GENETIC VARIANCE CONTRIBUTES TO OPIOID AND DOPAMINE RECEPTOR MODULATION OF SUCROSE AND FAT INTAKE AND SUCROSE-CONDITIONED PREFERENCES IN INBRED MOUSE STRAINS

by

CHERYL T. DYM

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2012

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Richard J. Bodnar

Date — Chair of Examining Committee

Maureen O'Connor

Date — Executive Officer

Anthony Sclafani

Benjamin Kest

Ralph Norgren

John Glendinning

Supervisory Committee

Abstract

GENETIC VARIANCE CONTRIBUTES TO OPIOID AND DOPAMINE RECEPTOR MODULATION OF SUCROSE AND FAT INTAKE AND SUCROSE-CONDITIONED PREFERENCES IN INBRED MOUSE STRAINS

by

Cheryl T. Dym

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

Whereas genetics and pharmacology influence nutrient consumption, the current dissertation used inbred mouse strains to examine genetic variance in the dopaminergic and opioid modulation of sucrose and fat. The first and second specific aims measured intake of a sucrose solution in 8 inbred and 1 outbred mouse strains following administration of opioid (naltrexone (NTX)), dopamine D1 (SCH23390), and D2 (raclopride) receptor antagonists. NTX inhibited intake strongly in C57BL/10 and C57BL/6, moderately in BALB/cJ, C3H/He, CD-1 and DBA/2, weakly in 129P3 and SJL/J, and not at all in the SWR/J mouse strains. SCH23390 attenuated sucrose intake across five (129P3/J, SJL/J), four (C57BL/6J, BALB/cJ), three (SWR/J, C3H/HeJ, C57BL/10J, DBA/2J) and two (CD-1) of the doses tested. Raclopride was wholly ineffective in attenuating intake. In the third specific aim, intake of a fat solution (Intralipid) was measured in 8 out of 9 prior strains following NTX and SCH23390 administration. NTX attenuated intake at four (DBA/2), three (SWR/J, SJL/J), two (CD-1, C57BL/10), one (C57BL/6, 129P3) and none (BALB/cJ) of the doses tested. SCH23390 reduced intake at five (DBA/2, SWR/J, CD-1), four (SJL, C57BL/6), three (129P3), one (C57BL/10) and none (BALB/cJ) of the doses tested. A high correlation was found in the strain-

dependent abilities of SCH23390 and NTX to suppress Intralipid, but not sucrose intake, suggesting differential pharmacological mechanisms responsible. The fourth specific aim investigated genetic variance in experiential factors by examining whether SCH23390 and NTX alter acquisition and expression of a sucrose-conditioned flavor preference (CFP) in BALB/cJ and SWR/J inbred mouse strains. Mice received either vehicle, SCH23390 or NTX prior to *acquisition*: alternate daily exposure to a sucrose solution mixed with one flavor (CS+/S) and saccharin solution mixed with another flavor (CS-/s) or *expression*: a two-bottle choice test with the two flavors mixed in saccharin. In expression, strong CS+ preferences were reduced by SCH in BALB and SWR mice and by NTX in SWR mice. In *acquisition*, CS+/S was reduced by SCH in both strains, and by NTX in BALB/cJ. Sucrose-CFP was reduced by NTX BALB/cJ mice and SCH in SWR/J mice. Taken together, future studies are needed to reconcile the divergent results between strains, pharmacological systems, and nutrients to fully understand their influence on nutrient consumption and CFP.

Acknowledgements

I would first like to thank to thank my advisor, Dr. Richard Bodnar. Without his tremendous patience and guidance, none of this would have been possible. I would also like to thank Dr. Anthony Sclafani. My career began with working in his laboratory as an undergraduate nearly 10 years ago and he was truly my inspiration. Many thanks also go to the people who have worked side by side with me over the years…Tammy Kraft, Veronica Bae, Alex Pinhas, Yakov Yakubov. My friends deserve so many thanks for their support and encouragement and for sticking by me even on all those nights when I just couldn't go out. I would also like to thank my family for their understanding and constant offers of assistance. Finally, I would like to thank my husband, Josh. Over these last crazy years, your appreciation of my nerdiness and your unwavering sense of humor is what helped push me push through. Thank you.

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CHAPTER ONE: INTRODUCTION:

SIGNIFICANCE AND SPECIFIC AIMS

Obesity has recently become a prevalent medical issue in our society, and therefore a topic of important study. Statistical analyses indicate that the combination of an overweight state and sedentary lifestyle now account for almost one-third of the total number of deaths in the United States (Mokdad et al., 2004). Childhood obesity has more than tripled in the past 30 years with the percentage of obese American children aged 6–11 years from 7% (1980) to nearly 20% (2008) (http://www.cdc.gov/healthyyouth /obesity/facts.htm). Over a comparable period, a dramatic increase in overall obesity in the United States such that in 2010, no state had a prevalence of obesity less than 20% (http://www.cdc.gov/ obesity/data/trends.html). Obesity results from an interaction between environmental and biological factors including excessive caloric intake, particularly in developed countries where cheap calorically-dense food is more readily available and accessible (Dragone, 2009). Other factors include genetic dispositions, neurochemical and hormonal influences, prenatal experiences, postnatal nutrition, and orosensory and postingestive signals that modulate eating and satiety processes (Smith and Dockray, 2006). Motivation for consumption by means of specific "needs" - specifically "liking" and "wanting" - also possibly play a role in the ingestion of fat and sugar (see review: Berridge and Robinson 1998). Therefore, intake of sucrose (Specific Aims 1, 2 and 4) and fat (Intralipid: Specific Aim 3) emerged as subjects of investigation in the present proposal. Moreover, genetic, pharmacological and environmental influences play major roles in palatable intake. One approach to examine genetic influences on ingestive behavior is through the systematic analysis of inbred mouse strains for intake of multiple ingestive stimuli including fats, as well as simple and complex carbohydrates. Therefore, this approach was utilized in all four

Specific Aims. Moreover, research over the past 30 years has implicated a number of neurotransmitter systems in mediating intake, particularly the dopamine (DA) and opioid receptor systems that are found to be intimately involved in the mediation of sucrose and fat intake. Thus, evaluation of these transmitters was examined in all four Specific Aims. Finally, in addition to the important role that innate, genetic components play in development of food intake (Specific Aims 1-3), environmental, and particularly learning, factors also contribute to intake through the development of conditioned flavor preferences (Specific Aim 4). Thus, the following Specific Aims combined the three influences of genetics, pharmacology and learning on ingestive behavior by examining how sucrose and fat intake is affected in inbred mouse strains treated with DA or opioid antagonists as well as through the learning paradigm of conditioned flavor preferences.

Specific Aim 1: Genetic variance has been observed in inbred mouse strains for intake of sucrose and the non-caloric sweeter, saccharin (Bachmanov et al., 1997, 2001; Blizard et al., 1999; Capeless and Whitney, 1995; Fuller, 1974; Inoue et al., 2004; Lewis et al., 2005; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Pothion et al., 2004; Reed et al., 2004; Stockton and Whitney, 1974; Tordoff et al., 2002) such that different mouse strains consume different amounts of sucrose, and are sensitive to different concentrations of sucrose. Further, general opioid antagonists potently reduce intake of palatable sweet sucrose and/or saccharin solutions (Apfelbaum and Mandenoff, 1981; Cooper, 1983; Lynch and Libby, 1983) in food restricted, ad libitum and sham-fed rats (Shabir and Kirkham, 1999; Weldon et al., 1996; Kirkham, 1990; Kirkham and Cooper 1988a, 1988b; Rockwood and Reid, 1982). *Specific Aim 1 examined whether systemic administration of the general opioid antagonist, naltrexone (NTX), dose-dependently reduces sucrose intake in eight inbred and one outbred mouse strains* previously

examined for sucrose intake and detection (Lewis et al, 2005). The following results were expected:

- NTX will dose-dependently reduce sucrose intake in all strains, confirming opioid mediation of this response.
- Genetic variance in overall sucrose intake will be observed across strains, confirming previous findings.
- Genetic variance will be observed in the dose-dependent inhibition of sucrose intake following NTX.

Specific Aim 2: In addition to the opioid system, DA has also been implicated in the reward value of sweet taste (e.g. Bello et al., 2003; Genn et al., 2004) and dose- dependently reduces the intake of sucrose and saccharin-glucose solutions in real-feeding rats (Sclafani et al., 1982; Tyrka et al., 1992; Tyrka and Smith, 1991,1993, Xenakis and Sclafani, 1981, 1982). *Specific Aim 2 examined whether systemic administration of DA D1 (SCH23390) and D2 (raclopride) receptor antagonists dose-dependently reduce sucrose intake across the same inbred and outbred mouse strains.* The following results were expected:

- Genetic variance in overall sucrose intake will again be observed across strains.
- SCH23390 will dose dependently reduce sucrose intake in all strains.
- Raclopride will dose dependently reduce sucrose intake in all strains.
- Genetic variance will be observed in the dose dependent inhibition of sucrose intake following SCH23390.
- Genetic variance will be observed in the dose dependent inhibition of sucrose intake following raclopride.

Specific Aim 3: In addition to sweet intake, differences in dietary fat intake are also associated with genetic variation across mouse strains (Alexander et al., 2006; Bachmanov et al., 2001; 2002b; Leibowitz et al., 2005; Lewis et al., 2007; Reed et al., 2003; Smith et al., 1999; 2000; West et al., 1992). Furthermore, both the opioid and dopaminergic systems have been implicated in modulating fat intake (Gosnell et al., 1990a; Schneider et. al, 1988; Schneider et al., 1989; Smith, 1995, Weatherford et al., 1988; Weatherford et al., 1990; Wise and Rompre, 1989; Zhang et al., 1998). *Specific Aim 3 examined whether systemic administration of a D1 receptor antagonist (SCH23390) or an opioid receptor antagonist (NTX) dose-dependently reduces fat intake across seven inbred and outbred mouse strains* previously examined for fat (Intralipid) intake and detection (Lewis et al, 2007). The following results were expected:

- Genetic variance in overall fat (Intralipid) intake will be apparent across strains.
- NTX will dose dependently reduce Intralipid intake across strains.
- Genetic variance will be observed in the dose dependent response to SCH23390 and Intralipid intake.
- Genetic variance will be observed in the dose dependent response to NTX and Intralipid intake.

