pavlovian conditioning and operant conditioning (Fantino and Storarz-Fantino, 2012). Both forms are avidly utilized for studies that evaluate preference behavior. Moreover there are

several types CFP's, including learned- safety, social-learning, as well as F-F and F-N. This dissertation focuses on F-F and F-N CFP's. In the laboratory setting when studying F-F or F-N induced CFP, associative Pavlovian learning processes result in the establishment of a CFP through repeated pairings of a neutral flavor (e.g.: cherry) with an already preferred flavor (e.g.: fructose) producing a "positive" conditioned stimulus (CS+), and the pairing of another flavor (e.g.: grape) with a less preferred flavor (e.g.: saccharin) resulting in a "negative" (or "less positive") conditioned stimulus (CS-) (Yiin et al., 2005). As expected, animals will usually display a preference, as noted by increased intake, for the CS+ over the CS- when given a choice between the two flavors presented side by side (Sclafani, 2002). Similarly, establishment of a flavor preference can occur when one flavor (administered orally) is associated with nutrient-rich intra-gastric infusions (CS+) and another flavor is associated with neutral intra-gastric infusions of water (CS-). Under these conditions, animals tends to exhibit strong preferences for the flavor associated with nutrient infusion over that which was associated with water infusion (Sclafani and Ackroff, 2006).

As discussed in the previous section (see Human Preferences Studies), some factors that may play a role in the development of CFP in animal subjects include age, gender, and state of energy homeostasis. As with humans, age of animals plays a significant role both the intensity of a tastant (Booth et al., 1982), and the neurochemical and anatomical systems that mediate learning, decision making, and memory processes (Marschner et al., 2005), vital to establishing and maintaining CFP. For instance, the number of taste buds and sensitivity to tastants, especially sweet and salty tastes, generally declines with age (Booth et al., 1982; Schiffman, et al., 1981). In rats tested between 7 and 24 months of age, it was found that younger rats more avidly developed preferences for positive flavor stimulants over negative flavor stimulants

following CFP procedures, whereas older rats failed to display preferences, possibly reflecting age-related deficits in the ability to develop and/or maintain associative processes related to CFP (Renteria et al., 2008). Additionally, a CFA study performed on weaning, adult, and older rats showed that age- related learning impairment was found in the older cohort of rats causing accelerated “forgetting" of the passive avoidance task necessary to evade administration of an aversive saccharin/lithium chloride solution, suggesting a deficit in retention (Guanowsky and Misanin, 1983).

Gender also plays a large role in animal food preference. Changes in food preferences occur across the female rat estrous cycle, as well as during pregnancy and lactation, suggesting a strong hormonal influence underlying perceived palatability and food consumption (Bowen, 1989; Geary, 2001). In reference to CFP induced by the sweet taste fructose, preferences are more robust in male rats then in female rats, such that male rats avoided unsweetened solutions and favored the fructose sweetened solution after training, whereas female rats displayed indifference to the solutions (Ackroff and Sclafani, 2004). Furthermore, females display preference for sucrose- and saccharin-sweetened solutions over those sweetened with sucralose, but have mixed preferences for sucralose paired with other sweeteners (Sclafani, 2004). Collectively, these data suggest the existence of gender differences in flavor preference. Fluctuating hormonal patterns as well as the overall influence of gonadal hormones cause the female rat to be a less reliable subject in the attempt to further our understanding of the neurochemical and neuroanatomical substrates that influence CFP. As such, the employment of the less hormonally-variable male rat appears to be a more useful experimental strategy.

As more comprehensively stated above, satiety and hunger are major contributors to the palatability and consumption of food. Energy deprivation is a highly motivating factor in the

consumption of food and stimulates an animal to seek out and intake nutrients. Food restriction may serve to increase an animal's behavioral response to salient stimuli, leading to increased chances of approach behavior to food substances (Peng, Ziff, and Carr, 2011). In CFP studies an animal in a hungry state is more likely than an animal in a non-hungry state to intake the target flavors, resulting in the hungry animal experiencing greater exposure to the flavor and therefore increased opportunity to develop learned associations. Furthermore, as demonstrated (Albertella and Boakes, 2006), food-restricted animals that receive food immediately or up to 120 minutes after CFP training do not display interruptions in preference maintenance for either Pavlovian or instrumental learning tasks. Thus, the utilization of non-satiated animals in CFP paradigms is a common practice due to increased motivation and exposure to the CS, in absents of adverse side effects.

2. Specific-Nutrient CFP: The role of Sugars, Starches, Fats.

The taste of a substance becomes associated with digestive factors like perceived fullness and nutrient content along with a variety of widespread neural actions that code for flavor, nutritional value, and the hedonic impact of a tastant. Associations between digestive and neural factors facilitate learning and discrimination between tastants, thereby allowing for the creation of a CFP or CFA (Touzani and Sclafani, 2009). In the case of a CFP, positive oral, post-oral, and associated neural actions result in the formation of a preference for a flavored item over a differently-flavored item. These preferences have been established in the laboratory setting with both flavors that are associated with post-oral actions, as well as with flavors presented with negligible post-oral impact (Sclafani et al., 1999; Touzani et al., 2010). Thus, conditioning of a flavor preference can occur due to the flavors associations with ingestive consequences or due to a hedonic reaction, in the relative absence of ingestive factors, elicited by the tastant alone. CFP

can be induced by a variety of nutritive substances like sugars, fats, starches, and substances linked to abuse, like alcohol and other psychotropic drugs. Although the ability for these substances to contribute to the formation of CFP is now firmly established (Touzani and Scalfani, 2009), much less is known about the neural mechanisms' responsible the acquisition and maintenance of this type of learning. Thus, it is unknown whether the same neural actions modulating conditioned preferences for sugars also modulate preferences for fats or starches. However, it is unlikely that a CFP for any of these nutrients could be conditioned if positive neural processes were not associated with intake. With more intensive research the scientific community may soon be able to determine if actions associated with the intake of different nutritive substances are governed by distinguishable neural processes, thereby greatly advancing our understanding of nutrient-specific flavor learning.

Sugar CFP: Sugars are highly attractive to humans and many other mammals presumably due to orosensory, postingestive and neural interactions encouraging the intake of this highly palatable energy dense substance (Menella, 2008; Touzani et al., 2010). Sweet tastes, elicited by stimulation of specific taste receptors by sugars and artificial non-caloric sweeteners, appear to indicate the presence of highly valuable carbohydrate calorie sources in foods (Yoshida et al., 2012). Recently, the molecular chemical mechanisms responsible for the conveyance of sweet tastes from the oral cavity to gustatory neural targets have been more fully revealed. Two G-protein coupled sweet taste receptors have been identified; these receptors are believed to function as heterodimers, with the T1R2 and T1R3 combination reacting to a variety of natural as well as artificial sweeteners in both humans and animals (Li et al., 2002; Montmayeur et al., 2001; Nelson et al., 2010), and the T1R1/T1R3 receptor heterodimer recognizing umami tastants (Li et al., 2002). Stimulation of the T1 and T2 receptors, via natural or artificial sweeteners,

induces increased activity in the gut as well as gustatory neural areas resulting in amplified glucose absorption. Moreover, Trpm5-/- mice, that lack the mechanism to "taste" sugars, retain the ability to develop a preference for sucrose solutions and glucose solutions, with increased DA efflux in the ventral striatum, indicative of consumption of rewarding foodstuff, present under both conditions (Ren et al., 2010).

In the development of CFP, not all sugars are created equal. Palatability and energy content are important factors in developing CFP for a sweet taste. For example, animals show preferences for solutions with higher sucrose concentrations over those with lower concentrations, with the amount of intake corresponding to the concentration of sucrose in sham feeding studies (Ackroff and Sclafani, 2004). It should be noted that this effect is not observed in real-feeding studies due to satiety. Conversely, animals show higher preference for non-nutritive saccharin solution at low concentrations and exhibit decreased intake of solutions as saccharin concentration is increased (Smith and Sclafani, 2002). Energy-dense sugars like glucose or sucrose are very potent in establishing robust CFP's in short-term sham-feeding or real-feeding F-N studies, respectively, whereas fructose, a sugar with minimal post-ingestive rewarding and satiating effects, lacks the ability to condition a flavor preference in short-term (30min daily sessions) IG infusion studies. Fructose, in comparison to glucose, produces only a small insulin response produces a minimal insulin response (Stanhope and Havel, 2010). Fructose however can elicit flavor preferences when intragastrically administered daily over long training sessions (Ackroff et al., 2001; Ackroff and Sclafani, 2004).

In putative F-F-mediated studies in which sucrose or glucose is utilized, sham-feeding is required to minimize the post-oral actions of these sugars (e.g., Yu et al., 1999, 2000a, 2000b). Fructose, although less preferred than sucrose, is more preferred than saccharin and is therefore

often utilized in short-term real-feeding F-F paradigms due to its effectiveness in establishing CFPs under these conditions (e.g., Baker et al., 2003, 2004; Golden and Houpt, 2007). Artificial sweeteners (usually non-caloric or minimally caloric) are often used in F-F and F-N CFP studies as the unconditioned stimulus for a so-called "CS-" flavor preference because they are generally less preferred (or in some cases rejected) relative to the natural sugars which act as the CS+ eliciting stimuli.

Starches: Although most animals can identify five basic tastants, behavioral and electrophysiological data indicate that rats not only possess the ability to taste polysaccharides, but can also discriminate between different polysaccharide molecules, indicating the possible existence of a polysaccharide taste receptor in these rodents (Sclafani, 1991; Sclafani, 2004). Several studies support this starch discrimination and receptor hypothesis. Rats readily display preference for corn starch powder over powdered cellulose in short-term trials. Food restricted rats and non-food restricted rats show a preference for starch powder over Polycose powder (a starch-derived polysaccharide) in short-term trials, and Polycose powder over starch powder in long- term trials. Rats also exhibit preference for amylopectin over amylose in both short- and long-term trails (Ramirez, 1991; Sclafani et al., 1987). Although rats show significant preference differences for different starch and starch-like substances, much of the effect of starch-induced CFP is likely due to rewarding nutritive post-oral actions. For instance, Lucas and co-workers showed that IG infusions of Polycose promotes conditioned preferences for flavors paired with Polycose (hydrolyzed starch) infusions over a differently flavored solution paired with IG water infusion (Lucas et al., 1998). Additionally, in two-bottle choice tests, food-deprived rats exhibited a significantly greater preference for flavors previously paired with Polycose than for differently-flavored solutions not previously paired with Polycose, but failed to

exhibit this preference if Polycose absorption was chemically delayed during training (Elizalde and Sclafani, 1988). Furthermore, preference induction is mediated by the solution's concentration of starch or starch-like substances. For instance, the positive postingestive effects of IG Polycose infusions occur at concentrations from 8% or 16%, but diminish at greater concentrations (32%), (Lucas et al., 1998). In real-feeding corn starch studies performed by Rameriz, it was found that Fischer rats displayed preference for solutions containing 0.5%, 1%, 5%, or 20% corn starch, but not 10% (Ramirez, 1993).

Additionally the highly-rewarding effects of starch have been evaluated in comparative feeding studies. For instance, in sucrose versus corn starch preference testing, rats preferred 0.5% sucrose over 0.5% starch on the first day of testing, but reversed preference to the starch solution on subsequent days (Ramirez, 1993). Furthermore, rats exposed to corn starch- or sucrose-paired solutions in one-bottle training trials displayed a significant preference for the starch-paired flavor over the sucrose-paired flavor in subsequent two bottle choice tests. These data indicate that starch has the unique ability to condition extremely powerful flavor preferences in rats, with starch-induced preferences mimicking or even exceeding those elicited by the highly-palatable sugar, sucrose. It is likely that these results are due to differential post-ingestive actions (Bonacchi et al., 2010). Taken together these data collectively indicate that starches, at desired concentrations, have strong rewarding effects, and are therefore able to promote robust CFP's.

Fats: Fat-rich foods, including nonnutritive fat substitutes (e.g.: mineral oil) are highly attractive and are capable of eliciting CFP's in animal models. This attraction is most likely mediated by the highly palatable nature of fats as well as positive nutritional actions (Acroff et al., 2005; Sclafani et al., 1999). Although it is currently unknown if there are specific taste

receptors for fats, as there are for the five basic tastants, it has been suggested that in rodents lingual CD36 may be involved in the orosensory identification dietary fat. Mice with CD36 knock down display decreased preference for the consumption of long-chain fatty acid during free choice feeding session in comparison to genetically-intact counterparts (Khan and Besnard, 2009). In a flavor preference study that attempted to minimize postingestive actions by utilizing short exposure time (e.g.: 5 minutes) to fat solutions, it was found that rats can develop preference for one fat over another. In these short trials, long-chain fatty acids were preferred to linoleic acid, and linoleic acid was preferred to oleic acid, indicating that olfactory and/ or gustatory cues contributed to fat perception in rats (Tsuruta et al., 1999). Furthermore, animals displayed a robust preference for flavors previously paired with higher levels (3.5%) of corn oil over the flavor previously paired with lower (0.9%) concentrations when exposed to two bottle choice testing (Dela Cruz et al., 2012). In F-N studies, it has been found that rats prefer flavors associated with IG fat infusions over those associated with water. Moreover, rats display differential preference for IG administered fats, with corn oil preferred over medium chain triglycerides by 75%, corn oil preferred to vegetable shortening by 64%, and corn oil and safflower oil equally preferred. Generally, IG infusions of high polyunsaturated and/or lower saturated fats are the most reinforcing. These data suggest that different fat sources can produce CFP's at varying intensities (Ackroff et al., 2005).

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

Definition of Flavor-Flavor (F-F) and Flavor-Nutrient (F-N) Conditioning: Food selection and preference are determined by both the orosensory aspects and post-ingestive nutrient actions of a substance. Orosensory perception of a food, such as taste, smell, texture, and temperature, is initiated by interactions between the food substance and oral/ olfactory

receptors that communicate associated qualities to central neural structures which generate the overall orosensory experience. Post-ingestive actions of a food refer to the positive or negative consequences associated with the pre and post-oral absorption of nutrients and other molecules contained within the food substance (Touzani and Sclafani, 2009). During normal eating situations, orosensory and post-ingestive actions usually operate concurrently; however in the laboratory setting, these components are separated and individually explored. Both warrant scientific investigation because orosensory and post-ingestive actions may be mediated by separate neural mechanisms within the same distributed brain network, and have different effects on mediating preference and consummatory behaviors.

Several methods are commonly utilized to investigate the respective roles of orosensory and post-oral effects of flavors and nutrients on consumption, reward, and preference. For example, investigation of orosensory effects of a food in the relative absence of post-oral consequences can be accomplished by the sham-feeding procedure, in which an intragastric fistula is surgically implanted allowing for consumed substances to be drained from the stomach, thereby minimizing nutritive effects (Weingarten and Watson, 1982; Yu et al., 1999, 2000a, and 2000b). Real-feeding procedures in which either a non-nutritive or minimally-nutritive substance is ingested can also be used to determine maximal effects of orosensory stimulation (Baker et al, 2003). Likewise, post-ingestive effects of a substance can be examined in absence of oral stimulation through direct intragastric infusions of nutritive or non-nutritive solutions (Sclafani, 2004; Ackroff, 2008). Individually, these two components have the ability to exert either positive or negative effects on food intake (Sclafani, 2001). For example, varying concentrations of a given nutritive or non-nutritive substance in a solution can differentially affect the amount consumed as well as the reward value of the stimuli in both sham-feeding and

real-feeding paradigms that uniquely target oral or post-ingestive effects (Sclafani, 2004; Smith and Sclafani, 2002). In addition to the orosensory and nutritional stimuli offered by a food, learning also plays a major role food avoidance and preference. Animals readily learn associations between the oral and post-oral effects of a food which can either act to increase or decrease intake (respectively indicating preference or avoidance) as well as alter previously-established preferences (Sclafani, 2004). This dissertation focus on the two types of learning involved in the mediation of preference and intake: F-F and F-N conditioning.

*Flavor-Flavor (F-F) Conditioning**:*** An experimental procedure utilized in the study of "pure" F-F learning (e.g., in the absence of flavor-nutrient learning) involves the training of animals with a novel CS+ flavor mixed into a preferred, non-nutritive solution (e.g., 0.2% saccharin) and a CS- flavor mixed into a less preferred, non-nutritive solution (e.g., 05% saccharin) (Fedochak and Bolles, 1987; Holman, 1975). F-F conditioning is then evaluated in a two bottle choice test with both the CS+ and the CS- flavors presented in saccharin solutions of the same concentration. Two related procedures can be used to establish and maintain F-F conditioning under sham-feeding and real-feeding conditions. In the sham-feeding paradigm, the animal is allowed to drink flavored sucrose (CS+) or saccharin (CS-) solutions, but the solutions are drained through an open gastric fistula before it reaches the small intestine, thus minimizing the contribution of post-ingestive actions to preference learning (Yu et al., 1999, 2000a, 2000b). In the real-feeding paradigm, the animal is presented with fructose instead of sucrose because fructose fails to support post-ingestive flavor preferences in short-term intake tests (Sclafani & Ackroff, 1994; Sclafani et al., 1993). Both fructose and sucrose are used in real and sham-feeding conditions, respectively, to insure the CFP is established maximally in response to the orosensory properties associated with flavor, rather than the post-oral actions of the nutrient. In

light of this, experiments contained within the present research proposal will investigate F-F learning in real-fed rats using a fructose (8%) and saccharin (0.2%) as the positive unconditioned stimulus (Baker et al., 2003, 2004; Golden and Houpt, 2007). Specifically, F-F conditioning will be produced in rats by mixing the CS+ flavor (e.g., unflavored cherry Kool Aid) into an 8% fructose solution that also contains 0.2% saccharin; a taste mixture that has been proven to be highly preferred to that of saccharin alone (Baker et al., 2003; Sclafani and Acroff, 1994). The CS- flavor (e.g., unflavored grape Kool Aid) will be mixed into a 0.2% saccharin solution. During two-bottle tests, the CS+ and CS- flavors will be presented in a common 0.2% saccharin solution.

Flavor-Nutrient (F-N) Conditioning: Several procedures are used to study F-N learning in rodents. The most straightforward is to pair the oral consumption of a CS+ solution (e.g., unflavored grape Kool-Aid in 0.2% saccharin) with an IG infusion of the nutrient (e.g., sucrose or glucose). The oral consumption of a CS- (e.g., unflavored cherry Kool Aid in 0.2% saccharin) solution is paired with IG water infusions. This procedure allows the post-ingestive consequences, but not orally-derived nutrients, to become associated with the consumed CS+ flavor. Rats acquire significant preferences for the CS+ flavor paired with IG infusions of various nutrients including carbohydrates, fats, proteins and complex foods (e.g., milk) (Sclafani,2004). F-N preferences can be quite strong in rats, and can actually convert inborn flavor aversions into preferences (Drucker and Sclafani, 1997). Nutrient-conditioned preferences are both very resistant to extinction and forgetting (Drucker and Sclafani, 1994), and can increase the absolute intake of the CS+ flavored solution when presented alone, thereby producing conditioned acceptance, and increasing body weight with repeated sessions (Sclafani,

2004). It is also important to note that nutrient conditioning can occur in the absence of a flavor cue (Ren et al., 2010; Sclafani & Ackroff, 2012).

4. Neurochemical Candidates in the Mediation of CFP

The acquisition and maintenance of CFP involves the collaborative actions of a variety of neurotransmitters ostensibly operating to mediate numerous CFP components, including food reward, hedonics, motivation, and learning. Studies (see reviews: Touzani et al., 2010; Sclafani et al., 2011) evaluating CFP and its related properties have investigated ; a) dopamine (DA), b) opioids, c) cannabinoid (CB), d) GABAergic and e) Glutamatergic (Glu) systems as major contributors in food reward and preference learning. The roles of the opioid and DA systems have been studied more extensively than the other neurochemical candidates, and both have been linked to modulating the functions of other implicated chemicals. This section will review all of the major neurochemicals believed to be involved in the conditioning of food consumption.

Dopamine: Extensive and complex theories link brain DA circuits to food reward, motivation, and learning (Berridge & Robinson, 1998; Berridge, 2007; Ikemoto et al., 1997; Smith, 1995). Although there has been much debate among the scientific community over the past two decades pertaining to the exact role of DA in preference learning, these data have consistently implicated the DA system as being a vital component in flavor conditioning, particularly in food reward processing and motivation. The rewarding aspects of DA pertaining to consumption are supported by findings that several brain sites involved in reward processing, including the NAC and prefrontal cortex displayed increased DA efflux in response to food consumption (Bassareo et al., 2002; Smith, 2004). Further, DA receptor antagonists reduced sham- and real- feeding of sugar solutions (Geary & Smith, 1985; Weatherford et al., 1990), with

effects analogous to reward reduction due to decreased sweetener concentration (Schotanus & Chergi, 2008; Smith, 1995; Wise, 1978**;** Xenakis & Sclafani, 1981). Furthermore, the role of DA in hedonic/reward mediation of food intake can be extrapolated from data that have identified DA as a neurotransmitter governing the hedonic aspects of drug reward (such as cocaine, amphetamines, and MDMA) (Arias-Carrion et al., 2010; Di Chiara & Imperato, 1988**;** Olive et al., 2001; Zemishlany et al., 2001), electrode self-stimulation in operant procedures (Cheer et al., 2007), and natural reward produced by sexual activity (Argiolas and Melis, 1995; Pfaus et al., 1995). On a motivational level, the mesocorticolimbic system has been implicated as the incentivizing/energizing component of incentive salience, with neural DA levels identified as "the most potent modulators of cue-triggered temptation" (Berridge, 2012). DA receptor antagonists have been found to reduce food-motivated operant responding at doses that are not correlated with reduced consumption (Ikemoto et al., 1997). Moreover, genetic studies have shown that “hyperdopaminergic” mice display enhanced motivation to obtain sweet rewards, presumably due to increased synaptic DA caused by DA transporter knockdown (Pecina et al., 2003).

DA signaling also plays an important role in food-related learning, particularly F-N and F-F preference conditioning. Intake of a bitter SOA solution stimulated NAC DA efflux in rats trained to prefer the SOA solution through pairings with IG nutrient infusions, suggesting that conditioned DA release is involved in F-N learning (Mark et al., 1991). Trpm5-/- mice who lack the ability to "taste" sugar exhibited increased striatal DA efflux in response to IG glucose infusions, and developed preferences for flavors paired with infusion of IG glucose over flavors paired with non-nutritive water infusions (Glass et al., 2009; Ren et al., 2010). DA receptors are also believed to modulate the potency of F-F conditioning to sweet tastes. Pairing the intake of

a flavored sucrose solution with a DA D2 receptor antagonist (raclopride) reduced the preference for that flavor relative to another flavored solution paired with saline treatment (Hsiao and Smith, 1995). Furthermore, DA signaling is also involved in flavor aversion learning, such that decreased DA NAC efflux occurs when conditioned aversions are accomplished to a particular taste or odor (Besson & Louilot, 1997; Louilot & Besson, 2000; Mark et al., 1991). Moreover, systemic and central injections of DA receptor antagonists impair flavor aversion learning (Caullies et al., 1996; Fenu et al., 2001; Huang and Hsiao, 2002; Risinger et al., 1999). Additionally, neural DA circuits are involved in other types of food-related learning including place preferences conditioned to sucrose or corn oil (Ågmo et al., 1995; Imaizumi et al., 2000) and the acquisition of food-motivated operant lever pressing in rats (Baldwin et al., 2002; Smith-Roe and Kelley, 2004). Taken together, these findings indicated that DA signaling appears to be involved in learning *where* and *when* to find food, *how* to obtain food, and *which* foods are nutritious or toxic.

Opioids: The neural opioid system is generally believed to govern the hedonic or reward- linked aspects associated with the consumption of palatable foods. Increased food and liquid solution intake has been observed in animals treated with general opioid agonists, and conversely, animals treated with general opioid antagonists display reduced intake (see review: Bodnar, 2004). Opioid mediation of feeding seems to be linked to palatability, with systemic general opioid injections increasing the consumption of palatable foods (like sucrose) more readily then less palatable tastants (Olszewski and Levine, 2007). For instance, subcutaneous naltrexone (NTX) preferentially reduces intake of more desired foods while leaving intake of a non-preferred foods less affected (Glass et al., 1996). The inhibitory effects of NTX were more evident in rats exposed to sweet-tasting chow then normal chow, such that decreased intake was

more robustly observed in the sweet chow group, with a low dose of NTX reducing sweet chow intake by 50% (Levine et al., 1995). In human studies, NTX administration reduced not only overall food consumption, but also significantly reduced subjective perceived pleasure ratings (Yeomans and Grey, 1997). However, in F-F and F-N studies, NTX failed to significantly alter either the acquisition or expression of sugar-mediated CFP, although opioid antagonist treatment substantially decreased the amount of solution intake. For instance, in F-F sham-feeding studies with sucrose, systemic NTX administration is associated with reduced intake of both CS+ (sucrose) and CS- (saccharin) flavors, but had no significant effect on the acquisition or expression of a sucrose-CFP (Yu et al., 1999). Correspondingly, systemic NTX failed to affect the acquisition or expression of a fructose-induced CFP in real-feeding rats (Baker et al., 2004), and in F-N studies, systemic NTX failed to alter the acquisition and expression of IG sucrose-induced CFP (Azzara et al., 2000).

As previously stated, it has been proposed that within specific neural "hotspots", including the rostrodorsal medial shell of the NAC and the posterior ventral pallidum, pharmaceutically-induced enhancement of opioid receptor activity by DAMGO is related to increased intake of sucrose solutions, as well as a two fold increase in oro-motoric facial expressions associated with the hedonic response of "liking" (Berridge et al, 2009). Additionally, rats exposed to choice tests between less preferred (banana) and more-preferred (chocolate) flavored pellets consumed a larger amount of chocolate-flavored pellets in both untreated conditions and under the influence of systemically administered NTX. Although systemic NTX failed to alter preference there was, as expected, a general decrease in the amount of pellets consumed. However, when NTX was centrally infused into the NAC, a reduction in consumption of the more preferred chocolate pellet occurred while consumption of the less

preferred banana flavored pellet remained stable (Wooley et al., 2006). This suggested that perhaps the NAC would be a site at which NTX would affect F-F- or F-N-CFP. Yet, neither fructose-CFP nor IG glucose-CFP was affected by NTX treatment into the NAC, even though sugar intakes were reduced by the opioid antagonist (Bernal et al., 2010).

NTX blocks the actions of most opioid receptor subtypes (Murrin, 2007). Studies on specific opioid subtypes have demonstrated that different receptors have distinct actions in the modulation of intake and food associated reward. For instance, NPY-induced feeding is significantly inhibited by intercerebral ventricular infusions of mu and kappa, but not delta opioid antagonists (Kotz et al., 1993). Mu and kappa antagonists directly administered into the NAC selectively decrease intake in rats during states of deprivation, glucoprivic-induced hunger, spontaneous intake, and sucrose feeding contingency situations (Bodnar et al., 1995). Similarly, direct administration of mu and kappa antagonists into the periventricular nucleus of the hypothalamus (PVN) reduced deprivation induced feeding whereas administration into the Ventral Tegmental Area (VTA) fails to show similar effects (Koch et al., 1995; Ragnauth et al., 1997). Moreover, there are differential μ and κ opioid antagonists' effects on Fos-like immunoreactivity in extended amygdala (Carr, 1999). Furthermore, in rats sham-fed a 20% sucrose solution general, mu, and kappa antagonism, but not mu-1 or delta antagonism, dose-dependently reduced solution intake in a fashion comparable to a 50% reduction in sucrose concentration. Equivalent results were found in both real-fed and sham-fed rats, suggesting the existence of a positive relationship between the orosensory properties associated with sucrose palatability and intake, and the mu and kappa opioid receptor subtypes (Leventhal et al., 1995). However, despite the powerful central effects of general or selective opioid antagonists, our

laboratories have failed to find significant evidence indicating opioid modulation of F-F- and F-N-CFP (Azzara et al., 2000; Baker et al., 2004; Bernal et al., 2010; Yu et al., 1999).

Cannabinoids: Over thirty years ago, Abel documented a correlation between marijuana usage, appetite stimulation, and desire to ingest palatable foods (Abel, 1975). More recent studies have identified Cannabinoid Receptor (CB) receptor activity as an influencing factor in perceived hunger and food selection. An effect presumably due to an amplification of the rewarding orosensory properties associated with intake (Cota et al., 2003; Kirkham and Williams, 2001). CB1 receptors in particular are believed to mediate the consummatory behavior associated with cannabinoid administration; with this receptor subtype widely spread throughout the brain at a neural density comparable to that of GABA or Glutamate (Freund et al., 2003). Antagonism of the CB1 receptor is associated with decreases in consumption of sucrose sugar, palatable sweetened foods, and alcohol (see review: Cota et al., 2003; Cota et al., 2006). Compelling evidence for the CB1 receptor in the modulation of food intake comes from data obtained from sated rats simultaneously treated with the CB1 receptor antagonist SR 141716 and anandamide (a CB1 agonist). These animals did not exhibit increased food intake associated with anandamide administration, suggesting that increased consumption associated with endocannabinoids may be mediated, in-part, by the CB1 receptor (Williams et al, 1999). Conversely, F-F studies found that systemic administration of CB-1 receptor inverse agonist AM-251 partially suppressed the expression of fructose induced CFP, but failed to significantly reduce the acquisition, suggesting partial CB1 receptor activity involvement in the modulation of F-F CFP (Miner et al., 2008) .

A "synergistic interaction between CB1 and opioid antagonists" may exist (Rowland et al., 2001; Cota et al., 2006), as endorsed by significant decreases in consumption following

simultaneously administered CB1 and general opioid antagonists, SR141716 and naloxone, at doses that have no effect on ingestive behavior when administered alone (Kirkham and Williams, 2001). Further, reductions in morphine induced c-fos immunoreactivity in various neural sites associated with food intake and reward, including the NAC shell, the PVN, dorsomedial and ventromedial portions of the hypothalamus, periventricular nucleus of the thalamus, both central and basolateral AMY, and the VTA, are observed in animals treated with systemic CB1 antagonists (Singha et al., 2004). DAMGO or Anandamide centrally infused into the NAC and ventral pallidum hedonic "hotspots" reveals a twofold amplification in the facial responses associated with "liking" (Berridge et al., 2009). Additionally, anandamide centrally infused into these "hotspots" has also been found to increase wanting responses, in a fashion comparable to that observed with opioid agonisim (Berridge et al., 2009; Smith and Berridge, 2005). Thus, further studies might discover an endocannabinoid-opioid interaction in mediating CFP.

In addition to the CB1/opioid interaction, there is substantial evidence suggesting an interaction between CB and DA receptor activity, such that administration of selective CB1 receptor antagonists is related to a reduction in NAC extra-cellular DA usually increased by consumption of palatable food (Melis et al., 2007). Administration of DA D_1 receptor antagonist SCH 23390 dose-dependently reduces intake in rats, the CB1 agonist Δ^9-tetrahydrocannabinol (THC) dose dependently increases intake, however combined SCH23390 and THC administration attenuated THC- induced consumption at SCH23390 doses that do not substantially effect feeding behavior (Verty et al., 2004). Collectively, these data suggest the presence of a CB-DA interaction mediating feeding behavior, and more specifically possible mesolimbic DA system involvement in the modulation of cannabinoid receptor action (Melis et

al., 2007; Verty et al., 2004). Further studies may identify endocannabinoid/DA interactions in CFP.

GABA: The effects of benzodiazepine (BNZ) on consumption, due to the binding of this pharmaceutical to the BNZ receptor site on the GABA receptor complex, have been linked to increases in food intake and perceived palatability (Cooper, 2005, 2007). In 1985 it was documented that rats sated with a palatable diet before drug administration exhibited increased food intake thirty minutes after exposure to BNZ agonists, substantially decreased intake after administration of inverse agonists, and BNZ antagonist administration caused a blocking of both agonists and inverse agonist effects (Cooper, 1985). In CFP studies Abecarnil, a partial agonist, increases the ingestion of a preferred sweetened solution in two bottle choice test, and significantly increased the consumption of a mash sweetened with sucrose (Cooper and Greenwood, 1992). Likewise, microinjection of midazolam (a $GABA_A$ agonist) into the IVth ventricle increased consumption of a pleasantly flavored mash, an effect that was blocked by systemic injection of flumazenil, a selective BNZ antagonist (Higgs and Cooper, 1996). Interestingly, in a recent study by Dwyer (2009), it was found that midazolam, a BNZ receptor agonist which increases sweet solution intake, administered during training decreased the CS+ preference compared to vehicle-treated rats (72% vs. 87%). This effect was attributed to the drug enhancing the palatability of the CS− saccharin solution during training as indicated by a selective increase in CS− intake. Furthermore, midazolam treatment failed to enhance the expression of the learned CS+ intake, although an increased overall CS intake was observed. 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; Dwyer, 2009; Yu et al., 1999).

A myriad of studies have documented site-specific GABA mediation of food intake, indicating the importance of GABAnergic processes in the distributed neural network mediating consumption. Central studies have demonstrated that GABA agonists induce feeding behavior when centrally infused into VTA (Arnt and Scheel-Kruger, 1979; Echo et al., 2002; Khaimova et al., 2004; Klitenick and Wirtshafter, 1988), NAC shell (Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Ward et al., 2000) and ventro-medial hypothalamus (Grandison and Guidotti, 1977; Kelly et al., 1977; Tsujii and Bray, 1991), but suppress food intake when injected into the central nucleus of the AMY and LH (Kelly et al., 1977; Minano et al., 1992). Recent studies have suggested that interactions among GABA receptor subtypes in neural locations implicated in the modulation of eating behavior may be crucial to the proper functioning of the greater neural network governing this phenomenon. It has been well documented that systemic administration of $GABA_A$ and $GABA_B$ agonists increase food intake (Ebenezer and Pringle, 1992; Garndison and Guidotti, 1977). Site-specific studies show a more complex $GABA_A$/ $GABA_B$ interaction. For example, feeding behavior induced through direct NAC infusion of baclofen, a $GABA_B$ agonist, is does-dependently blocked by administration of the $GABA_B$ antagonist baclofen into the VTA. Likewise, consumption induced by baclofen infusion into the VTA is blocked by antagonist administration into the NAC, indicating a bidirectional $GABA_B$ interaction between these locations. Moreover, $GABA_A$ antagonism of the VTA blocked NAC $GABA_B$ agonist induced feeding, but NAC $GABA_A$ antagonism failed to block VTA $GABA_B$ elicited consumption, indicating a unidirectional $GABA_A$/ $GABA_B$ interaction among these sites (Miner et al., 2010). Furthermore, opioid systems are believed to

play a crucial role in GABAnergic synaptic actions, such that NAC or VTA Baclofen treatment induced eating is differentially augmented by general and receptor specific opioid antagonists (Khaimova et al., 2004; Miner et al., 2012). Additionally, feeding elicited by central NAC $GABA_A$ agonist injections is attenuated by NMDA receptor agonists in the LH, and increased consumption due to AMPA antagonism of the NAC is reduced by administration of $GABA_A$ LH agonisim (Stanley et al., 2011). These data further suggest that GABAnergic effects on eating behavior are mediated by the actions of other neurotransmitters, with much research favoring substantial opioid system involvement. Future studies are necessary to examine GABA/opioid and GABA/DA interactions in CFP.

Glutamate: Glutamate has long been implicated as a neurotransmitter essential for the synaptic plasticity mechanisms believed to underlie learning and memory. Glutamate transmission within neural areas, including the VTA, NAC, AMY and mPFC is critical for reward-related learning, presumably due to interactions with DA transmission within these key sites (Kelley, 2004; Ranaldi et al., 2011). AP-5, a competitive NMDA antagonist, impairs acquisition but not the expression of operant conditioning in rats when injected directly into the VTA (Zellner et al., 2009), and is associated with reductions in NAC core c-fos expression in response to food-associated conditioned stimuli, when compared to animals treated with vehicle (Ranaldi et al., 2011). The involvement of glutamate signaling in flavor preference learning is suggested by a study investigating the effects of systemic administration of the non-competitive NMDA receptor antagonist, MK-801, on F-F conditioning by fructose. Comparable to direct VTA administration of AP-5 in operant reward paradigms, MK-801 blocked the acquisition but not the expression of fructose-based CFP and substantially reduced intake of the CS+ flavor during training (Golden and Houpt, 2007).

Moreover, in food restricted rats increased AMPA receptor insertion is observed in the NAC, a neurobiological process that also occurs when food restricted and non-food restricted rats are allotted a 10% sucrose solution. This change in the abundance of AMPA receptors and glutametergic transmission may be important in the mediation of synaptic plasticity, leading to increased regulation of the receptor (Peng, Ziff, and Carr, 2011), and thus modified behavioral responsiveness to reward stimuli. DA and glutamate may work together to modify gene expression and subsequent neuronal responsiveness and behavioral actions. In food restricted rats there may be an upregulation of DA1 receptor function in the NAC. DA1 agonisim with SKF-82958 during acute challenge produced greater phosphorylation of nuclear transcription factors including ERK ½ MAP kinase, factors related to CREB activation and subsequent gene transcription. Additionally food restricted rats exhibited increased phosphorylation of CaMKII and the NMDA NR1 subunit. This DA1 agonist effect on these nuclear transcription factors was found to be dependent on Glutamatergic NMDA receptor transmission, with MK-801 infusion blocking the intercellular phosphorylation that leads to gene transcription caused by DA agonisim (Carr, 2012). These data suggest that within the NAC NMDA receptor function may be mediated by DA1 activity. Thus, both the findings of Carr and Ranaldi, respectively, indicate that dopaminergic receptor actions function, in part, to regulate Glutamatergic actions and by doing so influence the downstream neuronal neural intracellular mechanisms' that allow for synaptic plasticity and learning.

5. DA Effects on Flavor-Flavor and Flavor-Nutrient CFP

Initial studies implicating DA involvement in both F-F and F-N induced CFP included the finding that intake of a bitter SOA solution stimulated NAC DA efflux in rats previously trained to prefer the SOA solution through repeated pairings with IG nutrient infusions (Mark et

al., 1994). Similarly, in sucrose-CFP studies, flavors associated with systemic injection of saline were significantly more preferred then flavors paired with the DA D2 receptor antagonist, raclopride (Hsiao and Smith, 1995). Our laboratories investigated the role of DA transmission on the acquisition and expression of F-N CFP (Azzara et al., 2001). In F-N acquisition studies with food-restricted rats receiving either saline, SCH23390 (200 nmol/kg), or raclopride (200 or 400 nmol/kg), respectively, prior to daily one-bottle training sessions with CS+ and a CS- solutions subsequent drug-free CS+ vs. CS- choice tests revealed significant preferences in the control , yoked-control, and DA D2 training groups, whereas DA D1-trained rats failed to consume more CS+ than CS-, suggesting an elimination the preference (50%). In evaluating DA D1 and D2 antagonist effects upon expression of F-N conditioning, SCH23390 (200 nmol/kg) and raclopride (200 or 400 nmol/kg) significantly reduced CS+ intake, but did not block the CS+ preference. The CS+ preference was only eliminated at higher drug doses which also greatly suppressed (by 60-90%) overall fluid intakes, suggesting that DA D1, but not D2 receptors are critically involved in the acquisition of a nutrient-CFP.