Specific Aim 4: Whereas Specific Aims 1-3 examined genetic variation in overall intake of palatable substances, they do not account for experiential factors which can greatly influence sugar intake and preference in inbred mouse strains (Sclafani, 2006a). As an example, naïve 129P3/J (129) mice display weaker preferences for dilute sucrose solutions than do C57BL/6J (B6) mice, but after experience with concentrated sugar solutions, the sugar preferences of the two strains are indistinguishable (Sclafani, 2006a). Learned preferences present another approach that demonstrates the importance of experience in regard to sugar intake. In the Conditioned

Flavor Preference (CFP) paradigm, a CFP is produced in rodents by adding a novel flavor to a preferred sugar solution (CS+) and a different novel flavor to a less-preferred saccharin solution (CS-) in sets of one-bottle training trials. The flavor associated with the CS+ solution is subsequently preferred to the flavor associated with the CS- solution when both are presented in saccharin solutions in two-bottle tests (Drucker, et al., 1994; see review, Sclafani, 1997). As observed for overall intake of palatable solutions, sugar-CFP in inbred mouse strains has also been found subject to profound genetic variation (Pinhas, et al. 2012). Moreover, in studies examining rats, CFP is differentially affected by pharmacological antagonists. Thus, systemic administration of DA D1, and to a lesser degree, D2 receptor antagonist interfered with both the initial acquisition and subsequent expression of sugar-CFP in oral (flavor-flavor) and post-oral (flavor-nutrient) paradigms in rats (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). Although an early study implicated the opioid system in sugar-CFP in rats (Mehiel, 1996), systemic administration of the opioid antagonist, NTX, failed to affect acquisition and expression of CFP elicited by intragastric sucrose infusions (flavor-nutrient: Azzara et al., 2000), CFP elicited by sucrose in sham-feeding rats (flavor-flavor: Yu et al., 1999), or CFP elicited by fructose in real-feeding rats (flavor-flavor: Baker et al., 2004). Although DA and opioid mediation of CFP has been examined extensively in rats, especially in determining the central sites of action (see reviews: Sclafani et al., 2011; Touzani et al., 2010), no systematic analysis of DA and opioid control of sucrose-CFP in inbred mice has been performed. *Specific Aim 4 examined whether systemic DA D1 receptor antagonism with SCH23390 or opioid antagonism with NTX alter acquisition and expression of a sucrose-CFP in* BALB *and SWR inbred mouse strains.* These two strains were selected based on their powerful and persistent flavor preferences (Pinhas et al., 2012). The following results were expected:

- As in rats, SCH23390 will reduce the acquisition of a sucrose-CFP in both strains of mice.
- As in rats, SCH23390 will dose-dependently reduce the expression of a sucrose-CFP in both strains of mice.
- Genetic variance will be observed in the ability of SCH23390 to reduce the acquisition of a sucrose-CFP in relation to its ability to affect sucrose intake in that strain.
- Genetic variance will be observed in the dose-dependent ability of SCH23390 to reduce the expression of a sucrose-CFP in relation to its ability to affect sucrose intake in that strain.
- As in rats, NTX will fail to reduce the acquisition of a sucrose-CFP in both strains of mice.
- Genetic variance will be observed in the ability of NTX to alter the acquisition of a sucrose-CFP in relation to its ability to affect sucrose intake in that strain.
- Genetic variance will be observed in the dose-dependent ability of NTX to reduce the expression of a sucrose-CFP in relation to its ability to affect sucrose intake in that strain.
- As in rats, NTX will fail to dose-dependently reduce the expression of a sucrose-CFP in both strains of mice.

Background:

A central premise of this dissertation is that genetics, pharmacology and learning play an interactive role in the intake of palatable substances by rodents. Many studies have examined one or two of these variables, but relatively few have examined all three variables, and their interactions. The Background section is organized so as to initially cover each of these issues singly, and then the interaction among the variables. Therefore, the first major section of the

Background will briefly review rodent models examining genetic influences on palatability. Rodent models of obesity will be initially reviewed followed by analyses of the use of inbred mouse strains in palatability studies. The second and third major sections of the Background will examine genetic variance in the specific palatable substances of sweets and fat respectively. As pharmacological manipulations play a major role in modulating intake of palatable solutions, the fourth major section in the background will examine the influence of the opioid and dopaminergic systems on sugar and fat intake, respectively. The fifth major section of the Background will review the influential role of learning on palatability by examining the history of CFP in regard to intake, as well as the pharmacology and genetic variance behind CFP. This will be followed by a rationale of the Specific Aims and their hypotheses (Part 6) for the present set of studies.

Section 1: Rodent Models of Obesity:

1.1. Obese Rodent Models: The first rodent models of obesity were genetic mutants of both outbred and inbred rodent strains. Ob/ob mice, discovered in 1949 (Ingalls et al., 1950), were typical B6 mice whose *ob* gene became mutated with the homozygotic offspring developed profound obesity, hyperinsulinemia and increased food intake. The underlying mechanism of action was identified nearly a half century later with the findings that this disorder resulted from a lack of functional leptin directly related to the development of obesity (Halaas et al. 1995; Friedman and Halaas, 1998; Zhang et al. 1994). Ob/ob mice were also used as an animal model for type II diabetes and have led researchers to understand the role leptin plays in food intake, body weight (Campfield et al. 1995; Halaas et al. 1995; Pelleymounter et al. 1995; Stephens et al. 1995) and metabolic rate (Pelleymounter et al. 1995). Another related mouse model, the db/db

mouse, is profoundly diabetic, an effect attributed to leptin resistance, and not to alterations in leptin levels (e.g., Chen et al., 1996).

Zucker rats were bred to be a genetic model for research on obesity and hypertension. There are two types of Zucker rat: a heterozygous lean Zucker rat, denoted as the dominant trait (Fa/Fa) or (Fa/fa); and the characteristically homozygous obese (or fatty) Zucker rat, represented as expression of its a recessive trait (fa/fa) (Bray and York, 1971; Kurtz et al. 1989; Takaya et al. 1997; Zucker and Antoniades, 1972; Zucker and Zucker, 1961). Obese Zucker rats have high levels of lipids and cholesterol in their blood, are resistant to insulin without being hyperglycemic, and gain weight from an increase in both the size and number of fat cells (Kava et al. 1990, Ikeda et al., 1986; Stern et al. 1972; Terrettaz et al. 1986,). Obesity in Zucker rats is primarily linked to their hyperphagic nature, which is accompanied by excessive hunger. However, food intake does not fully explain the hyperlipidemia or overall body composition (Kava et al. 1990; Kurtz et al. 1989).

1.2. Inbred Mouse Strains: When assessing the genetic contribution to any phenotype, comparisons of inbred mouse strains have also produced meaningful data. An inbred mouse strain is one that has been maintained by sibling (sister x brother) matings for 20 or more generations. Mice of an inbred strain are as genetically alike as possible, being homozygous at virtually all of their genetic loci. Each inbred strain possesses a unique genotype, and comparisons among strains can further isolate genotype differences as a function of behavioral differences (e.g., www.jax.org/jaxmice). Identifying divergent behavioral responses between strains can ultimately lead to chromosomal localization of that given behavior through quantitative trait loci (QTL) analyses. QTL analyses are used to localize chromosomal regions, and ultimately genes, critically involved in such differences. Furthermore, genetic models using

inbred mice can be potentially more instructive than using transgenic mouse models because they are not subject to possible confounding variables associated with mutations, such as developmental, compensatory, and multiple behavioral effects of the mutated gene (see review: Mogil and Grisel, 1998). In addition to inbred mice, a commonly-used outbred wild-type strain (e.g., CD-1) is also used in strain surveys which allows for direct comparisons of ingestive responses to inbred strains. In contrast to inbred strains, outbred mice are genetically heterogeneous with respect to one another, and therefore any variance in their behavioral responses cannot be attributed to genetic factors. Thus, the use of an outbred strain introduces an additional control when investigating the role of the genetic contribution to ingestive responses. Inbred mice have been used to examine the genetic variance involved in many aspects of food intake (see reviews: Reed et al., 1997; West and York, 1998). Given this dissertation's focus on genetic and pharmacological interactions in the modulation of palatable intake, the following sections will detail the role of murine genetic variance in sweet (Section 2) and fat (Section 3) intake.

Section 2: Genetic Variance in Sweet Intake:

Due to its high palatability and importance for energy utilization in nature, the consumption of sweet-tasting simple carbohydrates has specifically been analyzed for genetic variance (Reed et al. 1997; West and York, 1998). Behavioral murine strain differences have been consistently found for intake of sucrose and the non-caloric sweeter, saccharin (Bachmanov et al., 1997, 2001; Blizard et al., 1999; Capeless and Whitney, 1995; Fuller, 1974; Inoue et al., 2004; Lewis et al., 2005; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Pothion et al., 2004; Reed et al., 2004; Stockton and Whitney, 1974; Tordoff et al., 2002). In the consumption of 0.1% saccharin and 3% sucrose, genetic variance accounts for up to 78% and 83% of intake,

respectively (Ramirez and Fuller, 1976). In studies examining pairs of mouse strains, B6 mice displayed greater intake of five (0.005–1 M) glucose and sucrose concentrations than 101Bag/R1 mice (Stockton and Whitney, 1974), of a 0.1% saccharin solution than DBA/2J (DBA) mice (Fuller, 1974), and of low sucrose concentrations than 129 mice tested under naïve conditions (Bachmanov et al., 1997, 2001; Sclafani, 2006a,c; Sclafani and Glendinning, 2005; Tordoff et al., 2002), but not under conditions following behavior experience (Sclafani, 2006b). Lewis and co-workers (2005) examined twelve mouse strains across a range of nine sucrose concentrations (0.0001-20%) to determine potential genetic differences in the sensitivity to sucrose concentration, the magnitude of intake, and any alterations in corresponding chow intake. Inbred SWR/J (SWR), A/J (A) and B6 and outbred CD-1 mouse strains consumed the greatest amount (11–22 ml) of sucrose solutions relative to water in 24 h two-bottle preference tests, and inbred A, C57BL/10J (B10), SJL/J (SJL) and SWR mouse strains consumed the greatest (44– 56%) percentages of kilocalories as sucrose. In contrast, the AKR/J (AKR), CBA, C3H/HeJ (C3H) and DBA mouse strains consumed the least (7–8 ml) amount of sucrose, and displayed lower (20–30%) percentages of kilocalories consumed as sucrose (Lewis et al., 2005).