In F-F studies, two distinct paradigms were employed to reduce post-ingestive nutritive effects; sham-feeding with sucrose and real–feeding with fructose. Control, DA D1-trained (SCH23390: 200 nmol/kg, sc), DA D2-trained (raclopride (200 nmol/kg, sc) and yoked-control groups received training under sham drinking with a CS+ sucrose (16%) solution and a CS- saccharin (0.2%) solution (Yue et al., 2000a, 2000b). In drug-free CS+ vs. CS- choice tests, all groups significantly preferred the CS+ flavor, although the preference was stronger in the control group (80%) than in the DA D1 (66%), DA D2 (69%), and yoked-control (72%) groups. Drug effects on the expression of the conditioned CS+ preference were examined by treating the rats with saline, raclopride, or SCH23390 prior to the CS+ vs. CS- choice tests. Both DA

antagonists' selectively reduced CS+ intake and either attenuated or abolished the CS+ preference at doses ranging from 50 to 800 nmol/kg. These initial data suggested that DA antagonism is more effective in inhibiting the expression, relative to acquisition, of sucrose-CFP in sham-feeding rats. Because sham-feeding rats consumed considerably more CS+ sucrose than CS-saccharin, the modest preference displayed by the DA D1 and D2 groups may have represented, in part, an increased familiarity with the CS+ flavor rather than a preference shift based on its association with the sweet taste of sucrose. Consequently, our laboratories (Baker et al., 2003) then examined F-F fructose conditioning in which CS+/fructose and CS-/saccharin intakes were equated during training. In acquisition, control, DA D1-trained (SCH23390; 200 nmol/kg), D2- trained (raclopride: 200 nmol/kg) and additional yoked-control groups received one-bottle training with CS+/fructose (8%) and CS-/saccharin (0.2%) solutions. In the drug-free CS+ vs. CS- choice tests with saccharin (0.2%), the control and yoked-control groups displayed preferences for the CS+ solution, whereas neither the DA D1-trained nor D2-trained groups significantly preferred the CS+ to the CS-. In assessing drug effects on the expression of the fructose-CFP, the CS+ preferences were eliminated by SCH23390 (to 39%-55%), and significantly reduced to a lesser degree by raclopride (to 57%-74%), thereby indicating that both DA D1 and D2 receptors are involved in fructose-CFP, and that expression of fructose-CFP is more dependent upon DA D1 receptor activity.

6. Punitive Sites of Action in the DA Modulation of CFP

The neural actions that mediate sensory aspects of taste, food reward, and learning require activation of pathways that include both sub-cortical (i.e., brainstem, NAC, AMY, ventral tegmental area (VTA)), and more complex cortical (i.e., medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC)) structures (Berridge et al, 2009). The limbic and thalamo-

cortico pathways are believed to be involved in feeding behavior, with both emanating from the nucleus of the solitary tract (NTS) of the brainstem and bifurcating to individual neural paths (Berridge et al, 2009). Taste sensations are coded by the thalamo-cortical path. Information originating from receptors on the surface of the tongue, palate, and pharynx reach the NTS in the medulla via the facial, glossopharyngeal, and vagus nerves. From the NTS information is passed to the to the gustatory cortex (GC), then ascends through the parabrachial nucleus (PBN) of the pons and the ventro-postero-medial nucleus (VPM) of the thalamus onto cortical structures, including prefrontal structures like the OFC (Berridge et al., 2009; Menella, 2008). A second pathway also emerges from the NTS which is believed to encode food reward, in which information is sent to limbic forebrain structures including the hypothalamus, substantia innominata, and AMY (Berridge et al., 2009). At a rudimentary level, the involvement of these pathways in eating behavior is well-established, however the exact interactions (structural and neurochemical) among both the nuclei within individual paths and the greater neural network mediating food related actions remains vague. The following section will focus on DA receptor actions within the NAC, AMY and, mPFC in relation to conditioned flavor learning and related phenomena.

Nucleus Accumbens (NAC): The NAC receives direct DA input from the VTA via the meso-limbic DA circuit (see review: Swanson, 1982), and is considered to be a crucial structure in the DA mediation of natural and non-naturally occurring reward as well as reward related learning (Rotiman et al., 2004; Hajnal et al., 2004). Microdialysis studies have observed increased extracellular NAC DA in response to systemic as well as direct intra-cerebral amphetamine administration (Pothos et al., 1995; Vezina, 1993). Likewise, increased DA release has been observed after direct NAC self-stimulation; a behavior significantly reduced

following site specific DA D1 antagonist infusion (Cheer et al., 2007). In addition to drug or self-stimulation enhancement of NAC DA, there are a myriad of studies that implicate NAC DA in food reward and learning. Palatable foods, particularly sweet food or liquids, promote increased NAC DA efflux (Bassareo and Di Chiara, 1997; Bassareo and Di Chiara, 1999; Genn et al., 2004; Pothos et al., 1995), with parallel effects observed after IG glucose infusion (De Araujo et al., 2008; Ren et al., 2009). In a study performed by Hajnal et al. 2004, it was found that increased NAC DA efflux in response to varying sucrose concentrations occurred in a dose-dependent fashion. Furthermore, flavors previously paired with IG carbohydrate infusions (CS+) is related to a corresponding increase in DA NAC efflux, an effect that does not occur in response to IG water infusion paired CS- flavor (Mark et al., 1994).

Direct NAC infusions of DA antagonists have further elucidated the role of DA transmission in the acquisition and expression of F-F- and F-N-induced CFP. In F-N studies, acquisition of flavor preference is significantly impaired relative to vehicle-treated controls, following DA D1 (SCH23390, 12 nmol) antagonism of the NAC core or shell. However, impaired expression of F-N preferences was only exhibited at doses that greatly suppressed overall intake (Touzani et al., 2008). The elimination of the acquisition of IG glucose-induced CFP following NAC SCH23390 was quite similar to the elimination of IG sucrose-CFP following systemic DA D1 antagonism (Azzara et al., 2001). Surprisingly, DA1-like and D2-like NAC receptor antagonism at the same 12 nmol dose utilized in F-N paradigms, failed to prevent the acquisition of conditioned F-F preferences, but subsequently facilitated extinction of the preference. Expression of fructose-CFP was significantly and dose-dependently (12-48 nmol) reduced by both D1 (SCH23390) and D2 (raclopride) antagonists administered into the NAC shell (Bernal et al., 2008), though not to the same extent as the elimination of the expression of

fructose-CFP following systemic SCH23390 or raclopride (Baker et al., 2003). Thus, the NAC appears to be implicated in the DA modulation of F-F-CFP, but does not seem to be the primary structure mediating this response.

Amygdala: The AMY has long been implicated as a sight of action in conditioned flavor learning, including the acquisition of a flavor aversion (Gallo and Bures, 1991) as well as flavor preference learning (Gilbert et al., 2003; Touzani and Sclafani, 2005). Particularly, the AMY DA system seems to be substantially involved in the distributed brain network mediating CFP. Increased AMY DA efflux occurs in response to feeding and IG infusions of nutrient-rich substances (Heffner et al., 1980; Hajnal and Lenard, 1997), as well as to Pavlovian and instrumental reward learning (Baxter and Murray, 2002; Cardinal et al., 2002; Harmer and Phillips, 1999). Central injection of amphetamine into the AMY increases DA efflux and promotes learning of a CS related to food reward (Hitchcott et al., 1997). Additionally, intracerebral DA D1-like receptor antagonist SCH23390 injection into the basolateral or central nucleus of the AMY dose-dependently impaired instrumental learning for sucrose lever pressing **(**Andrzejewski et al., 2005).

Intra-cerebral injection of DA antagonists directly into the AMY has further clarified the role of AMY DA modulation of F-F and F-N induced CFP. Direct DA D1 antagonist SCH23390 infusion into the baso-lateral and central medial AMY at a 12nmol dose eliminated the acquisition of the F-N preference relative to controls, but only reduced the expression of F-N preference at doses that greatly suppressed overall intake (Touzani and Sclafani, 2009). Thus, this elimination of acquisition of F-N-CFP by DA D1 receptor antagonism mimicked this same antagonist's central effects in the NAC (Touzani et al., 2008) and its systemic effects (Azzara et al., 2001). This lent credence to the hypothesis that DA was modulating its effects upon F/N-CFP

through mediation of a distributed brain network (see review: Touzani et al., 2010). Again, a more complex picture emerged for central DA mediation of F-F-mediated fructose-induced CFP. The expression of fructose-CFP was significantly and dose-dependently (12-48 nmol) reduced by both D1 (SCH23390) and D2 (raclopride) antagonists administered the AMY (Bernal et al., 2009). However, the 12 nmol dose of SCH23390 that blocked acquisition of F-N-CFP did not significantly affect the acquisition of fructose CFP, but subsequently facilitated extinction of this response. Accordingly, the DA D2 receptor antagonist administered into the AMY at this same 12 nmol dose failed to affect the acquisition of fructose-CFP. Thus, like the NAC, the AMY is implicated in the DA modulation of F-F-CFP, but does not appear to be the primary structure mediating this response.

Medial Prefrontal Cortex: The mPFC is believed to be involved in higher-order complex behaviors, including reward experience, learning, and memory integration including the encoding of sensory stimuli (DeCoteau et al., 1997; Okamoto et al 2006a; Okamoto et al., 2006b). It has long been known that the mPFC is involved in reward as demonstrated by the speedy momentum at which rats learn to lever press for mPFC electrode self-simulation. Electrode self-stimulation studies performed in various areas of the prefrontal cortex have demonstrated that the mPFC is dissociable from adjacent cortical areas, with mPFC reward processing utilizing an organizationally distinct neural path than processing in other prefrontal divisions (Robertson et al., 1982; Robertson, 1989). mPFC involvement in learning and memory is believed to be modulated by a unilateral synaptic connection from the CA1 area of the hippocampus to the mPFC. This anatomical circuit was observed after microinjection of wheat germ agglutinin-horseradish peroxidase and verified by antidromic stimulation (Ferino et al., 1987). This modifiable connection is believed to modulate long-term potentiation and long-term

depression of synaptic firing, with both processes implicated in the phenomena of learning, including memory formation and consolidation (Laroche et al., 2000). The mPFC of rats has also been shown to serve a critical role in tasks requiring the maintenance of information over time (Vertes, 2006), including the integration of complex conditioned behaviors as evidenced by lesions placed in the mPFC facilitating the acquisition of conditioned responses on an autoshaping task, but inhibiting the acquisition of operant delayed spatial alternation tasks (Haaren et al., 1988). Furthermore, the mPFC has been shown to be related to food consumption and conditioned food behavior. Lesions placed in the mPFC, but not the posterior or lateral orbitofrontal cortex, impair food consumption in response to conditioned cues (Holland and Petrovich, 2005; Petrovich et al., 2007), and 6-hydroxydopamine lesions or knife cuts of the mPFC impair taste aversion learning (Hernandi et al., 2000; Schalomon et al., 1994). DA innervation of the mPFC has been implicated in conditioned eating behavior in that mPFC DA release increases when food-deprived rats are presented with food stimuli, (Feenstra and Botterblom, 1996) and by feeding and food-related cues in Pavlovian and instrumental learning tasks (Bassareo et al., 2002; D'Angio and Scatton, 1989; Hernandez and Hoebel, 1990; Izaki et al., 1999). Moreover, mPFC microinfusions of SCH23390 alone or combined with AP-5 impair the acquisition of sucrose-reinforced instrumental lever pressing (Baldwin et al., 2002). In F-N CFP studies, DA D1 antagonism in the mPFC at a dose of 12 nmol eliminated the acquisition a flavor preference conditioned by IG glucose, yet produced non-significant dose-dependent reductions in CS+ intake, without blocking overall CS+ preference (Touzani et al., 2010). Thus again, elimination of F-N- CFP acquisition by DA D1 receptor antagonism mimicked this same antagonist's central effects in the NAC (Touzani et al., 2008), in the AMY (Touzani and Sclafani., 2009) and its systemic effects (Azzara et al., 2001). This lent even further credence to

the hypothesis that DA has modulating effects upon F-N-CFP through mediation of a distributed brain network (see review: Sclafani et al., 2011).

Functional Anatomical Pathways of a Distributed Brain Network of DA Projection Sites: The mesocorticolimbic DA system has been implicated in the experience of reward as well as the modulation of reward related learning (Beninger and Miller, 1998; Cardinal et al., 2002; Koya et al., 2005). The mPFC is included in a network that is intimately and reciprocally connected with the NAC shell and AMY (Brog et al., 1993; Groenewegen et al., 1999; McDonald, 1991; McGeorge and Faull, 1989; Pinto and Sesack, 2002; Wright and Groenewegen, 1995; Wright et al., 1996), and like the NAC shell and AMY, the mPFC receives mesocorticolimbic DA from the VTA (Swanson, 1982). Anatomical studies show that the mPFC projects to the NAC and the AMY (Gabbot et al., 2006), with reciprocal connections to both areas. Furthermore, the mPFC has reciprocal connections with the Lateral Hypothalamus (LH), a neural site long implicated in the mediation of feeding behaviors, as well as the hippocampus, a location vital to the formation of memory (Ferino et al., 1987).

Pharmaceutical, electrophysiological, and behavioral studies have further documented neural circuits linking the mPFC to the NAC and AMY. Electrophysiological and behavioral studies have demonstrated activation of the AMY, mPFC, and NAC in response to reward-related tasks, including reward cue and seeking (Ishikawa et al., 2008). Activation of the mPFC and the NAC occurs in response to electrical stimulation of the AMY as well as to Pavlovian conditioned odors (McGinty and Grace, 2009). The NAC receives excitatory input from both the mPFC and AMY as evidenced by mPFC or AMY NMDA microinjection eliciting increased NAC neuronal action (McGinty and Grace, 2009). In turn, the NAC modulates mPFC activity. Pharmacological manipulations of the DA system in conjunction with in vivo

electrophysiological recording demonstrate that NAC DA receptor activation (tonic and phasic) modulates hippocampal and prefrontal cortical (including the mPFC) activity via both DA D1 and D2-like receptor action (Gotto and Grace, 2008). Furthermore, inactivation of the basolateral AMY via direct infusion of a $GABA_A$/$GABA_B$ agonist cocktail impairs conditioned cue-responding for sucrose pellet reward, results similar to those found with NAC DA antagonism via site specific microinjection. GABA blockade of the mPFC (dorsal and ventral portions) inhibits cue-induced reward seeking and induces disinhibition in responding to cues not previously related to the obtainment of reward; effects similar to GABA induced inactivation of the NAC. These data demonstrate the possibility of conjoined AMY and mPFC activity in the facilitation of cue-evoked reward-seeking behaviors (Ishikawa et al., 2008).

The interconnected nature of the mPFC, NAC, and AMY is further demonstrated by site-specific electrical stimulation studies. Single electrical pulse stimulation of the AMY is correlated to time-dependent mPFC induced spiking of NAC neurons, presumably due to mPFC-NAC glutamertigic connections, whereas repeated train activation of the AMY depresses mPFC to NAC communication. Interestingly, AMY-mediated depression of the mPFC was attenuated by SCH23390 blockade of D1-like receptors in the minutes after train stimulation ended (McGinty and Grace, 2009), just as long term potentiation induced by repeated tetanization of either the NAC or mPFC, neurons are modulated by DA D1-like receptors action augmentation (Besson and Louilot, 1997; Huang and Hsaio, 2002; Loretan et al., 2004; Otani et al., 2003; Schotanus & Chergui, 2008). Thus the mPFC, like the NAC and AMY, receives DA projections from the VTA and NAC, as well as glutamatergic and GABAnergic input from the AMY. In turn, the NAC receives major glutamatergic inputs from the mPFC, orbito-frontal cortex and AMY (Brog et al., 1993; McGeorge & Faull 1989; Touzani et al., 2007) and DA innervation

from the VTA (Swanson, 1982). Finally, the AMY receives both DA and glutamergic input from the mPFC and NAC, respectively. Thus, as proposed by Wickens (1993) and Beninger (1993), it is possible that DA actions in the AMY and NAC induced by nutrients or nutrient-associated cues act on DA D1-like receptors to promote preference learning by altering the effectiveness of activated glutamatergic synapses in these structures. Given the intimate anatomical and chemical connection between these structures, these relationships can be applied to the highly glutamatergic mPFC. Collectively, data collected from the experimental procedures discussed in this section implicate the mPFC as a brain region at which information received from prime neurological structures in the mediation of reward and food intake is integrated as an experience and transformed into memory. Thus, long considered a region of higher brain function, the role of the mPFC in eating behaviors is important to evaluate, especially when attempting to extend results of animal research to human populations.

7. Rationale and Specific Aims for the Present Study

NAC, AMY, and mPFC as Sites of Action: Our laboratories (Touzani et al., 2008, 2009) have recently evaluated the roles of the NAC and AMY in DA D1 antagonist-induced alterations in IG glucose F-N CFP. Similar to the ability of systemic DA D1 antagonism to eliminate an IG sucrose F-N CFP (Azzara et al., 2001), intracerebral administration of SCH23390 (12 nmol) into the NAC core (55%) or shell (61%) during acquisition significantly reduced F-N preferences relative to vehicle-treated controls (83-89%) and, SCH23390 administered directly into the NAC shell reduced the expression of F-N preferences, but only at doses that greatly suppressed overall intake (Touzani et al., 2008). Effects comparable to DA D1 systemic and NAC direct administration were observed with direct DA1 antagonist SCH23390 infusion into the baso-lateral and central medial AMY, such that the DA D1 antagonist (12 nmol, 55%) eliminated the

acquisition of the F-N preference relative to controls (82%), but only reduced the expression of F-N preference at doses that greatly suppressed overall intake. Thus, AMY sub-nuclei as well as the NAC core and shell appear to critically participate in the ability for DA signaling to mediate the acquisition and secondarily the expression of F-N preferences conditioned by IG sucrose and glucose.

Our laboratories (Bernal et al., 2008, 2009) also recently examined the roles of the NAC shell and AMY in DA D1 and D2 antagonist-induced alterations in F-F learning as measured by fructose-CFP. The expression of fructose-CFP was significantly and dose-dependently (12-48 nmol) reduced (62-63%) by both D1 (SCH23390) and D2 (raclopride) antagonists administered into either the NAC shell or AMY. Comparisons of untreated and yoked control rats with rats receiving SCH23390 or raclopride at the low 12 nmol dose into either the NAC shell (Bernal et al., 2008) or AMY (Bernal et al., 2009) during training revealed comparable magnitudes of fructose-CFP during the first two days of two-bottle choice testing. In contrast, the two control groups continued to display stable preferences over the next four days as observed previously (Ackroff & Sclafani, 2004). The DA D1-trained, and to a lesser degree, the DA D2-trained rats exhibited systematic and significant declines in the preference, suggestive of hastened preference extinction following DA D1 antagonist treatments in the NAC and AMY. However, these effects were less pronounced when compared to the elimination of fructose-CFP following systemic DA D1 and D2 antagonists (Baker et al., 2003). These data suggest that the AMY and NAC are vital to the expression of fructose induced CFP. Although it seems that the AMY and NAC may be less involved in acquisition, the hastened extinction observed following DA D1 and DA D2 administration suggest AMY- and NAC- induced reductions in the acquisition of fructose CFP may be more pronounced with more dramatic DA antagonism. Therefore, one of the main

objectives of this dissertation is to explore the effects of bilateral injections of Raclopride or SCH23390 administered into the NAC or AMY, at a higher 24nmol (12nmol per side), respectively.

As fully described in preceding sections, the mPFC is a neural site of interest in the mediation of feeding behaviors. Inquiries into the role of mPFC DA systems upon fructose induced F-F CFP is warranted due to this region's intimate and reciprocal connections with the AMY and NAC, as well as other regions of interest in the mediation of feeding behaviors. These neuroanatomical and neurochemical connections suggest that the mPFC may be part of the cooperative regional network of nuclei that mediate this type of learning. Therefore, to further our understanding of the greater neural network in DA CFP mediation, it is imperative to investigate the effect of DA D1 and D2 antagonism on the acquisition and expression of fructose-induced CFP.

Acquisition and Expression of CFP: F-F 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 display and maintain any learned preference. When evaluating the pharmacological effects of DA antagonists on the expression of CFP, rats will be trained to drink different training solutions in a series of 30 minute one-bottle daily sessions. Then, learned preferences will be assessed in a series of two-bottle choice tests preceded by central microinjections of either vehicle or drug. On the other hand, when evaluating the pharmacological effects of DA antagonists on the acquisition of CFP, rats will first be administered either vehicle or drug microinjections 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 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.