The genetic variation observed in saccharin and sucrose has allowed for the identification of trait-relevant genes using QTL analysis. Four studies (Blizard et al., 1999; Fuller, 1974; Inoue et al., 2004b; Lush, 1989) utilized QTL techniques to evaluate saccharin intake by crossing extreme responding sensitive B6 mice with subs-sensitive DBA or 129 mice. These studies revealed that both the saccharin preference locus (*Sac*) and the sweet taste receptor gene, *Tas1R3*, were localized to distal chromosome 4. Initially the Sac gene was offered as a means of elucidating the differences between preference responses of B6 strains and DBA strains for a 0.1% saccharin solution (Fuller, 1974). However, since the variation in preference among strains

for any one sweet substance is highly correlated with the variation in preference for the other sweet substances, the Sac gene was proposed as being responsible for detecting sweetness in general, and not just specifically saccharin (Lush, 1989). This finding was further validated by demonstrating that the sweet taste receptor recognized sweet tasting molecules as diverse as saccharin, sucrose, dulcin and aceseulfame-K (Nelson et al., 2001). The Sac gene has been mapped to distal chromosome 4 (Blizard et al., 1999) and linked to the Tas1R3 gene, which encodes the T1R3 receptor protein (Inoue et al., 2004b). When the T1R3 receptor binds to the T1R2 protein, the resultant heterodimer forms a sweet taste receptor and is responsible for sweetness detection in the taste buds of the oral cavity (Chandrashekar et al. 2006; Montmayeur and Matsunami, 2002; Nelson et al., 2001; Zhao et al., 2003). Sequence variants of the previously identified Tas1R3 taste receptor gene which codes for the protein associated with this preference, and their relevance to saccharin intake, were demonstrated by genotyping these polymorphisms in 30 inbred strains and comparing their allelic frequencies for these variants with their saccharin preference. This study concluded that the mouse Sac locus is identical to the Tas1R3 gene (Reed et al., 2004). A role for genetic variance in sucrose-CFP is described in detail in Section 6, and strains with strong and weak sensitivity to the Tas1R3 taste receptor gene are compared for their development of fructose-CFP (Pinhas et al., 2012).

***Section 3: Genetic Variance in Fat Intake*:**

In addition to sweetener intake, differences in dietary fat intake are also associated with genetic variation in mouse strains (Alexander et al., 2006; Bachmanov et al., 2001; 2002b; Leibowitz et al., 2005; Reed et al., 2003; Smith et al., 1999; 2000; West et al., 1992). Intake of 1-100% soybean oil solutions yielded higher preferences for fat in two-bottle preference tests in C57BL/6ByJ relative to 129 mice (Bachmanov et al., 2001), and elevated fat preferences were

observed in B6 mice relative to 129 and 129X1/SvJ mice (Sclafani, 2007). Lower preference thresholds in two bottle tests and higher licking activity of corn oil was observed in SWR mice compared to AKR mice (Smith et al., 2001). A self-selection strain survey of 12 inbred and one outbred strains provided separate and simultaneously available carbohydrate, fat and protein diets for 26-30 days. Whereas AKR and B6 strains self-selected the highest proportion of fat in the available macronutrient diet, which was directly related to epididymal fat, SWR and CAST/Ei strains consumed a great deal of fat that was inversely correlated with epididymal fat (Smith et al., 2000). Lewis and co-workers (2007) surveyed 11 inbred and 1 outbred strains in two-bottle choice test of water and nine different concentrations (0.0001-5%) of an emulsified soybean solution (Intralipid). Again, profound genetic variance in the sensitivity and magnitude of feeding responses was found with SWR (SWR) mice consumed the most (20.7 ml) Intralipid solution, and followed systematically by A, BALB, B10 and B6 mice (13.3-14.9 ml), then by SJL , AKR and 129 mice (9.4-12.5 ml), and finally by DBA , C3H and CBA/J (CBA) mice (6.7-9.2 ml).

In terms of the genetic mechanisms underlying fat preference and adiposity, QTL analyses of inbred mouse strains were successful in identifying several candidate loci (Reed et al., 2003; Smith-Richards et al., 2002). Crosses between fat-preferring B6 and carbohydrate-preferring CAST/EiJ mice in an F2 population yielded three loci responsible for fat intake: on chromosomes 8 (*Mnif1*), 18 (*Mnif2*) and X (*Mnif3*). In addition, two significant QTL for cumulative kilocalorie intake adjusted for baseline body weight, were found on chromosomes 17(*Kcal1*) and 18 (*Kcal2*). Without body weight adjustment, another significant kilocalorie locus appeared on distal chromosome 2 (*Kcal3*) (Smith-Richards et al., 2002). Moreover, a genome scan of a F_2 generation intercross between the C57BL/6ByJ and 129 mouse strains

(known to differ in body weight, body length and adiposity) identified loci on chromosomes 2, 4, 9 and 16 responsible for body weight, body length, and adiposity, with suggestive sex-dependent linkages on chromosomes 4 and 9 (Reed et al., 2003). The above studies together reveal that obesity is a complex trait influenced by genetics as well as its interactions with sex, age and diet for the determination of body size and adiposity in mice.

Despite their differences in taste and caloric consequences, a relationship in the genetic variance among strains for fat and simple carbohydrates has been observed. Strain-specific responses to nutritive (Intralipid) and non-nutritive (olestra oil) fats were similar in nature to that observed when testing for sucrose and saccharin (Sclafani, 2007). Moreover, Lewis and coworkers (2007), found a highly significant positive correlation (r=0.87) for the peak magnitude of sucrose intake and Intralipid intake was noted among 12 inbred strains. Significant positive correlations were also observed when comparing Intralipid (5%) intake with sucrose intake at concentrations of 5% (r= 0.82), 10% (r= 0.85) and 20% (r=0.88). An identical pattern of positive correlational effects was observed when one analyzed the percentage of kilocalories consumed as Intralipid (5%) relative to the percentage of kilocalories consumed as sucrose at concentrations of 5% (r= 0.81), 10% (r= 0.89) and 20% (r= 0.76). However, all studies do not support this relationship. No relationship was observed when measuring lick rates of Intralipid and sucrose solution ingestion in eight inbred mouse stains (Glendinning et al., 2008), necessitating further study of genetic variance involved in the connection between sweet and fat consumption

Observing genetic variance of a behavior is important for ultimately discovering which chromosomal locations and specific genes produce those behaviors. However, genetics alone do not modulate intake of palatable food substances. Neurochemical mediation also significantly influences intake. As the current dissertation examines the role that both genetics and

pharmacology play on food ingestion, the next section will examine neuropharmacological modulation on the intake of sucrose and fat.

Section 4: Neurotransmitter Control of Palatable Intake:

In addition to genetic variance in mediating sucrose and fat intake, research over the past 30 years has implicated a number of neurotransmitter systems in mediating intake. Among these, two major neurotransmitter systems, the opioid and DA systems, have been the focus of intense research. Indeed, a major theoretical evaluation of palatable intake, the Berridge model of "wanting" and "liking" examine these two systems (e.g., Berridge, 1996, 2009). The psychological construct, "Liking", refers to the affective aspect of ingestive behavior involving the hedonic and pleasurable experiences of eating, often measured in rats using the taste-reactivity test. "Wanting" is defined as the incentive salience or motivational disposition to eat, and is most often measured by a forced choice methodology (Finlayson et al., 2008). "Liking" and "wanting" have potential importance in the psychological understanding of taste preference and palatability. While not confirmed, physiological substrates have been introduced in order to maintain these psychological constructs (Berridge, 1996, 2009).

For instance, the opioid system has been implicated in the "liking", or assigning of hedonic quality, to food. An area in the rostrodorsal part of the medial shell of nucleus accumbens (NAC) was established as an area involved in the "liking" aspect of food reward when elicited by receptor agonists of the opioid and cannabinoid systems (Berridge, 2009). Additionally, central microinjections of mu-opioid agonists, such as DAMGO, in these same hotspots elicited further hedonic responses to sweet taste qualities (e.g., sucrose), and suppression of aversive reactions to bitter taste qualities (e.g., quinine) (Peciña, 2008).

The incentive process or “wanting” of food stimuli has been associated with the DA system (Berridge, 1996; Berridge and Robinson, 1998). Berridge and Robinson (1998) formed this conclusion based in part on their finding that 6-hydroxydopamine (6-OHDA) lesions dramatically reduced brain DA levels. Further support for this position is provided by data from two studies that used mice with significant mutations in normal DA function. Mice that lacked tyrosine hydroxylase in dopaminergic neurons, still displayed a strong preference for a sweet sucrose or saccharin solution over water (Cannon & Palmiter, 2003). In a related study, mice with a DA transporter (DAT) knockdown mutation preserving only 10% of DAT, accompanied by a 70% elevation of synaptic DA levels, performed significantly better in a runway task for a sweet food reward. In contrast, these mutant mice did not display enhanced orofacial hedonic reactions to the taste of sucrose (Peciña et al. 2003). Additionally, no significant increase in DA efflux in the NAC was observed during consumption of 4% sucrose by rats when shifted from 32%. In contrast, consumption of 4% sucrose by control rats was accompanied by a significant increase in DA efflux in the NAC (Genn et al. 2004).