In acquisition studies animals are exposed to 60 minute training and testing sessions, whereas in expression studies animals are exposed to 30 minute training and testing conditions. This time difference in exposure is utilized for several purposes. Firstly, in the event that the antagonist causes drastic intake reductions in acquisition paradigms animals are allotted more opportunity to come into contact with the solutions. Secondly, Baker et al, found that drug effects are similar from 30 minutes to 2 hours, therefore the 60 minute exposure time is within the appropriate window. Finally, the 60 minute session time is consistent with previous Bernal 2008 and 2008 studies evaluating the dopaminergic mediation of the acquisition of fructose induced CFP in the AMY and NAC.

Specific Aims and Hypotheses:

Specific Aim 1: Dopamine Receptor Antagonism in the Nucleus Accumbens and Acquisition of Fructose Conditioned Flavor Preferences: Acquisition of IG Glucose induced CFP was eliminated by a 12nmol dose of SCH23390 (Touzani et al., 2008). In contrast, acquisition of fructose-CFP was unaffected by the same 12nmol dose of SCH23390, but DA D1 and D2 antagonists hastened extinction of these responses (Bernal et al., 2008). It is conceivable that a higher SCH23390 or Raclopride dose administered into the NAC would alter the

acquisition of Fructose-CFP. Specific Aim 1 will evaluate the effect of a 24nmol dose of SCH23390 or Raclopride on the acquisition of fructose-CFP.

Hypothesized Outcome: It is expected that a higher dose of SCH23390 or Raclopride infused into the NAC will result in greater suppression of the CS+ intake during 2 bottle choice tests, blocking the acquisition of fructose induced CFP.

Specific Aim 2: Dopamine Receptor Antagonism in the Amygdala and the Acquisition of Fructose Conditioned Flavor Preferences: Acquisition of IG Glucose induced CFP was eliminated by a 12nmol dose of SCH23390 (Touzani et al., 2007). In contrast, acquisition of fructose-CFP was unaffected by the same 12nmol dose of SCH23390, but DA D1and D2 antagonists hastened extinction of these responses (Bernal et al., 2009). Specific Aim 2 will evaluate the effect of centralized infusions of a 24nmol dose of SCH23390 or Raclopride into the AMY on the acquisition of fructose-CFP.

Hypothesized Outcome: It is expected that a higher dose of SCH23390 or Raclopride infused into the AMY will result in greater suppression of the CS+ intake during 2 bottle choice tests, blocking acquisition of fructose induced CFP.

Experiment One: Experiment 1 was designed to analyze the effect of bilateral DA D1 or D2 antagonisms of the NAC (1A) or the AMY (1B) at a 24nmol dose on the acquisition of fructose conditioned F-F preferences in rats.

Specific Aim 3: Dopamine Receptor Antagonism in the Medial Prefrontal Cortex and Acquisition of Fructose Conditioned Flavor Preferences: Considering the evidence supporting the importance of mPFC DA in food intake and leaning the following two experiments will evaluate whether bilateral mPFC DA receptor antagonism with D1 SCH23390 or D2 Raclopride

will alter the acquisition of fructose induced CFP. As stated above, 12 nmol infusions of SCH23390 into the NAC or AMY effectively eliminated the acquisition of IG glucose CFP (Touzani et al., 2008, 2009) and in F-F studies both SCH23390, and to a lesser degree Raclopride, administered into either site resulted in a hastened extinction of an already acquired preference (Bernal et al., 2008, 2009). SCH23390 administered into the mPFC also eliminated IG Glucose-CFP (Touzani et al., 2010). Thus, corresponding with Specific Aims 1 and 2, Specific Aim 3 will focus on analyzing the effect of centralized infusions of a 24nmol dose of SCH23390 or Raclopride into the mPFC on the acquisition of fructose-CFP.

Hypothesized Outcome: It is expected that bilateral mPFC microinfusions of DA D1 or D2 antagonists, SCH23390 or Raclopride respectively, will block the acquisition of fructose induced F-F preferences in rats.

Experiment Two: Experiment 2 was designed to analyze the effect of bilateral DA D1 or D2 antagonisms of the mPFC at a 24nmol dose on the acquisition of fructose CFP in rats.

Specific Aim 4: Dopamine Receptor Antagonism in the Medial Prefrontal Cortex and Expression of Fructose Conditioned Flavor Preferences: DA receptor involvement in CFP varies in orosensory (F-F: DA D1 and D2 receptors) and post-ingestive (F-N: Da D1 receptor) involvement, as well as has differential effects upon acquisition (F-F and F-N) and expression (F-F) of these responses (Azzaria et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). Consistent with these systemic results, DA D1 antagonism in the NAC, AMY, and mPFC eliminated the acquisition, but minimally effected the expression of IG Glucose-CFP (Touzani et al., 2008, 2009, 2010). Whereas systemic DA D1 and D2 antagonists eliminated the expression of fructose-CFP, the same antagonists administered in the NAC (Bernal et al., 2008) or AMY

(Bernal et al., 2009) only significantly reduced, but did not eliminate, this response. To assess whether the mPFC is involved in the maintenance of this response, the last Specific Aim examined whether bilateral mPFC DA receptor antagonism with D1 SCH23390 or D2 Raclopride would dose-dependently reduce the expression of fructose induced CFP.

Hypothesized Outcome: It is expected that SCH23390 or Raclopride bilaterally injected into the mPFC will block the expression of fructose induced F-F preferences in rats.

Experiment Three: Experiment 3 was designed to analyze the effect of bilateral DA D1 or D2 antagonism of the mPFC on the expression of fructose conditioned F-F preferences in rats.

CHAPTER 2: GENERAL METHODS

Subjects: Adult male Sprague Dawley rats (250-300 g; Charles River Laboratories, Wilmington, MA) were individually housed in wire mesh cages at the Queens College vivarium. The vivarium was maintained at 21^{o}C and a 12:12 h light-dark cycle was utilized (lights on at 0800 h). Animals were provided with standard chow (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) and tap water ad libitum except where noted. The Queens College Institutional Animal Care and Use Committee approved all experimental protocol certifying compliance of all animals and procedures with the standards of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Surgery: Prior to surgery, all animals were pretreated with chlorpromazine (3 mg/kg, i.p.) and anesthetized with Ketamine HCl (120 mg/kg, i.m.). Animals were bilaterally implanted with chronic indwelling guide-cannulae (26-gauge, Plastics One, Inc., Roanoke VA) stereotaxically aimed (Kopf Instruments) at the NAC (Experiment 1), AMY (Experiment 1) or mPFC (Experiments 2 and 3) utilizing the following coordinates: NAC placements: incisor bar, +5 mm, 3.1 mm anterior to the bregma suture, 1.7 mm lateral and angled 10° towards the midline of the sagittal suture, and 6.8 mm below the surface of the skull; AMY placements: incisor bar, -3.3 mm, 2.8 mm anterior to the bregma suture, 4.3 mm lateral and angled 10° towards the midline of the sagittal suture, and 8.2 mm below the surface of the skull; and mPFC placements: incisor bar, 0 mm, 3.0-3.2 mm anterior to the bregma suture, 1.5 mm lateral and angled 10° towards the midline of the sagittal suture, and 3.5 mm below the surface of the skull. The cannulae were secured on the skull with three stainless steel screws and dental cement and occluded with stainless steel stylets when not in use.

Drug Administration: Microinjections for all three experiments consisted of either the D1-selective antagonist SCH23390 (Sigma Chemical Co.) or the D2-selective antagonist Raclopride (Sigma Chemical Co.) dissolved in 0.9% normal saline, or a vehicle solution (0.9% saline). Equimolar dose curves were utilized in all central studies with bilateral test does of 24 nmol (Experiments 1 and 2) or 12, 24, and 48 nmol (Experiment 3) to directly assess the potency of DA D1 and D2 antagonist effects in each paradigm. An ascending dose order was used to test half of the animals and a descending dose order was be used to test the remaining animals in Experiment 3. Subjects were bilaterally injected through a 33-gauge stainless steel internal cannula (Plastics One) extending 1 mm beyond the target site. The internal cannula were attached to 2 µl Hamilton syringes (Hamilton Company Reno, NV, USA) via PE-20 polyethylene tubing. Infusions (0.5 µl/side) were performed over a 2 min period with injectors remaining in place for an additional minute before removal. Behavioral testing occurred 10 minutes after the start of the drug or vehicle infusions.

Test Solutions: Pre-training solutions consisting of unflavored 0.2% sodium saccharin (Sigma Chemical Co.) dissolved in tap water were presented before the onset of training to familiarize the animal with the sipper tubes for all experiments. Training solutions consisted of either a 8% fructose (Sigma Chemical Co., St. Louis, MO) and 0.2% sodium saccharin mixture or a 0.2% sodium saccharin solution, each flavored with either 0.05% of unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY). The 8% fructose + 0.2% saccharin solution is preferred to the 0.2% saccharin solution; therefore, the more preferred fructose-paired flavor is referred to as the CS+ and the saccharin-paired flavor as the CS- (Sclafani and Ackroff, 2004). Half of the rats in each group have 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. Under one bottle training conditions, the fructose + saccharin-paired flavor is referred to as the CS+/F because of the addition of 8% fructose to the flavored 0.2% saccharin solution, and the saccharin-paired flavor as the CS-/s during training to indicate the presence of saccharin in the absence of fructose (Baker et al., 2003, 2004; Sclafani and Ackroff, 2004). In two-bottle testing procedures, the CS+ and CS- flavors are referred to as CS+/s or CS-/s to indicate the presence of saccharin only. The CS+/s during testing refers to same flavor as the CS+/F during training, and the CS-/s refers to the CS-/s flavor used in both training and testing conditions. All testing was conducted 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 varied across days using a left-right-right-left pattern in half of the animals in each condition, and a right-left-left-right pattern for the remaining animals. CS+/s and CS-/s solutions were counterbalanced across sessions to control for any position effects. Solutions were administered to animals in sipper tubes mounted on the front of the cage by a taut steel spring, positioned 3-6 cm above the cage floor. Solution intakes were measured to the nearest 0.1 g.

Pre-Training: Following recovery from surgery, rats were food restricted and maintained at 85-90% of their ad lib body weight with daily food rations; food restriction increases the rat's motivation to sample the solutions during training and testing. Prior to training and testing, rats were familiarized with the bottles used to administer all training and testing solutions by being trained to drink an unflavored 0.2% saccharin solution during 1-hr sessions over consecutive days. This procedure was repeated until all rats approach the sipper tubes with short (< 1 min) latency, which usually occurred within three days of exposure to pre-training conditions (e.g.,

Baker et al., 2003; Bernal et al., 2008, 2009). One hour after each pre-training session rats were given food rations.

One-Bottle Training: Following pre-training procedures, rats were exposed to either eight (acquisition) or ten (expression) days of 30 minute one-bottle conditioning trials in which 16 ml of the CS+/F and CS-/s were presented separately, with the CS+/F presented on odd-numbered days and the CS-/s presented on even-numbered days. On the last two days of training (either days 7 and 8 in acquisition studies or days 9 and 10 for expression studies) a second sipper tube containing water was presented alongside the CS+/F or CS-/s solution. The positions of the sipper tubes containing water or the CS solution were counterbalanced in a left/right fashion over both days to reduce the development of "side preferences". Intakes during training were limited to 16ml/session to minimize CS+/F and CS-/s intakes. Solution intakes were measured to the nearest 0.1 g. before and after training sessions. Animals were allotted food rations one hour after each training session

Two Bottle Testing: Following training, animals were exposed to either six (60 min/ day)daily 2-bottle choice sessions (acquisition paradigms, Experiments 1 and 2) or 8 daily (30 min/day) 2-bottle choice sessions (expression paradigms, Experiment 3) with unlimited (50 ml) access to the CS+/s and CS-/s solutions. The positions of the CS+/s and CS-/s solutions were counterbalanced across sessions in all groups as described above. Solution intakes were measured by weighing (0.1 g) the bottles before and after the two-bottle choice sessions. Animals were allotted food rations one hour after each training session

Histological Verification: At the end of the experiment, animals were overdosed with an anesthetic (Euthasol) and injected transcardially with potassium chloride (15 mg/ml, 0.9%

saline). Transcardiac perfusions were performed with 0.9% normal saline followed by 10% buffered formalin. Coronal 40-μm sections, stained with Cresyl violet, were examined with 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. Only animals with confirmed appropriate bilateral cannulae placements were included in the data analysis.

Data Analysis: In the acquisition studies, training intakes were averaged over the four CS+/Fs and four CS-/s sessions and were analyzed with a two-way 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 ANOVA was used to compare the CS intakes of D1, D2 and control groups (Group x CS x Test). Separate two-way ANOVAs were used to evaluate total CS intakes and percent CS+/s intakes of groups. When main or interaction effects were found, Bonferroni corrected comparisons ($p<0.05$) were used to detect significant effects. 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 D1 and D2 groups, respectively. Separate ANOVAs evaluated total intakes and percent CS+/s intakes as a function of dose for the two groups. When a main or interaction effect was found, Bonferroni corrected comparisons ($p<0.05$) were applied to detected significance.

Food Restriction: In all of the studies, animals were food-restricted to 85-90% of their body weight in order to motivate them to sample the solutions with short latencies. Animals were administered food rations 1 hour after each training/ testing session.

CHAPTER 3: DOPAMINE RECEPTOR ANATGONISIM IN THE NUCLEUS ACCUMBENS AND AMYGDLA ON THE ACQUSISTION OF FRUCTOSE CONDITIONED FLAVOR PREFERENCES.

Introduction

The NAC and AMY have both been implicated as crucial structures in the dopaminergic mediation of both natural and non-naturally occurring reward (Rotiman et al., 2004; Hajnal et al., 2004). DA efflux in the NAC is stimulated by palatable foods, particularly sweet food or liquids (Bassareo & Di Chiara, 1997; Bassareo & Di Chiara, 1999; Genn et al., 2004; Pothos et al., 1995), and, in intragastric studies, the post-ingestive effects of glucose stimulate increased NAC and AMY DA levels (De Araujo et al., 2008; Hajnal & Lenard, 1997; Heffner et al., 1980; Ren et al., 2009). Although DA release occurs in both the core and shell regions of the NAC, the release from the shell region in greater than that of the core. In this experiment the NAC shell is targeted for three reasons. First, Touzani 2008 found that acquisition of IG glucose CFP is equally reduced by DA1 antagonism in either the core or shell (Touzani et al., 2008). Secondly, the NAC shell was targeted in previous Bernal (2008) studies on the effect of DA1 and DA2 antagonism on the acquisition of F-F CFP, and the current experiment was specifically designed to investigate whether a higher, 24nmol, dose of these DA antagonists would be more effective in blocking fructose induced acquisition. Finally, in a series of studies performed by Carr et al, it was found that DA increases in the NAC shell region when an animal comes into contact with a novel or unexpected reward stimuli, but that DA in the core region is maximally released once the stimuli has already been conditioned (Carr, 2000). Therefore, in this experiment it is

appropriate to examine the effects of DA antagonism in the shell region because under acquisition conditions the stimuli are novel.

The AMY DA system seems to be involved in the distributed brain network mediating CFP. AMY involvement is believed to substantially contribute to conditioned flavor learning, including both the acquisition of a flavor aversion (Gallo, 1991), as well as flavor preference learning (Gilbert et al., 2003; Touzani and Sclafani, 2005). In this experiment both the basolateral and central nuclei of the AMY were specifically targeted for three reasons. Firstly, in a 2002 AMY leasioning study performed by Touzani and Sclafani it was found that deficits in F-N induced CFP was directly related to the size of the AMY leasion, with smaller leasions having less effect on preference then larger leasions (Touzani and Sclafani, 2002). Secondly, in 2009 Touzani found that centralized DA antagonist injection into either the basolateral or central nuclei of the AMY produced small effects on preference, whereas simultaneous DA antagonism of both areas produces a much larger effect on preference (Touzani et al., 2009). Lastly, both the basolateral and central nuclei od the AMY were targeted in previous Bernal (2009) studies on the effect of DA1 and DA2 antagonism on the acquisition of F-F CFP, and the current experiment was specifically designed to investigate whether a higher, 24nmol, dose of these DA antagonists would be more effective in blocking fructose induced acquisition.

Recent studies (Bernal et al., 2008, 2009) examined the role of NAC and AMY DA D1 and D2 antagonist-induced alterations in F-F learning. It was found that the expression of fructose-CFP was significantly and dose-dependently (12-48 nmol) reduced, but not eliminated, by either D1 (SCH23390) or D2 (raclopride) antagonists directly administered into the NAC or AMY. These effects were less pronounced when compared to the elimination of fructose-CFP observed following systemic administration of D1 or D2 antagonists (Baker et al., 2003),

thereby suggesting these two sites participate in the expression of fructose-CFP, but do not fully mediate this response (Bernal et al, 2008, 2009). These experiments also examined the effect of a 12 nmol dose of DA D1 or D2 antagonists SCH23390 or Raclopride respectively, administered bilaterally into the NAC or AMY upon the acquisition of fructose-CFP. NAC and AMY injections of SCH or RAC at the 12 nmol dose during training did not prevent the rats from acquiring a fructose-CFP. However, the drug-treated rats, unlike vehicle controls, exhibited a hastened extinction of the preference following DA D1 antagonism in either the NAC or AMY and following DA D2 antagonism in the NAC (Bernal et al., 2008, 2009). The 12 nmol dose of SCH and RAC was used in these experiments because this particular dose of SCH23390 was effective in blocking F-N CFP when administered into the NAC and AMY (Touzani et al., 2008; Touzani and Sclafani, 2009). However, it is possible that higher doses of the antagonists infused into the NAC or AMY would block acquisition of fructose-CFP. Given that expression of fructose induced CFP was dose-dependently reduced by both SCH23390 and RAC and acquisition of IG Glucose induced CFP was effectively eliminated by the 12nmol dose of SCH23390, further investigation of NAC and AMY DA D1 and D2 antagonism on the acquisition of fructose F-F preference is warranted. Therefore, the following experiment focused on evaluating the effect of a higher 24nmol dose of SCH23390 or RAC administered into the NAC or AMY on the acquisition of fructose-CFP.

Methods

Acquisition Procedure: All groups of rats in Experiments 1 (NACs and AMY) were matched for their intakes of an unflavored 0.2% saccharin solution prior to training. All rats in both experiments were given eight 1-bottle training sessions (60 min/day) with the CS+/F solution (16 ml) presented on odd-numbered sessions, and the CS- solution (16 ml) presented on

even-numbered sessions. On days 7 and 8, 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; water intake was negligible in these training trials. A 1-day break was placed between each of the four pairs of training trials to reduce the impact of repeated microinjections. Training intakes were limited to 16 ml/session to minimize the difference between CS+/F and CS- intakes.

In Experiment 1, two groups were given bilateral microinjections of the D1 antagonist SCH (24 nmol, 12 nmol/side, Sigma Chemical Co.) into the NACs (n= 8) or AMY (n= 10), ten minutes prior to each one-bottle training session. Two other groups were given bilateral microinjections of the D2 antagonist RAC (24 nmol, 12 nmol/side, Sigma Chemical Co.) into the NACs (n= 7) or AMY (n= 9) ten minutes prior to each one-bottle training session. A fifth group (Control, n= 7) received vehicle NACs (n= 3) or AMY (n= 4) microinjections throughout 1-bottle training and their intakes of the CS+ /F and CS- solutions approximated the mean 60-min intakes of the NACs and AMY SCH and RAC groups. Following training, all five groups were given six daily 2-bottle choice sessions (60 min/day) with unlimited (50 ml) access to the CS+ and CS- solutions; no drugs were administered prior to these sessions. The positions of the CS+ and CS- solutions were counterbalanced across sessions in all groups, 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: Figures (1A and 1B) displays schematic representations (Paxinos and Watson, 2009) of bilateral NAC (Panel A, n= 18) and AMY (Panel B, n= 23) placements of animals receiving vehicle, SCH23390 or raclopride in this acquisition experiment. All of the NAC placements were found within the shell region, and there was considerable

overlap among rats receiving vehicle, SCH23390 or raclopride. Moreover, the location of these placements was consistent with the previous acquisition studies examining fructose-CFP (Bernal et al., 2008) and IG glucose-CFP (Touzani et al., 2008). All of the AMY placements were found within the baso-lateral and central nuclei of the AMY, and there was considerable overlap among rats receiving vehicle, SCH23390 or raclopride. Moreover, the location of these placements was consistent with the previous acquisition studies examining fructose-CFP (Bernal et al., 2009) and IG glucose-CFP (Touzani et al., 2009a).