Taken together, while there is some neurobiological evident in relation to the Berridge (2009) definitions of “liking” and “wanting”, additional elucidation is needed in order to fully understand these psychological constructs. Thus, the following sections further examine the specific roles of opioid and dopaminergic neurotransmitter systems play in modulation of sugar and fat intake.

4a) Opioid Modulation of Intake of Sweet Substances: The inhibitory effects of the general opiate receptor antagonists, naloxone or NTX, appear most apparent when reducing sweet sucrose and/or saccharin solution intakes in rats (Apfelbaum and Mandenoff, 1981; Cooper, 1983; Levine et al., 1982; Lynch and Libby, 1983). This intake inhibition persists under

both food restricted and ad libitum conditions (Shabir and Kirkham, 1999; Weldon et al., 1996) as well as in sham-fed rats in which post-ingestive factors are minimized (Kirkham, 1990; Kirkham and Cooper 1988a, 1988b; Rockwood and Reid, 1982). Opioid antagonists act more potently when sucrose is preferred (Giraudo et al., 1993; Glass et al., 1996), reducing that aspect of feeding activated by sweet taste (Levine et al., 1995), and decreasing the length of the first meal without changes in eating rate (Glass et al., 2001). Opioid antagonists decrease the intake of sweet solutions much more effectively than water intake (Levine et al., 1982; Cooper, 1983; LeMagnen et al., 1980; Sclafani et al., 1982). Taste-reactivity measures predict the decreases in sucrose intake by opiate antagonists (Parker et al., 1992), and naloxone decreases motivation to respond to sucrose using a progressive reinforcement schedule (Cleary et al., 1996). Moreover, the inhibitory effects of the opiate receptor antagonists reduce sweet solution intake without affecting eating latency, suggesting effects upon the maintenance, rather than the initiation of intake (Kirkham and Blundell, 1984, 1986). Consistent with these results, central infusions of opioid agonists increase the intake of a saccharin solution, but not plain water (Zhang and Kelley, 2002), and consumption of sweet solutions increase brain beta-endorphin levels more than does the consumption of plain water (Yamamoto et al., 2000).

In examining specific opioid receptor subtype antagonists, mu- and kappa-, but not delta-selective receptor subtype opioid antagonists reduce sucrose intake in both real-feeding (Beczkowska et al., 1992) and sham-feeding (Leventhal et al., 1995) tests. Ventricular administration of selective mu and kappa, but not delta-1 receptor subtype opioid antagonists reduces intake of palatable sucrose diets (Arjune and Bodnar, 1990; Beczkowska et al., 1992; Calcagnetti et al., 1990; Islam and Bodnar, 1990). Ventricular administration of general, mu and kappa, but not mu-1 or delta opioid receptor subtype antagonists reduces sucrose intake in sham-

feeding rats by shifting effective sucrose concentrations to the right and producing delayed effects (Leventhal et al., 1995). In contrast, maltose dextrin intake was reduced by only mu antagonists in real-feeding rats and by kappa and delta-1 antagonists in sham-feeding rats, whereas only delta antagonists reduced saccharin intake in real-feeding rats (Beczkowska et al., 1993; Leventhal and Bodnar 1996). Investigation of antagonist administration to central sites found whereas general and mu, but not delta opioid antagonism in the NAC shell produce modest decreases in sucrose intake (Bodnar et al., 1995; Kelley et al. 1996), delta-2 receptor antagonism in the ventral tegmental area (VTA) marginally decrease sucrose intake (Ragnauth et al. 1997).

4b) Opioid Modulation of Fat Intake: In addition to sugar or sweetener consumption, fat intake has also been implicated as being modulated by the opioid system. Fat intake is significantly reduced in rats by general and selective mu and kappa opioid receptor subtype antagonists (Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008). This was demonstrated with dose-dependent decreases in the licks for Intralipid solutions (Higgs and Cooper, 1998). In rats, injection of the mu opioid agonist, DAMGO, into the NAC resulted in a four fold increase in the amount of fat diet ingested (Zhang et al., 1998). Morphine administration increased intake of a high fat diet to a greater degree in rats that preferred a high fat diet, indicating a role for self-selection (Gosnell et al., 1990a). Chronic mu, mu-1, delta-1, and delta-2 opioid receptor subtype antagonists also significantly decreased weight and intake of a fat source in rats during development of dietary obesity (Cole et al., 1995). Ventricular administration of selective mu and kappa, but not delta-1 opioid receptor subtype antagonists reduced intake of palatable fat diets (Arjune and Bodnar, 1990; Beczkowska et al., 1992; Calcagnetti et al., 1990; Islam and

Bodnar, 1990). Furthermore, several macronutrient diet selection studies suggest that brain opioid receptors are specifically involved in fat preference (Glass et al. 1999). Thus, the opioid neurotransmitter system plays a major role in the consumption of palatable substances, specifically sweets and fats.

4c) Genetic Variance in Opioid Modulation of Palatable Substances: Whereas many studies have examined the general role opioid pharmacology plays on the intake of sweets and fats, relatively few studies have addressed whether genetic variance affects the opioid modulation of palatable intake. Naloxone reduced the number of consumed sucrose pellets in an operant limited-feeding paradigm using DBA mice (Haywood and Low, 2001). Saccharin intake is reduced in opioid-deficient CXBK mice (Yirmaya et al., 1988), and mice deficient in either met-enkephalin or beta-endorphin showed reduced progressive ratio operant responses for food reinforcement (Haywood et al., 2002). However, no systematic changes in sucrose intake were observed in homozygous and heterozygous pre–pro-enkephalin knockout mice as compared to wild-type mice (Ragnauth et al., 2001), and mice deficient in beta-endorphin were hyperphagic and obese, yet showed normal orexigenic responses to exogenous opioids and normal anorectic responses to naloxone (Appleyard et al., 2003). Because evaluation of the genetic variance in opioid modulation in sucrose or fat intake has not been conducted, Specific Aims 1 and 3 will address these issues.

4d) Dopaminergic Modulation of Intake of Palatable Substances: In addition to the opioid system, DA has also been implicated in the reward value of sweet taste, primarily because sweet taste activates mesolimbic DA circuits that are implicated in the mediation of natural as well as drug rewards (e.g. Bello et al., 2003; Genn et al., 2004). DA acts at two major families of receptors, termed D1 and D2 that are blocked by selective antagonists. Both D1 and D2

receptor antagonists suppress intake of sweet solutions (Geary and Smith, 1985; Muscat and Wilner, 1989; Schneider et al., 1986a, 1986b, 1988, 1989; Smith and Schneider, 1988; Wise and Rompre, 1989; Xenakis and Sclafani, 1981). DA antagonists dose- dependently reduce the intake of sucrose and saccharin-glucose solutions more than water in real-feeding rats (Sclafani et al., 1982; Tyrka et al., 1992; Tyrka and Smith, 1991, 1993; Xenakis and Sclafani, 1981, 1982). Correspondingly, increases in extracellular DA levels have been found in the NAC following intraoral saccharin infusion (Mark et al., 1991). Examination of specific receptor subtypes revealed that D1 antagonists consistently block sweet solution intake, occurring under deprivation, sham feeding and ad libitum conditions (Hobbs et al., 1994; Terry, 1996), whereas administration of D2 receptor antagonists produce more varied responses. Whereas the D2 antagonist pimozide inhibited food intake at higher doses, it increased food intake at lower doses (Terry, 1996). Limited analyses of dopaminergic control of fat intake have been performed. Both systemic and central D1 and D2 antagonists dose-dependently decrease corn oil intake in sham feeding rats (Schneider et. al, 1988; Schneider et al., 1989; Smith, 1995, Weatherford et al., 1988; Weatherford et al., 1990; Wise and Rompre, 1989). Further, no extensive evaluation of a dopaminergic role in sucrose or fat intake across strains to examine genetic variance exists; these issues will be addressed in Specific Aims 2 and 3.

The vast majority of the studies examining genetic variance on pharmacological modulation of food intake measured general intake of sweets and fats. In contrast, virtually none have studied the role these factors play in a learned preference of palatable substances. This dissertation will examine genetics and pharmacology of modulated learned intake of sweets. Thus, the following section will define one form of learned intake of palatable substances though

the CFP paradigm. In addition, pharmacological influences on CFP as well as the few studies examining genetic variance on CFP will be explored.

Section 5: Conditioned Flavor Preferences (CFP): In addition to the important role that innate, genetic components play in development of food preferences, environmental factors play a strong role as well. The CFP paradigm has been used to demonstrate that learning is involved in food selection, specifically in the preference of food (see reviews: Capaldi, 1992; Sclafani, 1990, 1995, 1997). The CFP paradigm is typically viewed as a form of Pavlovian or classical conditioning. In one version of this paradigm, the novel cue flavor acting as the conditioned stimulus (CS+) is paired with an unconditioned stimulus (US) which is a nutritive source (e.g. glucose). A second cue flavor (CS-) is paired with a nonnutritive source (e.g. saccharin). The CS+ and CS- are offered to the animals in single-bottle exposures on alternate days with the nutritive and nonnutritive pairings administered respectively via the mouth or completely bypassing the oral route by using intragastric, intraduodenal or intravenous infusion. In this one-bottle acquisition phase, the animals learn to associate the cue flavor with the post-ingestive consequence of the nutrient and overall acceptance of the CS+ and CS- is assessed by measuring the absolute intake. During the expression phase, preference is evaluated by having the animal undergo a two-bottle test of both cue flavors minus any nutrient or non-nutrient pairing. Due to the post-ingestive consequences of the nutrient, animals typically display a preference for the CS+ over the CS- cues. The preference can be quite strong in rats, falling in the range of 97-99%, and is very resistant to extinction (Drucker, et al., 1994; see review, Sclafani, 1997). This type of CFP paradigm is referred to as flavor-nutrient conditioning.

A second form of conditioning, flavor-flavor conditioning, occurs when the CS+ does not result in a post-ingestive consequences and learning occurs mainly from associating the cues

with the hedonic orosensory properties of the food stimuli. Flavor-flavor conditioning can be established in two ways. One way is through the sham-feeding preparation in which animals are fitted with a gastric fistula (Van Vort and Smith, 1983, Weingarten and Watson, 1982). When the fistula is closed while the animal eats, the food is digested normally (real-feeding), and when the fistula is opened, this allows the stomach contents to empty (sham-feeding) and prevent post-ingestive learning (Young et al., 1974). However, one drawback to using the sham feeding preparation is that it may not block all neural and hormonal feedback from the gut because sham-feeing does not completely prevent food digestion and absorption (Sclafani and Nissenbaum, 1985a, 1985b). A second way to establish a flavor-flavor conditioned preference is by using a nutrient which is ineffective in producing a flavor-nutrient conditioned response but possesses highly palatable orosensory qualities. Ingestion of flavor immediately followed by nonnutritive saccharin solution results in learned preference for that flavor (Holman, 1975). The monosaccharide, fructose, is ineffective in producing CFP through intragastric infusions relative to glucose or sucrose (Sclafani and Ackroff, 1993; Sclafani et al., 1999). However, by combining a previously neutral flavor with a fructose solution a preference can be conditioned (Sclafani and Ackroff, 1994). Thus, in flavor-flavor conditioning, combining a previously neutral flavor with solutions that are inherently reinforcing due to their sweet taste or viscous consistency, preferences can be conditioned for stimuli that lack post-ingestive consequences (Ackroff and Sclafani, 1999, Sclafani and Ackroff, 1994).

5a) Role of DA Receptors in CFP: Initial evidence of DA's involvement in CFP involved the study of rats trained to drink a mildly bitter tastant paired with intragastric polycose (CS+) and a mildly sour tastant paired with intragastric infusions of water (CS-). After training, exposure to the CS+ and CS- without intragastric pairings resulted in significantly increased

extracellular DA levels in the NAC following voluntary intake of the CS+, but not CS- solutions (Mark et al., 1994). Administration of the D2 antagonist, raclopride reduced preference for a flavored 10% sucrose solution compared to a second flavor paired with saline (Hsiao and Smith, 1995). Systemic treatment with a D1 antagonist (SCH23390), but not a D2 antagonist (raclopride), blocked flavor-nutrient conditioning by intragastric sucrose infusions (Azzara et al., 2001). D1 and D2 receptor antagonists also interfered with preference expression during flavor-flavor paradigms. Rats treated systemically with D1 (SCH23390) or D2 (raclopride) receptor antagonists during sham-feeding training sessions subsequently displayed preferences for the sucrose conditioned CS+ flavor comparable to control animals (Yu et al., 2000a). However, both antagonists dose-dependently reduced an already-trained preference for the CS+ flavor, indicating that D1 and D2 signaling are involved in the expression of a conditioned preference (Yu et al., 2000a, 2000b). One possible limitation of the sham-feeding study was that the animals consumed substantially more of the flavored sucrose solution than the flavored saccharin solution during training and therefore were more familiar with the CS+ flavor. Therefore, in a subsequent study (Baker et al., 2003), a rationed portion of a distinctly-flavored fructose and saccharin solution (CS+) and another flavored saccharin solution (CS-) was employed in real-feeding rats. Systemic treatment with SCH23390 and, to a lesser degree, raclopride blocked acquisition of fructose-flavor conditioning, whereas both D1 and D2 antagonism significantly reduced the expression of a CS+ fructose-CFP (Baker et al., 2003). In examining, the neural substrates of these responses , intracerebral injections of SCH23390, and to a lesser degree, raclopride, significantly reduced the expression of a CS+ fructose-conditioned preference following administration into the amygdala (Bernal et al., 2007) and shell of the NAC (Bernal et al., 2008a) during a fructose-CFP. The acquisition of this preference was unaffected by NAC or

amygdalar dopaminergic antagonists, but the D1 antagonist hastened extinction of the learned response. D2 receptor antagonism in the medial prefrontal cortex was more effective than D1 antagonism in dose-dependently reducing the expression of fructose-CFP (Malkusz et al., 2008). SCH23390 administration into the shell and core of the NAC interfered with acquisition, but not expression of a flavor-nutrient CFP in rats (Touzani et al., 2008a).

5b) Role of Opioid Receptors in CFP: Although the opioid system is intimately involved in ad libitum intake of sweet stimuli, the role of opioids in learned responses have been mixed. Naloxone decreased motivation to respond to sucrose using a progressive reinforcement schedule (Cleary et al., 1996), interfered with acquisition and expression of odor paired preferences conditioned by intraoral sucrose or corn oil infusions in 6-day-old rat pups (Shide and Blass, 1991), and reduced acceptance of a flavor paired with intragastric carbohydrate infusions (Ramirez, 1997). In a flavor-flavor conditioning paradigm, rats were trained to drink a cue flavor mixed with preferred glucose, dextrose, or ethanol (CS+) and another cue flavor mixed with the less preferred saccharin (CS-). Treatment with naloxone during the single-bottle glucose training sessions interfered with acceptance and prevented preference of the preferred stimuli paired flavor (Mehiel, 1996). In contrast, the Sclafani and Bodnar laboratories have consistently found that systemic administration of the general opioid antagonist, NTX failed to affect acquisition and expression of sugar-conditioned CFP. NTX failed to alter either acquisition or expression of a CFP elicited by intragastric sucrose infusions (Azzara et al., 2000), or alter acquisition or expression of a CFP in elicited by sucrose in sham-feeding rats (Yu et al., 1999). In real-feeding rats exposed to a fructose solution paired with one novel flavor and a less preferred saccharin solution paired with a second flavor, NTX dose-dependently reduced fructose and saccharin intakes during training. Despite their reduced training intakes, the rats

treated with NTX displayed a preference for the fructose-paired flavor over the saccharin-paired flavor (Baker et al., 2004). This contradictory evidence may be a result of procedural differences especially for the positive result (Mehiel, 1996) in which rats were treated with naloxone only during exposure to the CS+ condition, thereby potentially affecting the quality of the CS+ making it less palatable and thereby equalizing it to the CS-. Central microinjection studies in the amygdala and NAC shell displayed at best modest decreases in the expression of a fructose-CFP following NTX, and no effects following injections into the NAC core significantly change CS+ preference (Bernal et al., 2008b). Moreover, infusions of NTX into either the core or shell regions of the NAC failed to alter the expression of CFP conditioned by the sweet taste of fructose or the post-ingestive consequences of IG glucose (Bernal et al., 2010, Touzani et al., 2008b).

5c) Genetic Variance using Inbred Mice in CFP: As discussed above, strain differences have been observed in the overall intake of noncaloric and caloric sweeteners. However, little research has been performed using the CFP paradigm in inbred mouse strains. In one experiment, B6, 129x1/SvJ, and 129 mouse strains were conditioned by drinking unsweetened and sweetened cue flavors that were paired with simultaneous intragastric infusions of either 16% sucrose or water. Both B6 and 129 mouse strains developed preferences for the sucrose-paired conditioned stimulus; however the CFP in the B6 mice was significantly stronger. This strain difference was possibly a result of the B6 mice drinking more of the CS+ during training than the 129 mice. When exposure to the CS+ and CS- was equalized in sweetened cue flavors, the B6 and 129x1/SvJ showed similar preferences. Since the intragastric infusions isolated the postingestive element of intake, the higher intake of the B6 mice was proposed as being a result of a stronger orosensory response to sucrose (Sclafani and Glendinning, 2005).

Pinhas and coworkers (2012) recently surveyed eight inbred and one outbred strains using a sucrose-CFP paradigm. Mice were exposed for 60 minutes to a 16% sucrose solution vs. a 0.05% saccharin solution paired with cue flavors. All nine inbred strains acquired and maintained a flavor preference. The SWR, DBA2/J, BALB, and SJL mice all showed strong preferences (86-94%) that persisted across six days of two-bottle tests. 129, C3H, and B10 (74-78%) displayed CFP with some variation in the temporal maintenance of the preference. B6 and CD-1 mice displayed a significant, though more modest initial CFP (71-75%) that displayed systematic temporal decrements over the six days of testing.

Section 6: **Rationale, Specific Aims and Hypotheses:** The foregoing sections presented a series of studies that evaluated genetic variance as well as dopaminergic and opioid neurotransmitter modulation of spontaneous sucrose and fat intake and sucrose-CFP. Based on these previous studies, four Specific Aims were proposed. .

- **Specific Aim 1:** Genetic variance in overall sucrose intake will be observed across strains, corroborating previous results from our laboratory (Lewis et al., 2005). Based on findings demonstrating that general opioid antagonists potently reduce intake of sucrose solutions (Apfelbaum and Mandenoff, 1981; Cooper, 1983; Lynch and Libby, 1983), Specific Aim 1 hypothesized that NTX will dose-dependently reduce sucrose intake in all strains. Finally, in combining these two ideas, genetic variance will be observed in the dose-dependent inhibition of sucrose intake following NTX. As of yet, genetic variance in the opioid modulation of sucrose intake has only been observed in a handful of studies (e.g. Haywood and Low, 2001; Yirmaya et al., 1988).
- **Specific Aim 2:** Confirming DA's implication in the reward value of sweet taste (e.g. Bello et al., 2003; Genn et al., 2004), both the D1 (SCH23390) and D2 (raclopride) receptor

antagonists will dose dependently inhibit spontaneous sucrose intake. Virtually no studies have examined the genetic variance in dopaminergic modulation of sucrose intake; however, many studies have observed genetic variance when utilizing inbred mouse strains across a host of pharmacological manipulations. Therefore, the present study hypothesized that genetic variance will be observed in the dose dependent inhibition of sucrose intake following SCH23390 and raclopride.

- **Specific Aim 3:** Genetic variance in overall fat intake will be observed across strains, confirming previous results from our laboratory (Lewis et al., 2007). Both the opioid and dopaminergic systems have been implicated in modulating fat intake (e.g. Gosnell et al., 1990a; Schneider et. al, 1988; Schneider et al., 1989; Smith, 1995), leading to the hypotheses that NTX and SCH23390 will dose dependently reduce Intralipid intake across strains. Furthermore, Specific Aim 3 hypothesized that genetic variance will be observed in the dose dependent response to NTX and SCH23390 upon Intralipid intake.