Experiment 1: Effects of DA antagonism in the NAC or AMY on the acquisition of Fructose-CFP.: During 1-bottle training, overall CS+/F intake (13.3 g) significantly ($F(1, 36) = 56.99$, $p<0.0001$) exceeded CS- intake (10.3 ml) and there was a significant interaction between groups and conditions ($F(4, 36) = 3.91$, $p<0.01$), but not among groups in CS intakes. CS+/F intake significantly exceeded CS- intake in the groups receiving SCH23390 in the NAC and raclopride in the NAC or AMY (Figure 2A). In contrast, CS+/F intakes failed to differ among groups, and CS intakes failed to differ among groups.

Following training, the rats were given three pairs of two-bottle preference tests without drug treatment. Significant differences were observed among tests ($F(2, 72) = 7.41$, $p<0.001$) and approached significance for the interactions between CS and groups ($F(4, 36) = 2.47$, $p=0.062$), but not among groups, CS conditions, and the remaining interactions. The groups did not differ significantly in their overall CS intakes during the tests. Within-group comparisons revealed that CS+ intake was significantly greater than CS- intake in Tests 1-3 for the vehicle Control group (Figure 2B), the AMY SCH23390 group (Figure 2C) and the NAC SCH23390 group (Figure 2D). CS+ intake was significantly greater than CS- intake in the first and third tests in the NAC raclopride group (Figure 2F). In contrast, the AMY raclopride group failed to

consume significantly more CS+ than CS- in all three Tests (Figure 2E). The CS+ intake of the vehicle Control group was significantly higher than that of the AMY raclopride group in Test 1 (Figure 2E), whereas the CS intake of the AMY raclopride group was significantly higher than the vehicle Control group in Tests 1 and 2 (Figure 2E). Significant differences in the percent CS+ intake were observed among groups ($F(4, 36) = 3.12$, $p<0.027$) and approached significance among tests ($F(2, 72) = 2.86$, $p= 0.064$), but not for their interaction. The percent CS+ intakes were stable across the three tests in the vehicle Control (70-80%, Figure 2B), the AMY SCH23390 (60-64%, Figure 2B), the NAC SCH23390 (63-73%, Figure 2D) and the NAC raclopride (64-69%, Figure 2F) groups, and in turn failed to differ from each other. In contrast, the percent CS+ intake remained near 50% in the AMY raclopride group (45-54%, Figure 2E) which was in turn significantly lower than the vehicle Control group (Figure 2B).

Figure 1A

NAC Placements

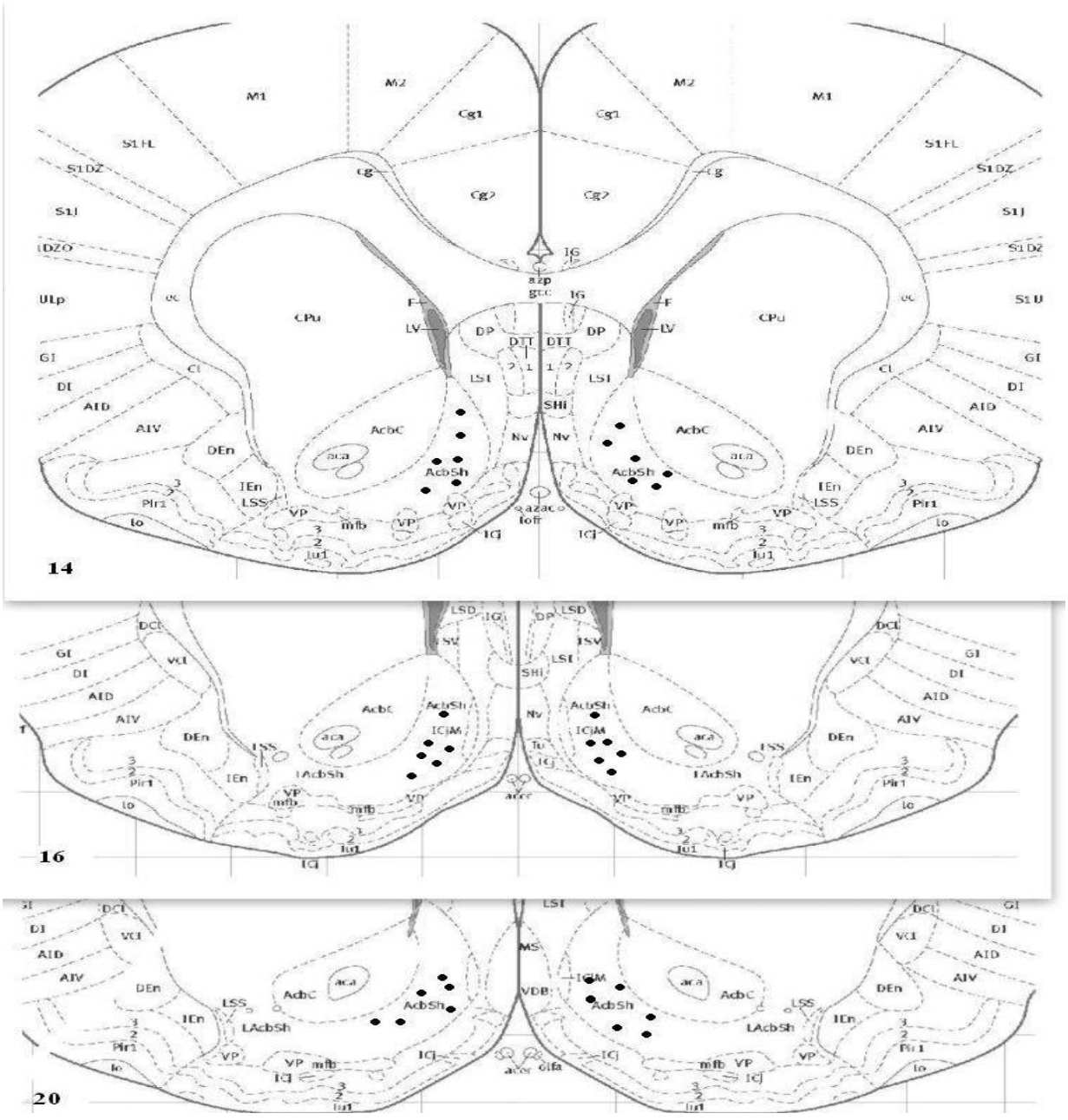


Figure 1B

AMY Placements

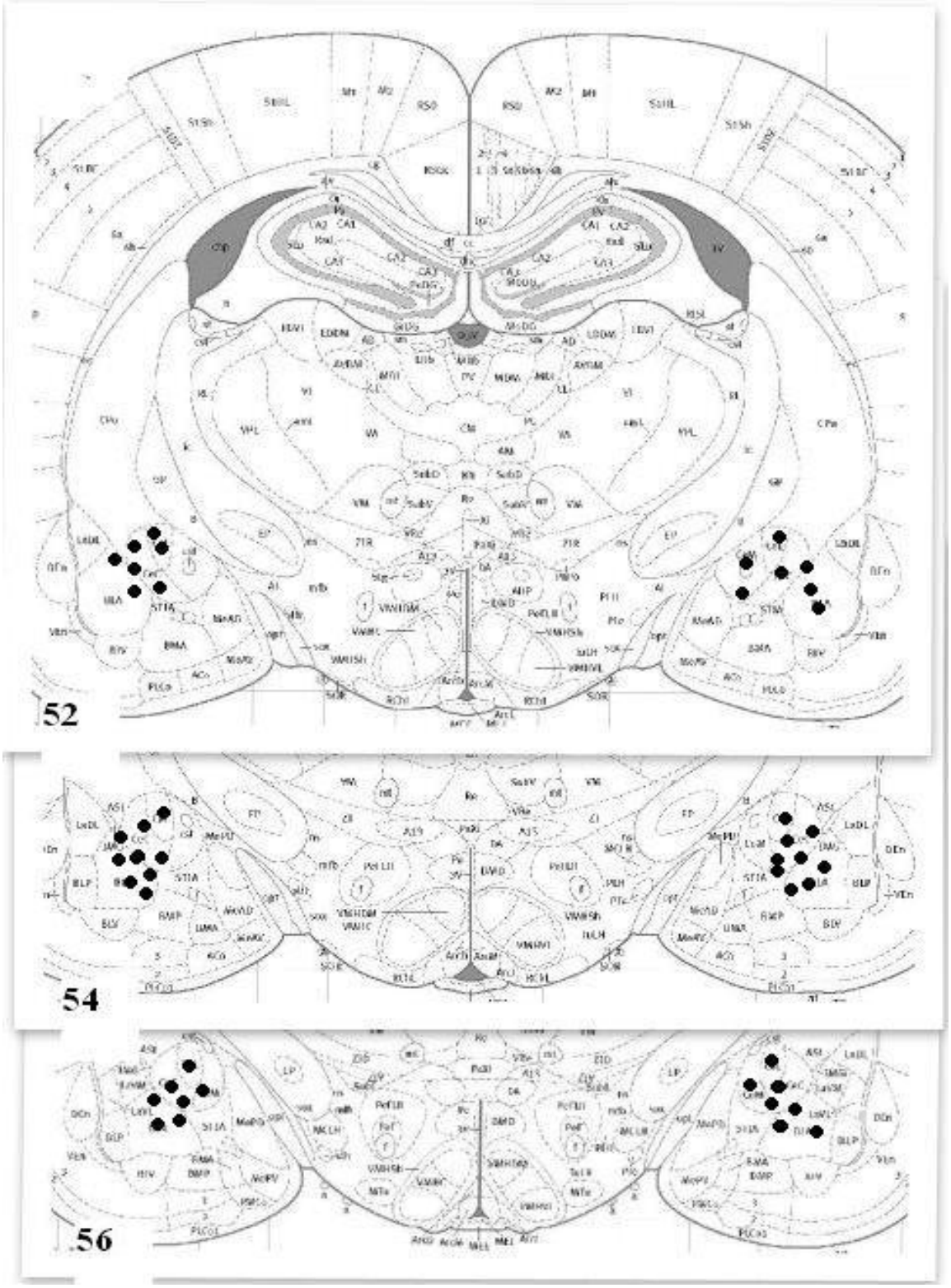


Figure 2

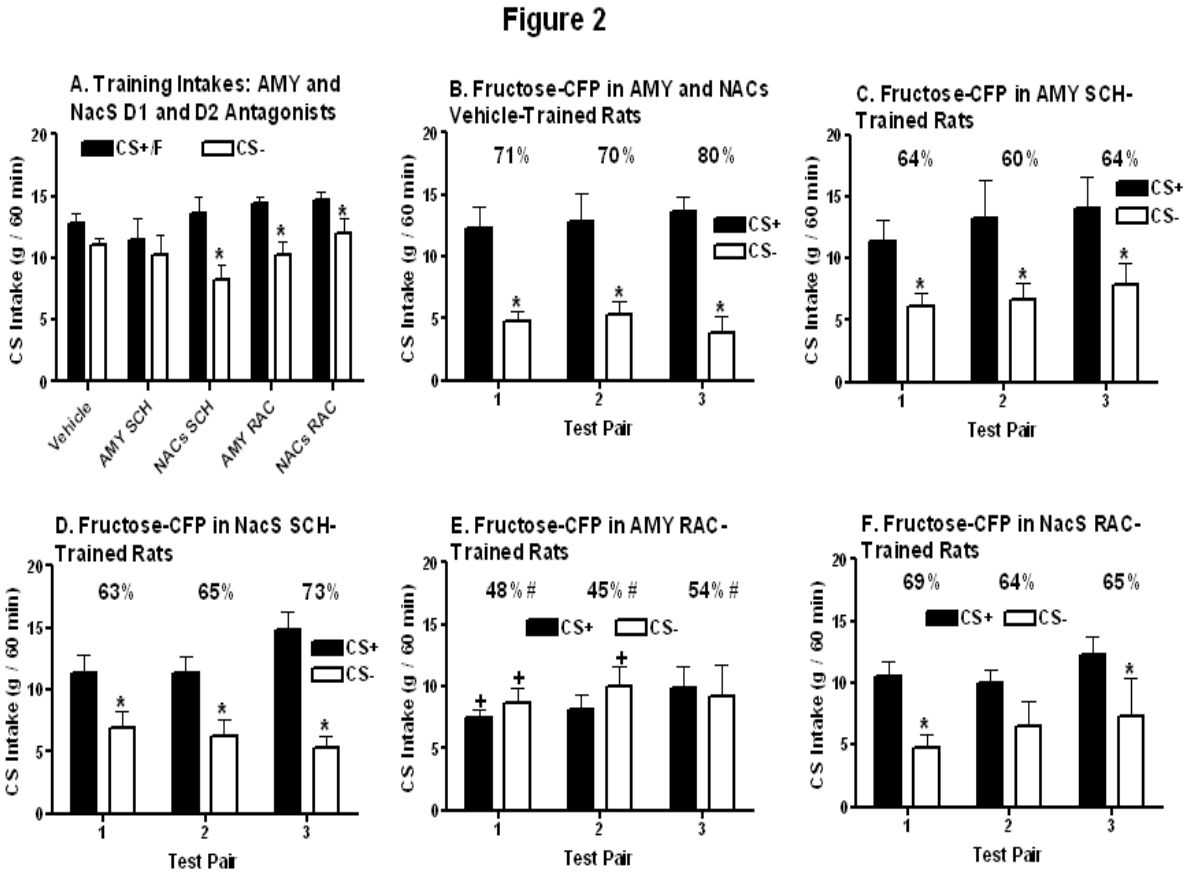


Figure 2
A. Training Intakes: AMY and NacS D1 and D2 Antagonists
CS+/F
CS-
CS Intake (g / 60 min)
Vehicle
AMY SCH
NACs SCH
AMY RAC
NACs RAC
B. Fructose-CFP in AMY and NACs Vehicle-Trained Rats
71%
70%
80%
CS+
CS-
Test Pair
C. Fructose-CFP in AMY SCH-Trained Rats
64%
60%
64%
D. Fructose-CFP in NacS SCH-Trained Rats
63%
65%
73%
E. Fructose-CFP in AMY RAC-Trained Rats
48% #
45% #
54% #
F. Fructose-CFP in NacS RAC-Trained Rats
69%
64%
65%

Discussion

Administration of the DA D2 receptor antagonist, raclopride, at a 24 nmol dose in the AMY during training totally blocked the acquisition of fructose-CFP (Figure 2E) in a manner similar to that observed following systemic raclopride treatment (Baker et al., 2003). In contrast, administration of either SCH23390, at a 24 nmol dose in either the NAC (Figure 2D) or the AMY (Figure 2C) or raclopride, at a 24 nmol dose in the NAC (Figure 2F) failed to significantly alter the magnitude of the acquisition of this preference. These data strongly suggest that DA D2 receptor signaling within the AMY is critical for the acquisition of fructose-CFP. It should be noted that the ability of raclopride in the AMY to block the acquisition of fructose-CFP occurred despite the fact that AMY raclopride rats consumed more CS+/F than CS- during training and that these intakes did not differ from those of vehicle rats (Figure 2A). It should also be noted that this effect is dose-dependent given that a lower (12 nmol) dose of raclopride administered into the AMY failed to alter the acquisition or persistence of fructose-CFP (Bernal et al., 2009).

Sugars can condition flavor preference based on both their oro-sensory (F-F; e.g., fructose-CFP) and viscerosensory (F-N; e.g., glucose-CFP) positive reinforcing signals (Sclafani, 1995). We previously demonstrated that dopamine D1-like receptor signaling within the AMY is critical for the acquisition of glucose-CFP (Touzani et al., 2009a). The results of Experiment 1 of this dissertation, however, revealed that only amygdalar D2-like receptor signaling is involved in the acquisition of fructose-CFP. Together, these findings indicate clearly that F-F and F-N preference learning are mediated by different neural processes. The D1-like receptor activation by endogenous DA released within the AMY during training in the glucose-CFP paradigm (presumably by the post-oral action of glucose) might strengthen the glutamatergic synapse on AMY neurons carrying the sensory information (flavor) promoting the acquisition of glucose-

CFP as proposed by a model of D1-like receptor interaction with glutamatergic receptors (Beninger, 1993). In the case of fructose-CFP, the sweet taste of fructose induces DA release in AMY that seems to act preferentially on D2-like receptors. The mechanism by which activation of D2-like receptors within the AMY promotes some forms of food-related learning is not presently known. It is generally admitted that D2 receptors activation induces a depressant effect on glutamatergic receptors. However, recent findings revealed DA D2 receptor excitatory mediation of glutamatergic receptors within the mPFC (Wang & Goldman-Rakic, 2004). Thus, one may speculate that during the acquisition of fructose-CFP, DA released within AMY by the sweet taste of fructose preferentially activates the D2 receptor subtypes that are present on the same neurons along with glutamatergic receptors, and this activation of D2 receptors, in turn, facilitates glutamatergic receptor transmission leading to a greater neuroplasticity and therefore promoting fructose-CFP learning.

The failure of raclopride or SCH23390 administration into the shell subdivision of the NAC to impair the acquisition of fructose-CFP is somewhat surprising given that sweet taste of sugar induces large increases of DA release in this striatal area (Hajnal & Norgren, 2001; Hajnal et al., 2004). Given that this stimulated DA release was measured in both the shell and core subdivisions, it is possible that DA receptors antagonisms in both regions would be more effective in impairing the acquisition of fructose-CFP. Further experiments are required to test this possibility.

CHAPTER 4: DOPAMINE RECEPTOR ANTAGONISIM IN THE MEDIAL PREFRONTAL CORTEX ON THE ACQUISITION OF FRUCTOSE CONDITIONED FLAVOR PREFERENCES

Introduction

Extensive theories link brain DA circuits to food reward, motivation and learning (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Smith and Berridge, 1995). Dopamine is believed to be one of the primary neurotransmitters involved in fructose induced F-F CFP, principally because sweet taste indices activation of the mesolimbic and mesocortical DA circuits, the same systems that are involved in the mediation of natural as well as drug reward (e.g., Genn et al., 2004; Hajnal et al., 2003). Consequently, DA receptor antagonism suppresses the intake of sweet solutions in rats (Geary and Smith, 1985; Xenakis and Sclafani, 1981), an effect presumably due to reductions in either hedonic evaluation (Schneider, 1989; Smith, 1995) or incentivizing properties (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone et al., 1997).

Systemic administration of DA D1 and D2 antagonists, SCH23390 and Raclopride respectively, blocked acquisition of fructose-CFP (Baker et al., 2003), although DA D1 antagonism had more robust effects than DA D2 antagonism. These data suggest that both D1 and to a lesser degree D2 receptor subtypes are vital for the acquisition of F-F CFP. To better understand the specific mechanisms by which systemic administration of DA antagonists impacts the acquisition of fructose induced CFP, it is important to locate specific neural sites of action. Currently, the central anatomical sites of action for dopaminergic modulation of fructose-conditioned F-F preferences are unknown. It is believed that a distributed brain network

mediates this type of learning (see reviews: Sclafani et al., 2011; Touzani et al., 2010); therefore several neural sites have been implicated as participating areas of interest, including the medial prefrontal cortex (mPFC). Sweet tastes increases overall DA levels in the prefrontal cortex, with DA efflux observed in response to intraoral infusion of a 20% sucrose solution (Bassareo et al., 2002). Furthermore, food consumption, odors and other related cues stimulate increased mPFC DA (Ahn and Phillips, 1999; Bassareo et al., 2002; Bassareo and Di Chiara, 1997; D'Angio and Scatton, 1989; Hernandez and Hoebel, 1990), and central microinfusions of SCH23390 alone or combined with AP-5 impair the acquisition of sucrose-reinforced instrumental lever pressing (Baldwin et al., 1997). Further, the mPFC, like the NAC and AMY, is a site at which DA D1 receptor antagonism eliminates the acquisition of CFP elicited by IG glucose (Touzani et al., 2008, 2009, 2010) in a highly-similar manner to the ability of systemic SCH23390 to eliminate the acquisition of CFP elicited by IG sucrose (Azzara et al., 2001). Taken together, these data indicate that the mPFC may be a region at which sensory information is integrated as an experience and transformed into memory. Thus, long considered a region of higher brain function, the role of the mPFC in eating behaviors is important to evaluate, especially when attempting to extend results of animal research to human populations. Considering the evidence supporting the importance of mPFC DA in food intake and learning, Experiment 2 evaluated whether bilateral mPFC DA receptor antagonism with D1 SCH23390 or D2 Raclopride will alter the acquisition of fructose-induced CFP.

Methods:

Acquisition Procedure: Rats with bilateral cannulae aimed at the mPFC were matched for their intakes of an unflavored 0.2% saccharin solution prior to training, and placed into 3 groups: D1, D2, and control groups. As in Experiment 1, all animals were then exposed to eight

1-bottle training sessions (60 min/day) with the CS+/F solution (16 ml) presented on odd-numbered sessions, and the CS-/s solution (16 ml) presented on even-numbered sessions. A 1-day break was placed between each of the first three pairs of training trials to reduce the impact of repeated microinjections. On days 7 and 8, a second sipper tube containing water was presented alongside the CS to familiarize the rats with the presence of two sipper tubes as used during the choice tests. Training intakes were limited to 16 ml/session to minimize differences between the CS+/F and CS- intakes. Two groups were given bilateral microinjections of SCH (24 nmol, n=17) or RAC (24 nmol, n=18), respectively, into the mPFC ten minutes prior to each one-bottle training session. The third group (Control, n=15) received vehicle mPFC microinjections throughout 1-bottle training, and their intakes of the CS+/F and CS- solutions approximated the mean 60-min intakes of the mPFC SCH and RAC groups. Following training, all three groups were given six daily 2-bottle choice sessions (60 min/day) with unlimited (50 ml) access to the CS+ and CS- solutions; no drugs were administered prior to these sessions. The positions of the CS+ and CS- solutions were counterbalanced across sessions in all groups, 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 (3) is a schematic representation (Paxinos and Watson, 2009) displaying the bilateral mPFC cannula placements of all 72 animals receiving vehicle, SCH23390, or raclopride in both acquisition and expression experiments. All cannulae in were located within the mPFC, and there was considerable overlap between rats receiving vehicle, SCH23390, or raclopride. Moreover, the location of these placements was consistent with the previous acquisition studies examining IG glucose-CFP (Touzani et al., 2010).

Experiment 2: Effect of bilateral DA D1 or D2 antagonisms of the mPFC on the Acquisition of Fructose-CFP: During 1-bottle training, overall CS+/F intake (14.2 g) significantly ($F(1, 47) = 87.65$, $p<0.0001$) exceeded CS- intake (11.3 ml), and there were no group differences in CS intakes (Figure 4A).

Following training, the rats were given three pairs of two-bottle preference tests without drug treatment. There were significant interactions between CS and groups ($F(2, 47) = 8.83$, $p<0.0001$), groups and tests ($F(4, 94) = 7.03$, $p<0.0001$), and CS and tests ($F(2, 94) = 23.31$, $p<0.041$), but not among groups, CS conditions, and tests. The groups did not differ significantly in their overall CS intakes during the tests. Within group comparisons revealed that the Control group (Figure 4B) consumed significantly more CS+ than CS- in Tests 1-3. In contrast, the SCH23390 and raclopride groups failed to consume significantly more CS+ than CS- in all three Tests (Figures 4C, 4D). The CS+ intake of the Control group was significantly higher than that of the SCH23390 group in Test 1 (Figure 4C), and significantly higher than that of the raclopride group in Tests 1 and 2 (Figure 4D). Analysis of the percent CS+ data revealed significantly greater percent CS+ intakes in the Control group compared to the SCH23390 and raclopride group across all three tests ($F(2,47)= 7.99$, $p<0.001$) The percent CS+ intakes were stable across the three tests in the Control group (67- 68%, Figure 4B), but remained near 50% in the SCH23390 (50-56%, Figure 4C) and raclopride (52-55%, Figure 4D) groups.

Figure 3

mPFC Placements (Acquisition and Expression Study)

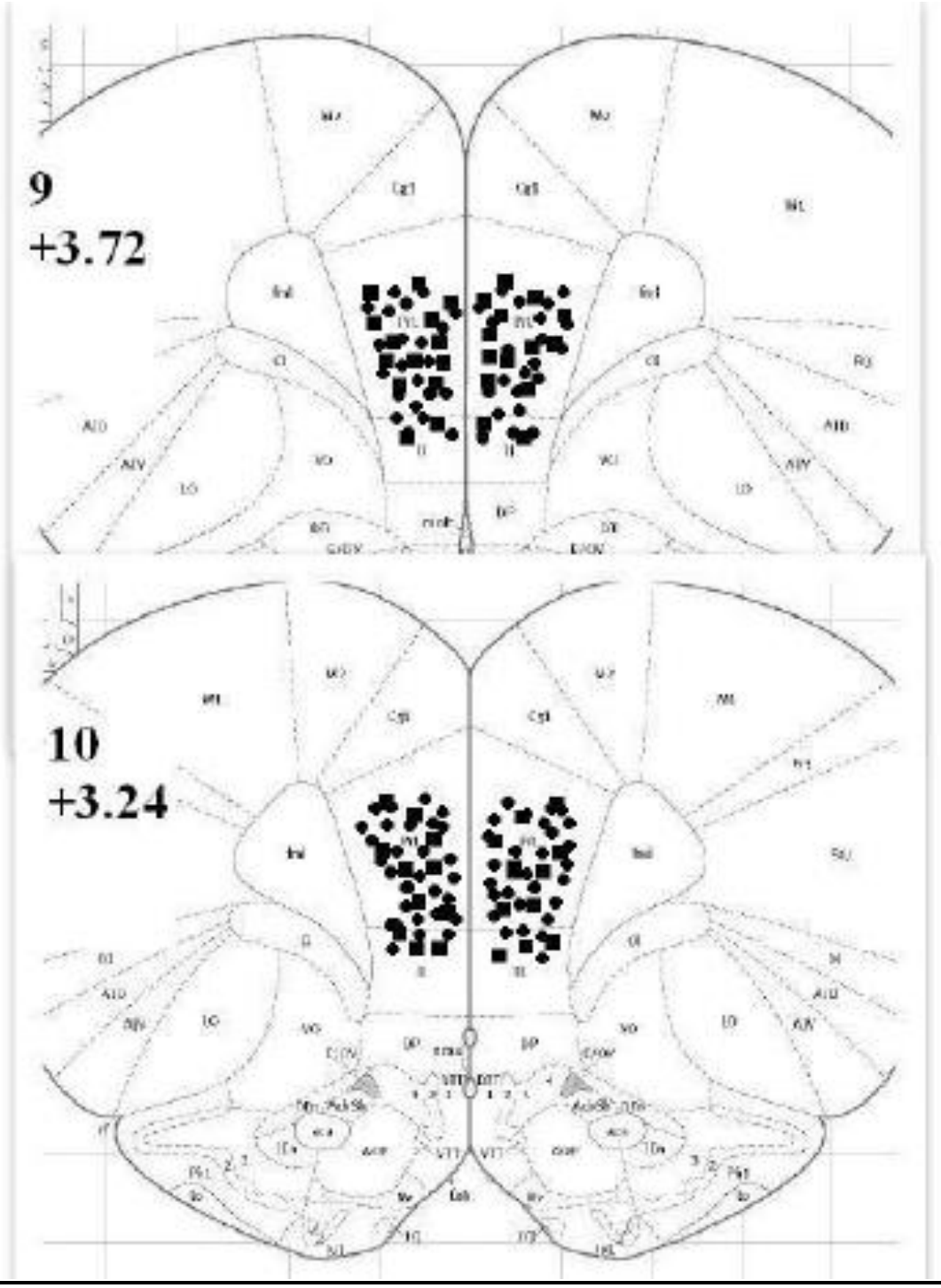
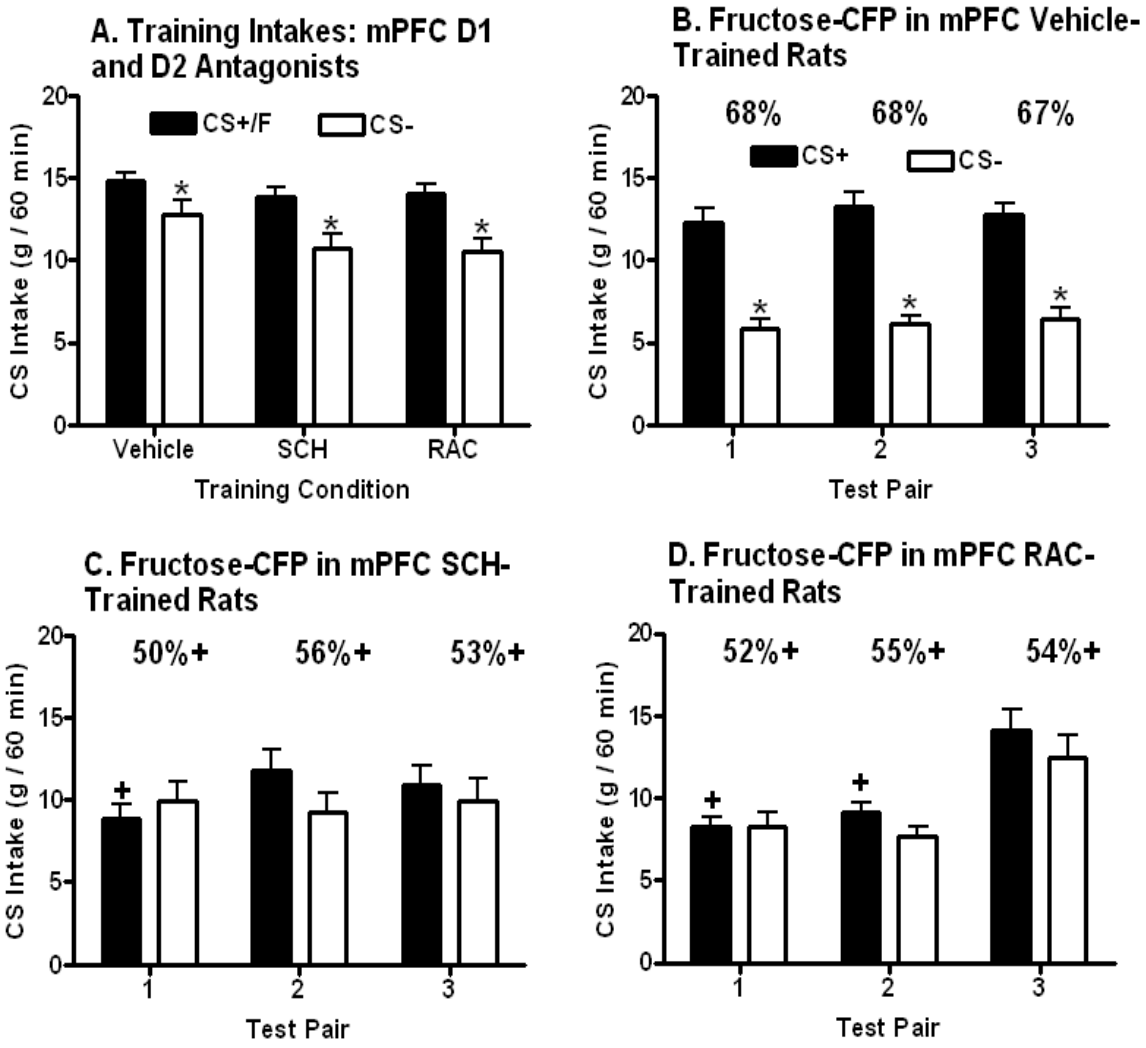


Figure 4


A. Training Intakes: mPFC D1 and D2 Antagonists
CS+/F
CS-
CS Intake (g / 60 min)
Vehicle
SCH
RAC
Training Condition
B. Fructose-CFP in mPFC Vehicle-Trained Rats
68%
68%
67%
CS+
CS-
CS Intake (g / 60 min)
Test Pair
C. Fructose-CFP in mPFC SCH-Trained Rats
50%+
56%+
53%+
CS Intake (g / 60 min)
Test Pair
D. Fructose-CFP in mPFC RAC-Trained Rats
52%+
55%+
54%+
CS Intake (g / 60 min)
Test Pair

Discussion

Administration of DA D1 or D2 receptor antagonists in the mPFC during training, at a dose that did not affect the expression of fructose-CFP (See Experiment 3), totally blocked the acquisition of the preference in a manner similar to that observed following systemic DA D1 and D2 antagonist treatment (Baker et al., 2003). The drug effects on acquisition of fructose-CFP were not due to the inability of the rats to detect, process or discriminate odor stimuli during training because the DA antagonists did not prevent the rats in the subsequent expression experiment from exhibiting a strong preference for the CS+ flavor during the two bottle tests. It should be noted that the ability of either SCH23390 or raclopride in the mPFC to block the acquisition of fructose-CFP occurred despite the fact that neither treatment affected one-bottle CS+ or CS- intakes during training (Figure 4A).

The ability of SCH23390 administration within the mPFC to block the acquisition of fructose- CFP mirrors the effect of D1 antagonism on glucose-CFP (Touzani et al., 2010) suggesting that the establishment of F-F and F-N learning may be mediated by the same D1 receptor neurobiological process within this cortical area. As proposed previously (Touzani et al., 2010), DA D1 receptor signaling in the mPFC may be involved in strengthening the representation of the CS+ flavor and SCH23390 treatment during the acquisition prevented this from occurring. The complete blockade of fructose-CFP acquisition by intra-mPFC administration of raclopride on the other hand further indicates that fructose-CFP and glucose-CFP are mediated by subtly different neurophysiological processes. It is possible that the meso-mPFC DA system is involved in the acquisition of F-F preference learning by updating (Cohen et al., 2002) or strengthening the representation of the CS+ flavor in the mPFC following its association with the reinforcing actions (sweet taste) of sugars. The strengthened representation

of the CS+ flavor should further guide the animal in selecting the appropriate motor response that leads to a maximum reward. Future studies are necessary to examine the relationship between mPFC DA mediation of fructose-CFP and glucose-CFP.

CHAPTER 5: DOPAMINE RECEPTOR ANTAGONISIM IN THE MEDIAL PREFRONTAL CORTEX ON THE EXPRESSION OF FRUCTOSE INDUCED FLAVOR PREFERENCES

Introduction

As described in Chapter 4, mPFC DA circuits are of particular interest in evaluating CFP. mPFC DA involvement in consummitory behaviors (Ahn and Phillips, 1999; Bassareo et al., 2002; Bassareo and Di Chiara, 1997; D'Angio and Scatton, 1989; Hernandez and Hoebel, 1990), learning (Baldwin et al., 1997), and sensory integration make this region a prime candidate in contributing to the greater neural network governing CFP. In systemic F-F studies on the expression of CFP both DA D1 and D2 antagonists blocked the expression of a previously acquired preference, with a marked reduction in the magnitude of expression following DA D2 antagonism, and elimination following DA D1 antagonism (Baker et al., 2003). Bernal and co-workers (2008, 2009) demonstrated that DA D1 and DA D2 antagonists administered into the NAC or AMY significantly and dose-dependently reduced, but did not eliminate the expression of fructose-CFP, suggesting that another site might mediate these effects on the maintenance of this preference. The highly dopaminergic mPFC has long been regarded as a region of higher brain function, and considering the evidence supporting the importance of mPFC DA in food intake and leaning, evaluating the role of the mPFC in eating behaviors is imperative to a more complete understanding of the CFP phenomena. Therefore, the following experiment evaluated whether bilateral mPFC DA receptor antagonism with D1 SCH23390 or D2 Raclopride will alter the expression of fructose induced CFP.

Methods:

Expression Procedure: Rats were be given eight 1-bottle training sessions (30 min/day) with 16 ml of the CS+/F solution presented on odd-numbered days, and 16 ml of the CS- solution presented on even-numbered days. On days 9 and 10, the animals were given access to a second sipper tube containing water to familiarize the rats to the presence of two sipper tubes, as used during choice testing. 30 min training session are utilized for F-F expression studies, as opposed to 60 minutes sessions for F-F acquisition studies, because in acquisition studies the animals are being pharmacologically influenced and may require more time to adequately acquire a preference then in expression training with non-pharmacologically influenced rats. Training intakes were limited to 16 ml/session to minimize the difference between CS+/F and CS- intakes. Left-right positioning of the CS solution and water sipper tubes were counterbalanced across both days. Following training, the rats were given eight days of 2-bottle choice test sessions (30 min/day) with unlimited (50 ml) access to the CS+ and CS- solutions. Solution intakes during the training and testing were measured by weighing (0.1 g) the bottles before and after the 30-min sessions.

Ten minutes prior to the two-bottle test sessions rats were given bilateral injections (0.5 µl/side) through a stainless steel internal cannula (33-gauge, Plastics One) extending 1 mm past the guide cannula by using a Hamilton microsyringe connected by polyethylene tubing to the internal cannula. For the first two sessions of choice tests, all animals were administered a vehicle (0.9% saline) microinjection. Based on individual CS+ and CS- intakes during the first two days of choice testing, the rats were then divided into two matched groups. The DA D1 group were treated with SCH23390 at total doses of 12 (6 nmol/side), 24 (12 nmol/side) and 48 (24 nmol/side) nmol. The DA D2 group were similarly tested, but with microinfusions of the

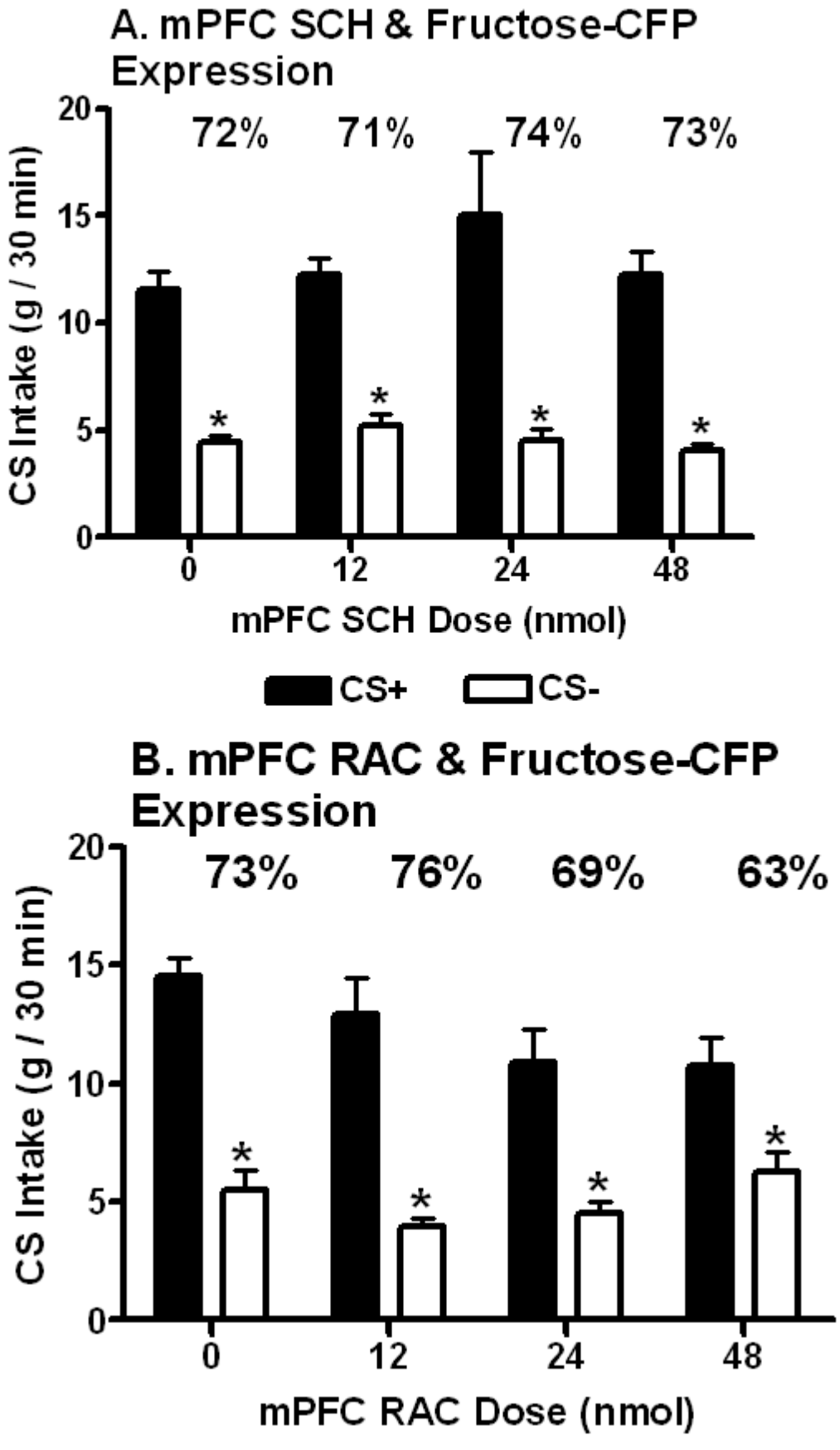
D2-like receptor antagonist Raclopride (Sigma Chemical Co.) at total doses of 12, 24, and 48 nmol. In each group half of the rats were tested with an ascending dose order, and the remaining rats were tested with a descending dose order. All animals were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups to reduce the impact of repeated infusion on neural tissue.