- **Specific Aim 4:** Specific Aim 4 examined the experiential factors that influence sugar intake through a sucrose-CFP paradigm. Sugar-CFP in inbred mouse strains is also subject to profound genetic variation (Pinhas, et al. 2012). In addition, rat studies have observed differential affect on CFP using various pharmacological antagonists. Systemic administration of a D1 receptor antagonist interfered with both the acquisition and expression of sugar-CFP (e.g. Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). Therefore, as in rats, it was hypothesized that SCH23390 will reduce the acquisition and the expression of a sucrose-CFP in two selected strains of mice. In contrast to dopaminergic influence on sucrose-CFP, prior studies generally fail to support a role for opioids mediation of sucrose-CFP (Azzara et al., 2000; Baker

et al., 2004; Yu et al., 1999). Similarly, in the proposed study, it was hypothesized that NTX will fail to reduce the acquisition and expression of a sucrose-CFP in both strains of mice.

CHAPTER TWO: GENERAL METHODS

Subjects

Outbred (CD-1, Charles River Laboratories, Wilmington, MA) and eight strains of inbred BALB, C3H, B6 , B10, DBA, SJL, SWR and 129 (The Jackson Laboratory, Bar Harbor, ME) male mice (6 weeks of age) were initially acclimated to the Queens College vivarium for 1 week in group (5 per cage) housing. Ten to twelve mice of each inbred strain completed testing in each of the first three paradigms. All strains were used in Specific Aims 1-3, whereas inbred BALB and SWR male mice were used in Specific Aim 4. The animals were housed individually in plastic cages (30×20×15 cm) with stainless steel tops throughout the entire study, and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22 °C. All animals were provided with chow (Lab Diet Mouse Chow 5015) and water ad libitum throughout the experiment, except when conducting the experimental testing.

Testing apparatus

Accurate measurement (± 0.2 ml) of the presented solutions were insured by using a sipper tube apparatus constructed from a leur slip tip syringe (10 ml, 0.2 ml gradations, Pharmaseal Laboratories, Glendale, CA), silicone sealant (All-Glass Aquarium Co., Inc., Franklin, WI), a rubber cork and a straight sipper tube (63 mm in length, 8 mm in width, Lab Products, Seaford, DE). The sipper tube apparatus is built by removing and drilling a hole into the tip of the syringe, inserting the cork and sipper tube into the hole, and securing them with the sealant that also prevents leakage. The sipper tube was firmly secured to the stainless steel top of the cage by a taut metal spring (100 mm) with clips at each end that affixed to the cage top so that the gradations and meniscus are easily visible.

Solutions

For Specific Aims 1 and 2, the mice received a 10% sucrose (Domino Sugar) solution, whereas in Specific Aim 3, the mice received a 5% Intralipid (Baxter Labs) solution. The concentrations were chosen based on previous effectiveness to elicit intake (Lewis et al., 2005, 2007). The Intralipid solution is prepared by diluting 20% Intralipid with water (Baxter Healthcare Corporation, Deerfield, Illinois). For Specific Aim 4, mice received a pre-training solution consisting of an unflavored 0.2% sodium saccharin solution (Sigma Chemical Company, St. Louis, MO). The two training solutions will be comprised of a 16% sucrose (Domino Sugar) and 0.05% sodium saccharin mixture (Sigma) and are both flavored with 0.05% unsweetened orange or lemon-lime Kool-Aid (Kraft Foods, White Plains, NY). Two-bottle preference test solutions present the orange and lemon-lime flavors in a 0.05% saccharin solution. The sucrose paired flavor is referred to as the CS+/S, and the saccharin-paired flavor as the CS−/s, because 16% sucrose is preferred to 0.05% saccharin, a 0.2% saccharin solution mixed with the preferred (CS+/s) and less-preferred (CS-/s) flavors is used during testing. These parameters were chosen on the basis of previous effectiveness in producing CFP (Pinhas et al., 2012).

Statistics

Specific Aims 1-3: A pooled vehicle score comprising these sessions was assessed for each time point for each animal in each strain. The first types of analyses assessed drug-induced changes in cumulative sucrose intake across strains using two three-way randomized block analyses of variance with the nine strains as a between-subjects variable, the pooled vehicle and drug doses as one within-subject variable, and the six test times as the second within-subject variable. Bonferroni comparisons ($P<0.05$) evaluated significant drug effects within groups in this and subsequent analyses. The second types of analyses evaluated individual strain-specific effects of each drug across the dose conditions and six test times upon cumulative sucrose intake using

two-way repeated measures analyses of variance. Because the mouse strains may differ in their 2 h baseline sucrose intakes, the third types of analyses evaluated between-strain differences by transforming the intakes following drug treatments into percent intakes (intake/baseline intake×100), and performing three-way randomized- block analyses of variance with the nine strains as a between-subjects variable, the drug doses as one within-subject variable and the six test times as the second within-subject variable. The fourth type of analyses examined strain-specific differences in the potency of each drug that produced significant dose-dependent inhibitory effects such that post-drug intake difference scores were calculated by subtracting solution intake following each drug dose condition from pooled baseline intake for each animal in each strain. Then linear regression analyses was performed for each time point for each strain with the drug dose as the independent variable and the difference scores for each mouse in each strain as the dependent variable to determine the dose that would inhibit sucrose intake by 50% (ID50). A sensitivity ratio reflecting the potency fold shift of each drug's-induced inhibition of sucrose intake (ID50) was calculated by obtaining the quotient between the least sensitive strain at each time point relative to the most sensitive strain. The fifth type of analyses examined potential differences in narrow-sense trait heritability between strains for those drug doses that previously produced significant inhibitory effects. A better variable for estimating heritability of drug effects would be relative sucrose intake (% of baseline) because of baseline differences in sucrose intake across strains. Heritability was determined by comparing the between-strain sum of squares to the total sum of squares for each percent vehicle baseline score for the most effective drug doses. Because animals are isogenic (i.e., genetically identical) within individual inbred strains, between-strain variance (measured by the sum of squares between-subjects score)

provided a measure of additive genetic ('allelic') variation (VA), whereas within-strain variance ('error variance', measured by the sum of squares error score) represents environmental variability (VE). Thus, using all eight inbred strains, an estimate of narrow-sense heritability (h2) for each trait was obtained using the formula: h2=VA/(VA+VE). Finally, a sixth type of analysis assessed whether differences in the magnitude of DA D1 and D2 receptor antagonist inhibition varies as a function of strains that possessed the sweet-sensitive form of the Tas1R3 receptor (B6, B10, SJL and SWR) relative to the sweet-sub-sensitive form (BALB, C3H, DBA and 129). Two-way analyses of variance were performed on the percentage of vehicle baseline intake score at 30 min for the most effective drug doses.

Specific Aim 4: In the expression study, training intakes were averaged over the five CS+/Suc and five CS-/Sac sessions for each strain, and evaluated for differences. Intakes during the preference tests were averaged over the two sessions at each dose and evaluated with two-way analyses of variance (ANOVA, CS condition vs. Dose) for each strain and drug condition. Separate ANOVAs evaluated percent CS+/s intakes as a function of dose for the strains and drugs.

In the acquisition study, training intakes were averaged over the five CS+/Suc and five CS-/Sac sessions for the three (Veh, SCH23390, NTX) training groups and were analyzed with a two-way ANOVA (CS conditions x Groups). Intakes during the preference tests were averaged over sessions 1 and 2 (referred to as Tests 1 and 2) to control for side position effects. A three-way ANOVA was performed to compare the CS intakes of the vehicle, SCH23390 and vehicle training groups (Group x CS x Test). Separate two-way ANOVAs evaluated percent CS+/s intakes of the four groups. When main or interaction effects were found, Neuman-Kuels corrects comparisons ($p<0.05$) to detect significant effects.

CHAPTER THREE: GENETIC VARIANCE CONTRIBUTES TO NALTREXONE-INDUCED INHIBITION OF SUCROSE INTAKE IN INBRED AND OUTBRED MOUSE STRAINS

Introduction

The inhibitory effects of the general opiate receptor antagonists, naloxone or NTX, appear most apparent when reducing sweet sucrose and/or saccharin solution intakes in rats (Apfelbaum and Mandenoff, 1981; Cooper, 1983; Levine et al., 1982; Lynch and Libby, 1983), persisting under food- restricted or ad libitum conditions (Shabir and Kirkham, 1999; Weldon et al., 1996), acting more potently when sucrose is preferred (Giraudo et al., 1993; Glass et al., 1996), reducing that aspect of feeding activated by sweet taste (Levine et al., 1995), and decreasing the length of the first meal without changes in eating rate (Glass et al., 2001). Naloxone reduces sucrose intake without affecting eating latency, suggesting effects upon the maintenance, rather than the initiation of intake (Kirkham and Blundell, 1984, 1986). Opioid antagonists appear to affect the hedonic and/or orosensory characteristics of sucrose because naloxone continues to inhibit sucrose intake in vagotomized (Clarkson et al., 1982) and sham-feeding (Kirkham and Cooper, 1988a,b; Rockwood and Reid, 1982) rats. Taste-reactivity measures predict the decreases in sucrose intake by opiate antagonists (Parker et al., 1992), and naloxone decreases motivation to respond to sucrose using a progressive reinforcement schedule (Cleary et al., 1996). Although naloxone interferes with odor preferences conditioned by intraoral sucrose infusions in 6-day-old rat pups (Shide and Blass, 1991), NTX fails to alter learning about the flavor- nutrient consequences given that it failed to alter the acquisition or expression of flavor-conditioned sucrose preferences in sham-fed rats, flavor-conditioned

fructose preferences in real- feeding rats, and flavor-nutrient conditioned sucrose preferences (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999).

Through the use of inbred mouse strains, genetic variation has been observed for sucrose and saccharin intake (Bachmanov et al., 1997, 2001; Blizard et al., 1999; Capeless and Whitney, 1995; Fuller, 1974; Inoue et al., 2004; Lewis et al., 2005; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Pothion et al., 2004; Reed et al., 2004; Stockton and Whitney, 1974; Tordoff et al., 2002), accounting for 78% and 83% of the genetic variation associated with consumption of 0.1% saccharin and 3% sucrose, respectively (Ramirez and Fuller, 1976). In studies examining pairs of strains, B6 mice displayed greater intake of five (0.005–1 M) glucose and sucrose concentrations than 101Bag/R1 mice (Stockton and Whitney, 1974), of a 0.1% saccharin solution than DBA mice (Fuller, 1974), and of low sucrose concentrations than 129 mice tested under naïve (Bachmanov et al., 1997, 2001; Sclafani, 2006a,c; Sclafani and Glendinning, 2005; Tordoff et al., 2002), but not experienced (Sclafani, 2006b) conditions. Although some studies (Lush, 1989; Pothion et al., 2004; Reed et al., 2004) examined large numbers of mouse strains for sucrose and saccharin intake, they typically employed either single or multiple supra- threshold concentrations. Our laboratory (Lewis et al., 2005) examined twelve mouse strains across a range of nine sucrose concentrations to determine potential genetic differences in sensitivity, magnitude and alterations in corresponding chow intake. Inbred SWR, A and B6 and outbred CD-1 strains consumed the greatest amount (11–22 ml) of sucrose in 24 h preference tests, and inbred A, B10, SJL and SWR strains consumed the greatest (44–56%) percentages of kilocalories as sucrose. In contrast, the AKR , CBA, C3H and DBA strains consumed the least (7–8 ml) amount of sucrose, and displayed lower (20–30%) percentages of kilocalories consumed as sucrose. Interestingly, higher-consuming (A, B6, B10,

CD-1, SWR, SJL) strains displayed pronounced compensatory chow intake decreases as the percentage of kilocalories consumed as sucrose increased, whereas less-consuming (AKR , C3H, DBA) strains failed to adjust their chow intake even at high sucrose concentrations. These data provide convincing evidence of marked genetic variance in sucrose intake.

Relatively few studies have addressed whether there is genetic variance in opioid modulation of intake of sweet substances. Naloxone reduced the number of consumed sucrose pellets in an operant limited-feeding paradigm using DBA mice (Haywood and Low, 2001). Saccharin intake is reduced in opioid-deficient CXBK mice (Yirmaya et al., 1988), and mice deficient in either met-enkephalin or beta-endorphin showed reduced progressive ratio operant responses for food reinforcement (Haywood et al., 2002). However, no systematic changes in sucrose intake were observed in homozygous and heterozygous pre–pro-enkephalin knockout mice as compared to wild-type mice (Ragnauth et al., 2001), and mice deficient in beta-endorphin were hyperphagic and obese, yet showed normal orexigenic responses to exogenous opioids and normal anorectic responses to naloxone (Appleyard et al., 2003). To examine potential genetic variance in the opioid modulation of sucrose intake, the present study tested eleven inbred (A, AKR , BALB, CBA, C3H, B6 , B10, DBA, SJL, SWR, 129) and one outbred (CD-1) mouse strains for differences in the ability of systemic administration of the general opioid receptor antagonist, NTX to dose-dependently (0.01–5 mg/kg) and time-dependently (5–120 min) decrease intake of a 10% sucrose solution.

This work has been published in the journal, Brain Research (Dym et al., 2008) and presented at the Society for Neuroscience meeting (Dym et al., 2006)

Experimental Procedure

Subjects: Outbred (CD-1, Charles River Laboratories, Wilmington, MA; n = 10) and inbred A, AKR , BALB, CBA, C3H, B6, B10 , DBA , SJL , SWR and 129 (Jackson Laboratories, Bar Harbor, ME; n = 10 each) male mice (6 weeks of age). For housing conditions see: General Methods.

Sucrose Intake Procedure: All procedures were approved by the Queens College Institutional Animal Care and Use Committee. At the start of the testing procedure at 2–9 h into the light cycle, chow and water were removed from the cage, and each animal was provided with an approximate 8 ml ration of a 10% sucrose solution in the sipper tube that left about 2 ml of air to allow easy licking access to the sucrose (see: General Methods for detailed description of Testing Apparatus). Sucrose intake was measured by reading the meniscus of the sucrose solutions along the gradations after 5, 15, 30, 60, 90, and 120 min whereupon the sipper tube was removed and food and water immediately returned. Each animal was exposed to only one sucrose bout per day until a criterion minimum of 1 ml was consumed over three consecutive exposures; three to six trials were typically needed to reach criterion for the successful strains in tests conducted approximately three times each week. For the three strains that failed to reach criterion, individual mice were tested anywhere from 7 to 20 times in efforts to reach criterion intake. This criterion was employed to avoid “floor effects” of antagonist treatment. Following this initial baseline treatment, each mouse (n =7–10) of each strain received an intraperitoneal vehicle (0.3 ml distilled water/30 g body weight) injection, and sucrose intake was assessed as described previously. Following determination that each mouse met the minimal 1 ml criterion sucrose intake following vehicle treatment over 1–2 exposures, they were tested at minimal 72 h intervals across an identical sucrose intake time course following intraperitoneal injection of NTX hydrochloride (Sigma Chemical Co., St. Louis, MO) at total doses of 0.01, 0.1, 1.0 and

5.0 mg/kg. Half of the animals of each strain, matched for vehicle sucrose intake, received an ascending series of NTX doses, whereas the remainder received a descending series of NTX doses. Following all NTX treatments, sucrose intake was reassessed following a second vehicle injection. Analyses of sucrose intake under baseline and the two vehicle treatments failed to reveal any differences, and therefore, these data were pooled as a representative baseline treatment for each strain.

Results

Strain differences in sucrose intake following vehicle control injections: Whereas nine of the strains (BALB, C3H, B6, B10, CD-1, DBA , SJL , SWR, 129) reached the1 ml criterion during baseline and vehicle testing, mice in three strains (A [5–120 min range: 0.3–0.8 ml], AKR [5–120 min range: 0.2–0.5 ml], CBA [5–120 min range: 0.2–0.4 ml]) failed to meet this criterion even after 7–20 exposures, and therefore did not continue in the study. Significant differences in sucrose intake were observed among the nine mouse strains ($F(8,80) = 14.01$, $P < 0.0001$), among vehicle and NTX doses ($F(4,40) = 49.65$, $P < 0.0001$), across test times ($F(5,20) = 459.65$, $P < 0.0001$), and for the interactions between strains and doses ($F(32,320) = 2.14$, $P < 0.001$), between strains and times ($F(40,160) = 10.51$, $P < 0.0001$), and between doses and times ($F(20,80) = 14.14$, $P < 0.0001$), but not among strains, doses and times ($F(160,640) = 1.18$, ns). Outbred CD-1 mice consumed significantly greater amounts of sucrose following vehicle injections than the eight inbred groups across the entire time course. A rank-order of the cumulative 2 h sucrose intake among the eight inbred strains was: DBA > 129 > BALB ~ SWR > SJL > C3H ~ B6 ~ B10 . To adjust for baseline differences in sucrose intake across strains, the effects of NTX across doses and times were evaluated within each strain as well as an evaluation of the percent baseline values across strains, doses and times.

Strain differences in naltrexone-induced inhibition of sucrose intake: Marked strain-specific effects were observed in the dose- dependent and time-dependent ability of NTX to significantly reduce sucrose intake; the data are presented in the order of most potent to least potent strain effects. B10 mice displayed significant differences in sucrose intake among doses ($F(4,35) = 19.00$, $P < 0.0001$), across times ($F(5,20) = 67.57$, $P < 0.0001$) and for the dose and time interaction ($F(20,175) = 4.14$, $P < 0.0001$) with significant reductions in sucrose intake noted following the 0.01 (30–90 min), 0.1 (15–120 min), 1 (5–120 min) and 5 (5–120 min) mg/kg doses (Figure 1D). Similarly, B6 mice displayed significant differences in sucrose intake among doses ($F(4,45) = 7.98$, $P < 0.0001$), across times ($F(5,20) = 52.70$, $P < 0.0001$) and for the dose and time interaction ($F(20,225) = 1.86$, $P < 0.017$) with significant reductions in sucrose intake noted following the 0.01 (60–120 min), 0.1 (15–120 min), 1 (30–120 min) and 5 (15–120 min) mg/kg doses (Figure 1C). 129 mice displayed significant differences in sucrose intake across times ($F(5,20) = 104.21$, $P < 0.0001$), but not among doses ($F(4,45) = 2.37$, $P > 0.067$) or for the dose and time interaction ($F(20,225) = 1.19$, $P > 0.26$) with significant reductions in sucrose intake noted following the 0.01 (120 min), 1 (120 min), 5 (15–30, 90–120 min), but not 0.1 mg/kg doses (Figure 1I). SJL mice displayed significant differences in sucrose intake across times ($F(5,20) = 88.00$, $P < 0.0001$) and for the dose and time interaction ($F(20,175) = 2.51$, $P < 0.0007$), but not among doses ($F(4,35) = 2.28$, $P > 0.081$) with significant reductions in sucrose intake noted following the 0.1 (90 min), 1 (60–90 min), 5 (60–120 min), but not 0.01 mg/kg doses (Figure 1G). DBA mice displayed significant differences in sucrose intake among doses ($F(4,35) = 9.09$, $P < 0.0001$), across times ($F(5,20) = 136.65$, $P < 0.0001$) and for the dose and time interaction ($F(20,175) = 2.90$, $P < 0.0001$) with significant reductions in sucrose intake noted following the 1 (60–120 min), 5 (30–120 min),

but not 0.01 or 0.1 mg/kg doses (Figure 1F). Outbred CD-1 mice displayed significant differences in sucrose intake among doses ($F(4,30) = 3.39$, $P < 0.021$), across times ($F(5,20) = 126.07$, $P < 0.0001$), but not for the dose and time interaction ($F(20,150) = 1.30$, $P > 0.19$) with significant reductions in sucrose intake noted following the 1 (60–120 min), 5 (60–120 min), but not 0.01 or 0.1 mg/kg doses (Figure 1E). C3H mice displayed significant differences in sucrose intake across times ($F(5,20) = 102.91$, $P < 0.0001$), but not among doses ($F(4,35) = 2.06$, $P > 0.11$) or for the dose and time interaction ($F(20,175) = 1.58$, $P > 0.061$) with significant reductions in sucrose intake noted following the 1 (120 min), 5 (30–120 min), but not 0.01 or 0.1 mg/kg doses (Figure 1B). BALB mice displayed significant differences in sucrose intake among doses ($F(4,45) = 4.05$, $P < 0.007$), across times ($F(5,20) = 257.84$, $P < 0.0001$), but not for the dose and time interaction ($F(20,225) = 1.43$, $P > 0.11$) with significant reductions in sucrose intake noted following the 5 (30–120 min), but not 0.01, 0.1 or 1 mg/kg doses (Figure 1A). In contrast, SWR mice displayed significant differences in sucrose intake across times ($F(5,20) = 126.47$, $P < 0.0001$), but not among doses ($F(4,50) = 2.05$, $P > 0.10$) or for the dose and time interaction ($F(20,250) = 0.93$, $P > 0.55$) with reductions in sucrose intake failing to occur at any of the NTX doses (Figure 1H). To assess inter-strain differences in NTX-induced inhibition of sucrose intake in the presence of baseline differences in sucrose intake following vehicle treatment, percent of baseline values were determined for each strain at each NTX dose and at each time point.