Results

Histological Verification: Figure (3) is a schematic representation (Paxinos and Watson, 2009) displaying the bilateral mPFC cannula placements of all 72 animals receiving vehicle, SCH23390, or raclopride in both the acquisition and expression experiments. All cannulae in were located within the mPFC, and there was considerable overlap between rats receiving vehicle, SCH23390, or raclopride. Moreover, the location of these placements was consistent with the previous expression studies examining IG glucose-CFP (Touzani et al., 2010).

Experiment 3: Effect of DA D1 or D2 antagonisms of the mPFC on the Expression of Fructose-CFP: During 1-bottle training, the mean intake of the CS+/F solution significantly exceeded that of the CS- solution for both the SCH23390 and raclopride groups (SCH23390: 12.4 vs. 8.9 g/30 min, $t(13) = 5.69$, $p<0.0001$ and raclopride 11.4 vs. 8.6 g/ 30 min, $t(7) = 4.01$, $p<0.005$). In the 2-bottle preference tests conducted following drug treatment, overall, CS+ (SCH23390: 12.8 g and raclopride: 12.3 g) intakes significantly exceeded CS- (SCH23390: 4.6 g and raclopride: 5.0 g) intakes (SCH23390: $F(1,13)= 61.14$, $p<0.0001$ and raclopride: $F(1,7)= 162.61$, $p<0.0001$), and intakes for both SCH23390 and raclopride groups failed to significantly differ as a function of drug dose or for the interaction between CS conditions and drug doses. CS+ intake was significantly higher than CS intake following the vehicle and all SCH23390 and raclopride doses (Figures 5A, 5B), and there were no significant differences in percent CS+ preferences (Figure 5A).

Figure 5


A. mPFC SCH & Fructose-CFP Expression
72%
71%
74%
73%
CS Intake (g / 30 min)
20
15
10
5
0
*
0
12
24
48
mPFC SCH Dose (nmol)
CS+
CS-
B. mPFC RAC & Fructose-CFP Expression
73%
76%
69%
63%
CS Intake (g / 30 min)
mPFC RAC Dose (nmol)

Discussion

Administration of DA D1 or D2 receptor antagonists in the mPFC during testing failed to affect the expression of fructose-CFP. Unlike the systemic ability for DA D1 and D2 treatment to block the expression of previously acquired fructose included CFP (Baker et al., 2003) and sucrose –CFP in sham-fed rats (Yu et al., 2000a), with non- significant preference reduction (73-63%) observed at the highest dose (48nmol). These results were somewhat surprising given that DA D1 or D2 antagonism of the mPFC (See Experiment 2) completely blocked the acquisition of fructose induced CFP, and bilateral DA D1 or D2 administration into the NAC or AMY reduced, but not completely block, expression of fructose-CFP (Bernal et al., 2008, 2009). However, the inability for mPFC antagonism to alter F-F CFP expression with SCH23390 or raclopride is similar to F-N IG- glucose findings in which mPFC D1 receptor antagonism failed to alter the expression of glucose-CFP (Touzani et al., 2010).

This failure of SCH or RAC administration into the mPFC to reduce the expression of fructose-CFP was accompanied by the absence of inhibitory antagonist effects upon total intake of the CS per se, which is in contrast to drastic significant reductions in total intake following systemic DA D1 or D2 receptor antagonism (Baker et al., 2003) and the smaller, but significant reductions in total CS intake following DA D1 or D2 receptor antagonism in the NAC (Bernal et al., 2008) or AMY (Bernal et al., 2009). Hence, these data collectively indicate that DA D1 and D2 receptor signaling in the NAC and AMY, but not the mPFC participate in, but do not exclusively control the expression of fructose-CFP.

CHAPTER 6: GENERAL DISCUSSION

This final chapter will review the effects of direct DA D1 and D2 antagonism on the NAC, AMY, and mPFC during the acquisition and expression of fructose-CFP, in relation to the hypothesis posited for Specific Aims 1, 2, 3, and 4 respectively. This will be followed by a general discussion of the following topics: A) The role of the dopaminergic system in F-F and F-N processes related to CFP; B) Proposal of a distributed neural network mediating dopaminergic functioning in relation to CFP; C) Future directions of proposed research; and D) Implications of animal based CFP pharmacology on human ingestive behaviors.

I.A. The role of DA D1 and D2 antagonism in the NAC on the acquisition of fructose-CFP

The impetus for the present dissertation, which examines the role of DA mediating fructose-CFP, was in part due to extensive and complex theories linking DA circuits to food reward, motivation, and learning (Berridge & Robinson, 1998; Ikemoto& Glazier, 1997; Smith, 1995). For example, conditioned DA released within the NAC has been found to be involved in F-N learning. This is evidenced by data showing that NAC DA efflux occurs in response to the presentation of food substances, and that intake of bitter SOA solution stimulates NAC DA efflux in rats previously trained to prefer a SOA solution paired with IG nutrient infusion (Bassareo & Di Chiara, 1997; Mark et al., 1991). Additionally, recent F-F and F-N studies evaluating the effect of DA antagonism of the NAC have yielded important information regarding dopamine induced augmentation of CFP acquisition and expression. Touzani et al. (2008) found that intracerebral administration of SCH23390 (12 nmol) into the NAC core or shell during acquisition significantly reduced F-N preferences relative to vehicle-treated controls (55%, 61% and 83-89%, respectively); however, expression of F-N preference was only reduced at doses that greatly suppressed overall intake by NAC shell SCH23390 administration. In a

parallel study examining fructose conditioned F-F preferences, Bernal et al. (2008) found that the expression of a fructose-CFP was dose-dependently (12-48 nmol) reduced by both SCH23390 (62% at 48nmol) and raclopride (63% at 24nmol) administered into the NAC shell (2008). Furthermore, both SCH23390 and raclopride at a 12nmol dose failed to alter the acquisition of a fructose-CFP, but unlike control rats, animals treated with a DA antagonists during training lost their CS+ preference over repeated 2 bottle preference testing, with reductions from 70-73% during the first pair of tests to 57-60% by the third pair of tests. The observed hastening of extinction suggests that D1 and/or D2 receptors may function to modulate the acquisition of fructose CFP. The role of DA action within the NAC can be more thoroughly evaluated by increasing D1 and D2 receptor antagonism. Given that expression of a fructose-CFP was dose-dependently attenuated by D1 and D2 antagonism in F-F studies, and the acquisition of CFP in F-N studies was significantly reduced at the 12nmol SCH23390 dose, but only hastened extinction in F-F paradigms, it is important to further evaluate the role of NAC DA functioning on the acquisition of conditioned F-F preferences. Thus, the *First Specific Aim* was formulated to examine whether bilateral direct NAC shell injections of D1 or D2 receptor antagonists at a higher 24nmol dose (12nmol per side) would eliminate the acquisition of fructose-CFP.

The hypothesis (**Specific Aim 1**) that direct bilateral administration of D1 or D2 receptor antagonists into the NAC Shell 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+ F-F 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, D1 and D2 antagonists, SCH23390 and raclopride, administered into the NAC shell at a 24 nmol dose failed to significantly alter the magnitude of

fructose-CFP acquisition. The D1 group displayed increased preferences for the CS+ over three two-day choice tests, ranging from an average preference of 63% to 73% during the first and third choice tests, respectively. In contrast, the D2 group displayed stable preferences over the three two-day choice tests with an average preference of 69% and 65% during the first and third choice tests, respectively. These data, combined with the data gathered from Bernal et al. (2008) and Touzani et al. (2008), strongly suggest that the NAC Shell is *not* a critical site for the acquisition of F-F fructose-CFP, but is vital to the acquisition of IG glucose F-N CFP. Given the fundamental differences between conditioning a preference to a flavor alone (F-F) and conditioning a preference for a flavor associated with positive intragastric consequence (F-N), it is reasonable to assert that NAC DA may be involved in different aspects of CFP. Acquisition of IG Glucose CFP was significantly reduced by DA D1 antagonism at a 12 nmol dose in the NAC (Touzani et al., 2008), but the same antagonist at the same (Bernal et al., 2008) and a higher 24 nmol (present study) NAC dose either failed to significantly alter, or at best hastened, the extinction of the acquisition in fructose- induced CFP. These data suggest that NAC DA may be more involved in the viserosensory modulation of CFP than the orosensory component of this behavior.

I.B. The role of DA D1 and D2 antagonism in the AMY on the acquisition of fructose-CFP

The role of AMY DA signaling in relation to feeding behavior has been well documented (see Background). Increased AMY DA efflux occurs in response to feeding and IG infusions of nutrient rich substances, as well as in response to Pavlovian and instrumental learning (Baxter and Murray, 2002; Cardinal et al., 2002; Harmer and Phillips, 1999; Hajnal & Lenard, 1997; Heffner et al., 1980), with AMY D1-like receptor antagonism dose-dependently impairing instrumental lever-pressing for sucrose **(**Andrzejewski, 2005). Additionally, recent F-F and F-N

studies evaluating the effect of AMY DA antagonism have yielded important information regarding dopamine induced augmentation of the acquisition and expression of CFP. Touzani et al. (2009) found that intracerebral administration of the D1 antagonist, SCH23390 (12 nmol) into the baso-lateral or central AMY during acquisition significantly reduced previously established F-N preferences (55%) relative to controls (82%), but only reduced the expression of this preference at doses that greatly suppressed overall intake. In the parallel fructose induced CFP F-F study conducted by Bernal et al. (2009), it was found that the expression of fructose-CFP was significantly reduced at the 48 nmol dose of SCH23390 (66%) or raclopride (68%), compared to vehicle (77%), but not at the 12 or 24 nmol total doses. Acquisition of this preference failed to be altered by both SCH23390 and raclopride at a 12nmol, but unlike control rats, DA antagonist treated animals lost their CS+ preference over three consecutive two-day, 2 bottle choice testing, from 70% during the first test to 57% by the third test. The observed hastening of extinction suggests that AMY D1 and/or D2 receptors may function to modulate the acquisition of fructose CFP. The role of DA D1 and D2 action within the AMY can be more thoroughly evaluated by increasing dopaminergic receptor antagonism. Given that expression was significantly attenuated by D1 and D2 antagonism in F-F studies, and eliminated by 12nmol SCH23390 in F-N studies, but only hastened extinction in F-F paradigms, it is important to further evaluate the role of AMY DA on the acquisition of F-F preferences. Thus, the *Second Specific Aim* was formulated to examine whether bilateral direct AMY injections of DA D1 or D2 receptor antagonists at a higher 24nmol dose (12nmol per side) would eliminate the acquisition of fructose-CFP.

The hypothesis (**Specific Aim 2**) that direct bilateral administration into the

AMY of D1 or D2 receptor antagonists would block the *acquisition* of fructose-CFP was *partially confirmed.* As described above, systemic treatment with SCH23390 or raclopride completely prevented the acquisition of a fructose-conditioned CS+ F-F preference (Baker et al., 2003). Administration of the D2 receptor antagonist, raclopride, at a 24 nmol dose in the AMY during training totally blocked the acquisition of fructose-CFP, resulting in preferences ranging from 48% to 54% during the first and third two-bottle test. These results mimic the complete elimination of acquisition observed following systemic raclopride treatment (Baker et al., 2003). In contrast, administration of the 24nmol SCH23390 dose into the AMY fails to significantly alter the acquisition of this preference, with percent CS+ intake remaining stable over the three two bottle tests (64% CS+ intake during tests one and three). It should be noted that the ability for raclopride in the AMY to block the acquisition of fructose-CFP occurred despite the fact that treated rats consumed more CS+/F than CS- during training and that these intakes did not differ from those of vehicle rats. It should also be noted that this effect is dose-dependent given that a lower (12 nmol) dose of raclopride administered into the AMY failed to alter acquisition of fructose-CFP (Bernal et al., 2009). These data strongly suggest that D2, but not D1, receptor signaling within the AMY is critical for the acquisition of fructose-CFP. Again, given the fundamental differences between conditioning a preference to a flavor alone (F-F) and conditioning a preference for a flavor associated with positive intragastric consequence (F-N), it is reasonable to assert that AMY DA1 and D2 receptors may be involved in these different aspects of CFP. Acquisition of IG Glucose CFP was significantly reduced by DA D1 antagonist at a 12 nmol dose in the AMY (Touzani et al., 2009), but administration of the same antagonist at the same (Bernal et al., 2009) or a higher 24 nmol (present study) dose in the AMY both failed to significantly alter the acquisition of fructose- induced CFP, suggesting that AMY DA D1

receptor function may be more involved in the viserosensory modulation of CFP than the orosensory component of this behavior. Systemic studies revealed that DA D1 receptor antagonism significantly alters the acquisition of sugar-CFP in both F-F and F-N paradigms (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). However, the DA D2 receptor antagonism effects on acquisition of a sugar-CFP are limited to F-F conditioning, with systemic DA D2 antagonism blocking the acquisition of CFP induced by orosensory aspects alone (F-F), but having no effect on the acquisition of F-N IG Glucose induced preferences (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). DA D2 antagonism in the AMY dose-dependently reduced the acquisition of fructose-CFP with the lower 12 nmol dose failing to exert effects (Bernal et al., 2009) but, the higher 24 nmol dose abolishing the acquisition of this response in the present study. Thus, AMY DA D1 receptor action seems to be intrigual for the acquisition of IG Glucose F-N CFP but not F-F CFP, whereas AMY DA D2 receptor action is important for proper acquisition of F-F, but not F-N, CFP.

I.C. The role of DA D1 and D2 antagonism in the mPFC on the acquisition of fructose-CFP

Direct intracerebral injection of either SCH23390 and raclopride injections at a 12 nmol dose into the NAC and AMY (Bernal et al., 2008, 2009) were less effective than systemic injections in reducing the acquisition of fructose-CFP (Baker et al., 2003), indicating that brain sites outside the NAC and AMY contribute to the DA modulation of fructose-CFP acquisition. A prime candidate for this modulation is the mPFC (see background). The mPFC is included in a network that is intimately and reciprocally connected with the NAC and AMY (Berendse et al., 1992; Brog et al., 1993; Groenewegen et al., 1999; McDonald, 1991; McGeorge and Faull, 1989; Sesack et al., 1989; Wright and Groenewegen, 1995; Wright et al., 1996), and like the NAC and AMY, the mPFC receives mesocorticolimbic DA projections from the VTA (Swanson, 1982).

DA innervation of the mPFC has been implicated in conditioned eating behavior in that mPFC DA release increases when food-deprived rats are presented with food stimuli (Feenstra and Botterblom, 1996), and by feeding and food-related cues in Pavlovian and instrumental learning tasks (Bassareo et al., 2002; D'Angio and Scatton, 1989; Hernandez and Hoebel, 1990; Izaki et al., 1999). Finally, in F-N studies DA D1 antagonism with a 12nmol dose of SCH23390 injected directly into the mPFC eliminated the acquisition of IG glucose CFP, but failed to significantly augment the expression of F-N-CFP (Touzani et al., 2010). Thus, the *Third Specific Aim* was formulated to examine whether bilateral mPFC injections of DA D1 or D2 receptor antagonists would impair the acquisition of fructose-CFP.

The hypothesis (**Specific Aim 3**) that direct bilateral administration into the AMY of D1 or D2 receptor antagonists would block the *acquisition* of fructose-CFP was *confirmed.* Administration of DA D1 or D2 receptor antagonists in the mPFC during training, at a dose that did not affect the expression of fructose-CFP, completely blocked the acquisition of this preference in a manner similar to that observed following systemic D1 and D2 antagonist treatment (Baker et al., 2003). Both mPFC SCH23390 and raclopride treated animals exhibited indifference to the CS+ solution, with SCH23390 treated animals displaying 50% CS+ consumption during the first two-bottle choice test and a 53% CS+ consumption during the third choice test. Likewise, raclopride treated animals displayed a 52% CS+ consumption during the first test and 54% CS+ consumption by the third test. The drug effects on acquisition of fructose-CFP were not due to the inability of the rats to detect, process or discriminate odor stimuli during training because the DA antagonists did not prevent the rats in the subsequent expression experiment from exhibiting a strong preference for the CS+ flavor during the two-bottle tests. It should be noted that the ability of either SCH23390 or raclopride in the mPFC to block the

acquisition of fructose-CFP occurred despite the fact that neither treatment affected one-bottle CS+ or CS- intakes during training. These findings suggest that the mPFC is a neural location vital to the acquisition of F-F fructose induced CFP.

I.D. The role of DA D1 and D2 antagonism in the mPFC on the expression of fructose-CFP

As stated above, the mPFC is included in a network that is intimately and reciprocally connected with the NAC and AMY (Berendse et al., 1992; Brog et al., 1993; Groenewegen et al., 1999; McDonald, 1991; McGeorge and Faull, 1989; Sesack et al., 1989; Wright and Groenewegen, 1995; Wright et al., 1996), and like the NAC and AMY, the mPFC receives mesocorticolimbic DA projections from the VTA (Swanson, 1982). Additionally, DA innervation of the mPFC has been implicated in conditioned eating behavior (Bassareo et al., 2002; D'Angio and Scatton, 1989; Feenstra and Botterblom, 1996; Hernandez and Hoebel, 1990; Izaki et al., 1999).

Systemic administration of SCH23390 or raclopride effectively eliminated the expression of a previously acquired preference in F-F CFP studies (Baker et al., 2003). Moreover, both SCH23390 and raclopride centrally injected into either the NAC or AMY resulted in significant reductions in CS+ preference during 2 bottle choice tests (Bernal et al., 2008, 2009). Although site specific NAC and AMY SCH23390 administration had a minimal effect on F-N expression (Touzani et al., 2007, 2008), it is important to further evaluate the role of DA D1 and D2 receptor antagonism on F-F CFP given the significant effects on expression in both systemic and site specific NAC and AMY studies. Data from the aforementioned F-F preference studies combined with the elimination of acquisition observed with both D1 and D2 antagonism of the mPFC warrants further investigation into the role of mPFC DA D1 and D2 receptor function on the expression of F-F fructose-CFP. Thus, the *Fourth Specific Aim* was formulated to examine

whether bilateral direct mPFC injections of DA D1 or D2 receptor antagonists would impair the expression of fructose-CFP.

The hypothesis (**Specific Aim 4**) that direct bilateral administration into the mPFC of D1 or D2 receptor antagonists would block the *expression* of fructose-CFP was *not confirmed.* Whereas systemic SCH23390 eliminated the expression of the CS+ preference (39-55%) at all doses tested (50–800 nmol/kg) compared to the vehicle preference (77%) (Baker et al., 2003), SCH23390 administered directly into the mPFC failed to alter fructose-CFP, with CS+ intake preferences remaining stable across all doses (72%: vehicle; 73%: 48 nmol). Similarly, systemic raclopride dose-dependently eliminated the expression of fructose-CFP, but direct injection of raclopride into the mPFC reduced, but failed to eliminate the expression of fructose-CFP (73%: vehicle; 63%: 48 nmol). This finding is similar to the inability of mPFC D1 receptor antagonism to alter the expression of glucose-CFP (Touzani et al., 2010), suggesting that the mPFC is *not* a site vital to the *expression* of F-N or F-F CFP.

II. Dopaminergic modulation of flavor-flavor and flavor-nutrient CFP.

Conditioned preference learning in relation to food intake is determined by interrelated orosensory and post-ingestive factors, which are studied through F-F and F-N conditioning paradigms. F-F conditioning occurs when preference for target flavor (e.g., cherry) is established through repeated pairings with an already preferred flavor (e.g., sweetness). F-N conditioning occurs when a preference for a target flavor is established through pairing the consumption of that flavor (e.g., grape) with positive postingestive nutrient actions (e.g.: glucose). During normal eating situations orosensory and post-ingestive actions usually operate concurrently, however in the laboratory setting these components can be separated and individually explored. Both warrant scientific investigation because the orosensory and post-ingestive actions that are

believed to modulate F-F and F-N CFP seem to be mediated by separate neural mechanisms within the same distributed brain network, and have different behavioral effects on mediating preference and consummitory behaviors.

F-N and F-F conditioning are both quite potent and enduring, with CFP for nutrients proving to be more robust (>90%: e.g., Azzara et al., 2000, 2001; Touzani et al., 2008, 2009) then CFP developed in response to a flavor accompanied by negligible nutrient action (~75%: e.g., Baker et al., 2003, 2004; Yu et al., 1999, 2000a, 2000b). F-F conditioning occurs in responses to orosensory stimulation alone, but F-N conditioning involves both orosensory stimulation and the addition of nutrient absorption through the gut. Thus, F-N conditioning employs gut-brain chemical interactions to modulate the acquisition and expression of this type of CFP. Because of the essential differences and similarities between these types of CFP, it is reasonable to assert that modulatory actions at a neural level involve both convergent and divergent chemical activity, as well as differential employment of nuclei involved in the brain network governing conditioned feeding behaviors. As this dissertation is based on the involvement of AMY, NAC, and mPFC dopaminergic substrates in the modulation of CFP, the following discussion will be limited to the role of DA D1 and D2 receptor actions in the acquisition and expression of F-F and F-N conditioning.

Collaboration between the laboratories of Drs. Richard Bodnar and Anthony Sclafani has yielded data essential to the understanding of dopaminergic F-F and F-N CFP modulation. Systemic studies have shown that administration of DA D1 and D2 receptor antagonists block both the acquisition and expression of F-F conditioning (Baker et al., 2003; Yu et al., 2000a, 2000b), whereas systemic administration of D1 receptor antagonist SCH23390, but not D2 antagonist raclopride, block the acquisition, but not the expression of F-N CFP (Azzara et al.,

2001). These data clearly indicate that in F-F CFP DA D1 and D2 like receptors are critically involved in both acquisition and expression, but that DA plays a more limited role in F-N conditioning, with DA D1, but not D2 receptor actions, being vital for the acquisition, but not expression, of this type of CFP. Thus, in subsequent F-N site specific studies only DA D1 antagonism is utilized, whereas both DA D1 and D2 antagonism is employed in F-F paradigms.

Central studies on the NAC, AMY, and mPFC have further illuminated differential F-F and F-N dopaminergic modulation. Bilateral administration of DA D1 antagonist SCH23390 into the NAC significantly reduces the expression of fructose-induced CFP (Bernal et al., 2008), but fails to cause significant reductions in expression of F-N CFP, except at doses that suppress overall intake (Touzani et al., 2008). Similarly, NAC SCH23390 treatment at a 12nmaol dose completely blocks F-N acquisition, but an identical dose of SCH23390 or raclopride only hastens extinction of F-F acquisition (Bernal et al., 2008), with a higher 24nmol dose having no effect (Malkusz et al., 2012). Microinfusions of DA antagonists administered directly into the AMY have generated similar results. AMY DA D1 SCH23390 administration significantly reduces, but fails to eliminate, CS+ preference in two bottle choice tests, but only effects F-N expression at doses that significantly alter overall CS intake, with results comparable to that observed with NAC DA antagonism (Touzani et al., 2009). Conversely, 12 nmol SCH23390 administration into the AMY significantly reduced CS+ preference in acquisition of CFP , but failed to significantly alter acquisition in F-F studies at a higher 24nmol dose, and only hastened extinction with a lower 12nmol (Bernal et al., 2009; Malkusz et al., 2012, Touzani et al., 2009). However, DA D2 antagonism of the AMY at a 24nmol raclopride dose completely eliminated the acquisition of fructose induced CFP (Malkusz et al., 2012). This effect is dose dependent given that 12nmol infusions of either SCH23390 or raclopride into the AMY only hastened the

extinction of fructose CFP (Bernal et al., 2009), and significantly altered acquisition of F-N IG glucose induced CFP (Touzani et al, 2007). Moreover, mPFC DA D1 and D2 antagonism in F-F studies, and DA D1 antagonism in F-N studies, completely blocked the acquisition of a flavor preference, but antagonism of either receptor failed to significantly alter expression in either paradigm (Malkusz et al., 2012; Touzani et al, 2010). These data suggest that the mPFC is a vital site for the acquisition, but not expression, of F-F CFP, with effects mimicking results derived from systemic studies.

Therefore, the experiments that comprise this dissertation combined with previous (Bernal et al., 2008, 2009) studies indicate that the central neurobiological mechanisms underlying acquisition and expression of fructose-CFP are distinct with the AMY and mPFC mediating the acquisition of this F-F-mediated response, and the NAC and AMY mediating the expression of this preference. Further studies are necessary to evaluate the interaction between these respective pairs of sites on the two processes underlying fructose-CFP. The ability of SCH23390 administration within the mPFC to block the acquisition of fructose- CFP mirrors the similar drug effect on glucose-CFP (Touzani et al., 2010) suggesting that the establishment of F-F and F-N learning are mediated by the same D1 receptor neurobiological process within this cortical area. As proposed previously DA D1 receptor signaling in the mPFC might be involved in updating (Touzani et al., 2010), or strengthening the representation of the CS+ flavor and SCH23390 treatment during the acquisition prevented it. The complete blockade of the acquisition of fructose-CFP by intra-mPFC administration of raclopride on the other hand further indicates that fructose-CFP and glucose-CFP are mediated by subtlety different processes. It is possible that the meso-mPFC DA system is involved in the acquisition of F-F preference learning by updating (Cohen et al., 2002) or strengthening the representation of the CS+ flavor

in the mPFC following its association with the reinforcing actions (sweet taste) of sugars, an association that requires intact DA D2 signaling in the AMY. The updated representation of the CS+ flavor should guide the animal in selecting the appropriate motor response that leads to a maximum reward.

III. Proposal of an interactive distributed brain network mediating CFP

The acquisition and maintenance of CFP involves collaborative actions between numerous anatomical sites and their neurotransmitters within a distributed neural network that operates to process, integrate, and direct numerous CFP components of food reward including hedonics, motivation, and learning. Studies evaluating CFP and related properties have implicated several major contributors in food related reward and preference learning, This includes neurochemical modulators such as DA, opioids, glutamate, cannabinoids, and GABA, as well as anatomical sites such as the NAC, VTA, AMY, LH, and mPFC (See: Background). As stated throughout, the specific mechanisms by which the proposed neural sites and chemicals interact to regulate feeding behavior and related conditioning remains largely elusive. The following discussion will focus on dopaminergic interactions within and among the anatomical sites and neurochemical systems proposed to mediate the acquisition and expression of CFP.

The neural actions that mediate sensory aspects of taste, food reward, and learning require activation of pathways that include both sub-cortical and more complex cortical structures (Berridge, 2009). The mesocorticolimbic DA system has been implicated as a dominating force in regulating interactions among various relevant neuroanatomical substrates. There are a variety of structures that regulate the positive and negative feedback loops involved in CFP. For example, the NAC, AMY, mPFC and VTA are all involved in a reciprocal network that not only modulates tonic VTA DA firing intensity, but also functions to modulate local

activity within and between these neural locations. The modulation of these nuclei, via self-modulation or modulation provided via positive and negative feedback from associated nuclei is necessary for the efficient processing, acquisition, and maintenance of CFP related components (in addition to the many other possible functions in which this network may be involved). As demonstrated by Experiment 1 and 2 of this dissertation, proper D2 functioning of both the mPFC and AMY is necessary for the acquisition of fructose-CFP. These results indicate that the AMY and mPFC work together to mediate this aspect of CFP, and further attests to the collaborative nature of the nuclei proposed within this network. Thus, to understand the mechanisms of action that allow this network to maintain proper functioning and promote CFP related processes, it is important to understand how the various neural components interact.

The VTA is a primary structure in the mesocorticolimbic system and as such, projects dopaminergic signals to widespread neural locations. Although this site contains a high density of DA neurons (50-60%), there is also a sizable GABAergic neuronal population, as well as a more limited glutamatergic presence. Activation of GABAergic and gultamatergic neurons in the VTA is believed to regulate the tonic firing activity of dopaminergic fibers, which in turn regulates the intensity of dopaminergic output to various neural locations (Alcaro et al., 2007). The VTA is reciprocally connected (either directly or indirectly) to many neural structures such as the mPFC, NAC, AMY, and hippocampus, as well as a variety of hypothalamic nuclei (including the LH via the mesodiencephalic DA complex) (Alcaro et al., 2007; Geisler et al., 2007; Lammel et al., 2008), which in turn, project glutamatergic or GABAergic afferents back to the VTA, thereby contributing to the modulation of VTA DA firing rates. NAC neurons receive input from both the dopaminergic neurons of the VTA and the glutamatergic neurons of the hippocampus, AMY, and mPFC. When activated by these inputs, the medium spiny GABAergic

NAC neurons release GABA onto the inhibitory ventral pallidum cells, resulting in the disinhibition of this structure, and thus increased VTA DA firing onto target sites. This VTA excitation and DA efflux can be further magnified if the NAC receives concurrent AMY and mPFC stimulation (McGinty, 2009).

The AMY receives direct VTA dopaminergic input, and in return sends limited direct and more substantial indirect glutamatergic output back to the VTA (Haber et al., 1997; Zahm et al., 1999). Moreover, the AMY receives glutamergic, GABAergic and dopaminergic input from the mPFC, NAC, VTA, LH, and substantia nigra (Fudge et al., 2000; Ono, 1985). Importantly, the AMY sends both glutamate and GABA projections to the mPFC, and NAC (Brog et al., 1993; MvGeorge & Faull 1989; Touzani et al., 2007). Additionally, excitation of the AMY via electrode stimulation increases firing rates of cells in the mPFC and NAC (Matsuda, 1993), which, though direct anatomical connections, modulates activity within the VTA.

Although primarily glutamatergic, the mPFC has a large DA and GABA neuronal presence. It has been suggested that within the mPFC GABA neurons mediate interactions between glutamate and DA neurons, thereby modulating mPFC transmitter release onto target sites (Drejer et al., 1987; Drejer and Honoré, 1987; Bowery et al., 1987; De Blas et al., 1988; Santiago et al., 1993; Yonezawa 1998). The mPFC primarily sends glutamatergic, and more limited dopaminergic, projections to other prefrontal areas as well as to the entire amygdaloid complex, NAC, thalamic nuclei, lateral (as well as medial, dorsal and ventral) hypothalamus, hippocampus, and VTA (Takagish et al., 1991). In return the mPFC receives dopaminergic, glutamatergic, and GABAergic inputs from a variety of locations, including the VTA, AMY, NAC, and other subcortical and cortical nuclei. Furthermore, the prefrontal cortex and VTA communicate directly with GABA-containing neurons of the VTA projecting to the prefrontal

cortex (Carr et al., 2000), and prefrontal cortex neurons synapsing onto dendrites of DA and non-DA neurons in the VTA (Carr et al., 2000). Moreover, electrode stimulation of the prefrontal cortex elicits excitation and inhibition-excitation patterns in VTA DA and non-DA neurons (Tong et al., 1998).

Notably, both the mPFC and VTA have reciprocal connections with the LH and hippocampal neurons (Ferino et al., 1987). Electrode stimulation of CA3 hippocampal neurons causes increased DA firing rates in VTA cells, and decreased firing rates for VTA GABA neurons, resulting in increased VTA dopaminergic output. Activation of CA3 glutamatergic cells results in VTA GABA interneuron inhibition, via increased lateral septum inhibition, resulting in increased VTA DA efflux (Luo, et al., 2011), and subsequent increased DA action in VTA projection sites, including the AMY, NAC, and mPFC.

In addition to the glutamate-GABA-dopaminergic interactions mediating the activities within and between nuclei in the proposed distributed neural network, evidence suggests opioid and cannabinoid modulation of DA transmission as well. It has been suggested that opioid receptor activity may regulate mesolimbic DA neuronal firing. For example, VTA mu and NAC delta1 and delta 2 opioid receptor activation increases NAC DA efflux (Devine et al 1993; Di Chiara and Imperato 1988 ; Hirose et al., 2005; Spanagel et al 1990; Spanagel et al 1992; Leone et al 1991; Pontieri et al 1995; Yoshida et al 1999). Interactions between CB and DA receptor activity has also been proposed, such that administration of selective CB1 receptor antagonists are related to a reduction in NAC extra-cellular DA usually increased by consumption of palatable food (Melis et al., 2007). These data suggest the additional presence of opioid-DA and CB-DA interactions in the mediation of feeding behavior (Melis et al., 2007; Verty et al., 2004).

Collectively, the data presented in this section along with the data presented in the Background section, although limited, suggest that the integration, acquisition, and maintenance of CFP and CFP related functions relies on the concerted actions of the nuclei and chemical transmitters proposed within this distributed brain network. Further research is necessary to uncover the exact intricate and complex mechanisms' by which nuclei and neurotransmitters proposed within, as well as outside of, this distributed neural network collaborate to mediate CFP.

IV. Future Directions

Dopaminergic signaling within the NAC, AMY, and mPFC is crucial to CFP generated through both F-F and F-N paradigms. These nuclei may be part of a distributed brain network which employs a variety of additional sites and neurotransmitters to effectively support CFP functions. There are several areas in which further scientific exploration is necessary to advance our understanding of role of DA in CFP modulation within the proposed neural network, including; a) Investigating the possibility of DA D1 and D2 synergistic interactions within the NAC, AMY and mPFC; b) Explore the effect of a 12nmol dose of D1 and D2 antagonists on the mPFC; c) Explore possible DA interactions with other neurochemical modulators (e.g.: Glutamate, GABA, etc...) within the AMY, NAC, and mPFC and; d) Investigate the role of DA in the acquisition and expression of CFP in locations outside the currently proposed neural network.

One area of interest for future investigations into the mechanisms' by which CFP is mediated, includes exploring the functional interactions between the NAC, AMY, and mPFC. Differential DA D1 and D2-like receptor actions in the NAC, AMY, and mPFC on the acquisition and expression of F-F and F-N CFP have been documented by the laboratories of

Drs. Bodnar and Sclafani. To further illuminate the role of DA in F-F conditioning it is imperative to explore possible synergistic interactions between DA D1 and D2 actions within these nuclei. This can be accomplished by either utilizing combined cocktails of SCH23390 (24nmol) and raclopride (24nmol) into a respective location (e.g.: NAC), or through administration of SCH23390 or raclopride simultaneously into multiple locations (e.g.: D1 antagonism of the NAC and simultaneous D2 antagonism of the AMY).

Future F-F acquisition studies should aim to investigate the effect of a 12nmol dose of either SCH23390 or raclopride in the mPFC. Given that the 24nmol dose had a robust effect and eliminated the acquisition of preference when injected into the mPFC, it seems that this site may be more sensitive to the effects of DA antagonism then either the NAC or AMY. Thus, studies evaluating the dose response curve of the mPFC in respect to DA antagonism in warranted.

Another area of interest includes exploring possible interaction of DA with other neurotransmitters on acquisition and expression of CFP. For instance, it has been suggested that both cannabinoid and opioid receptor activity may regulate DA firing within the mesolimbic DA pathway (Devine et al 1993; Di Chiara and Imperato 1988 ; Hirose et al., 2005; Spanagel et al 1990; Spanagel et al 1992; Leone et al 1991; Pontieri et al 1995; Yoshida et al 1999), it has also been suggested that DA signaling is greatly mediated by GABA and glutamate transmission. One possible way of further examining the influence of these neurotransmitters on DA modulation of acquisition and expression of CFP is to simultaneously inject a DA antagonist and opioid or cannabinoid antagonist (or antagonists that work of different neurochemical receptors) as a combined cocktail into a specific neural location (e.g.: NAC) to determine whether the augmentation (if any) on CFP is different than that observed with DA antagonism alone.

Exploration of dopaminergic actions in locations outside of the NAC, AMY, and mPFC in the acquisition and expression of F-F and F-N CFP is essential to gain a more complete picture of the neuroanatomical nuclei involved in the CFP distributed brain network "puzzle". Candidates include a number of locations activated in human FMRI studies on food processing and reward, including non-medial portions of the prefrontal cortex like the orbitofrontal and dorsolateral cortices, the insula, and ventral pallidum (Del Parigi et al., 2002; Holsen et al., 2005; Jackobson et al., 2010; Smeets et al., 2006).

The proposed studies would greatly expand our understanding of the specific mechanisms' by which anatomical sites and chemical transmitters collaborate to mediate CFP and CFP related processes.

V. Implications.

As discussed in the introduction and background sections at the beginning of this dissertation, the increased incidence of obesity along with related medical complications is considered to be a major health crisis in America and in developed countries around the world today (Abelson and Kenned, 2004; World Health Organization, 1997). Despite significant advances in our understanding of the biological underpinnings of energy regulation over the past decade, the “epidemic of obesity rages on, oblivious to scientific advances” (Filer, 2004), and changes in the environment rather than our biology seem to be driving force (Hill et al., 2003). Orosensory attraction to a food is based on dynamic, complex interactions between the internal state of the individual, the given environment, and learning. Behavioral studies in humans and laboratory animals demonstrate that while there are some innate preferences (sweet taste of sugar), most food preferences are learned through experience (Blake et al., 2008; Rozin and Zellner, 1985; Ruegg et al., 1997). However, it is important to note that information gained

through research performed on an animal model might not be generalized to the human subject in totality. *"*An animal model with biological and/or clinical relevance in the behavioral neurosciences is a living organism used to study brain-behavior relations under controlled conditions, with the final goal to gain insight into, and to enable predictions about, these relations in humans and/or a species other than the one studied, or in the same species under conditions different from those under which the study was performed" (van der Staay, 2006). Whether this goal is fully attained in the study of CFP employing the rat is yet to be determined, nevertheless information derived from utilization of the rat brain can help in a more generalized understanding of how neurological chemical and anatomical systems collaborate to execute functions related to learning and feeding behavior, even if evidence acquired from the rat yields only partially relevant to the functions of the human neural system. Moreover, the experiments comprising this dissertation are short-term studies on preference conditioning and merely suggest that there may be a link between the short-term conditioning of a flavor preference to long term preferential behaviors. It is yet to be determined if a substantial link between the conditioning of a short-term flavor preference influences general long-term ingestive behaviors, and then thereby contributes to the observed obesity epidemic in human populations.

Over the past several decades great strides in our understanding of food intake and neural reward circuitry have been made. The studies comprising this dissertation were performed to enhance our understanding of dopaminergic transmission within specific neural locations involved in the development and maintenance of food preference. Understanding of the neural mechanisms' underlying food intake and preference development in rats may further insight into human eating behavior, and possibly be applicable to the clinical treatment of obesity and support decline in this worldwide epidemic.

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