Strain differences in percent baseline values in naltrexone-induced inhibition of sucrose intake: Significant differences in percent baseline values of sucrose intake following NTX were observed among the nine mouse strains ($F(8,80) = 9.17$, $P < 0.0001$), among the NTX doses ($F(3,30) = 46.29$, $P < 0.0001$), across test times ($F(5,50) = 6.15$, $P < 0.0002$), and for the

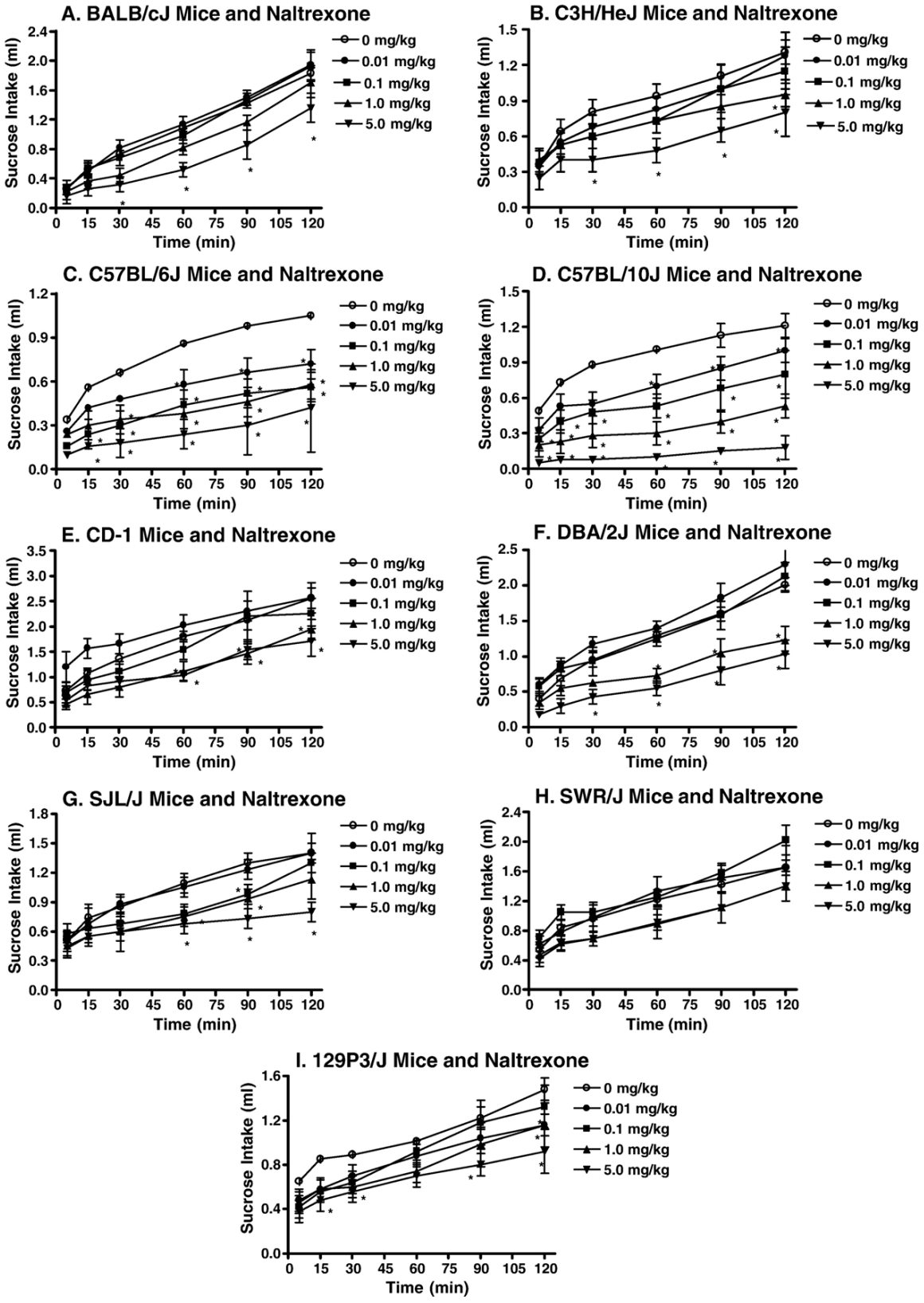
A. BALB/cJ Mice and Naltrexone
B. C3H/HeJ Mice and Naltrexone
C. C57BL/6J Mice and Naltrexone
D. C57BL/10J Mice and Naltrexone
E. CD-1 Mice and Naltrexone
F. DBA/2J Mice and Naltrexone
G. SJL/J Mice and Naltrexone
H. SWR/J Mice and Naltrexone
I. 129P3/J Mice and Naltrexone
Sucrose Intake (ml)
Time (min)
0 mg/kg
0.01 mg/kg
0.1 mg/kg
1.0 mg/kg
5.0 mg/kg

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Figure 1. Alterations in cumulative sucrose intake (mean±SEM) across a 120 min time course following vehicle and four NTX doses in nine mouse strains. Although it would be preferable to have identical y-axis scales in all figures, the different Y-axes were employed to allow for clearer reading of the NTX-induced effects within each strain. The asterisks (*) denote significant reductions in sucrose intake for that NTX dose relative to corresponding vehicle values for that particular mouse strain (Tukey comparisons, $P < 0.05$).

interaction among strains, doses and times ($F(120,1200) = 1.78$, $P < 0.0001$), but not for the interactions between strains and doses ($F(24,240) = 2.26$, ns), between strains and times ($F(40,400) = 1.41$, ns), and between doses and times ($F(15,150) = 1.68$, ns). Table 1 summarizes the differences in NTX-induced inhibition across doses, strains and test times using percent baseline sucrose intake scores. B10 mice displayed significantly greater degrees of inhibition than SWR mice following the 0.01 (60 min), 0.1 (5–120 min), 1 (60–120 min) and 5 (15–120 min) mg/kg NTX doses, than BALB mice following the 0.1 (90 min), 1 (60–120 min) and 5 (30, 90–120 min) mg/kg NTX doses, than DBA mice following the 0.01 (5, 30 min) and 0.1 (5, 60 min) mg/kg NTX doses, than CD-1 mice following the 0.1 (30 min) and 5 (30, 90–120 min) mg/kg NTX doses, than 129 mice following the 1 (60–90 min) and 5 (30–120 min) mg/kg NTX doses, than SJL mice following the 5 (30–60 min) mg/kg NTX dose, and than C3H mice following the 1 (60 min) and 5 (120 min) mg/kg NTX doses. B6 mice displayed significantly greater degrees of inhibition than SWR mice following the 0.01 (60 min), 0.1 (5–60, 120 min), and 5 (30–60, 120 min) mg/kg NTX doses, than BALB mice following the 0.1 (15–30, 90–120 min) mg/kg NTX dose, than DBA mice following the 0.01 (5, 30–120 min) and 0.1 (5–120 min) mg/kg NTX doses, than CD-1 mice following the 0.01 (30 min) and 0.1 (90 min) mg/kg NTX doses, and than 129 mice following the 5 (60 min) mg/kg NTX dose. 129 mice displayed significantly greater degrees of inhibition than SWR mice following the 0.1 (5–15 min) NTX dose, than DBA mice following the 0.01 (5 min) and 0.1 (5 min) mg/kg NTX doses, and than CD-1 mice following the 0.01 (15 min) mg/kg NTX dose.

Strain differences in the ID50 of naltrexone-induced inhibition of sucrose intake: Table 2 summarizes the ID50 values for NTX's ability to inhibit sucrose intake across the 120 min time course. In agreement with the strain differences in NTX-induced inhibition in the percent of

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Table 1. NTX inhibition of sucrose intake across strains, doses and test times as a measure of percent baseline intake

Strain	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
(A) NTX 0.01 mg/kg						
BALB/cJ	1.26	1.29	1.12	1.04	1.03	1.05
C3H/HeJ	1.00	0.86	0.85	0.88	0.89	0.95
C57BL/6J	0.83	0.80	0.76	0.67	0.67	0.67
C57BL/10J	0.69	0.71	0.60	0.66	0.73	0.81
CD-1	1.61	1.43$^{\#}$	1.19*	1.13	1.06	1.00
DBA/2J	2.15*$^{,+,\#}$	1.30	1.28*$^{,+}$	1.10*	1.14*	1.14*
SJL/J	1.00	1.04	1.05	0.99	0.96	0.99
SWR/J	1.35	1.06	1.07	1.08*$^{,+}$	1.06	1.02
129P3/J	0.74	0.69	0.80	0.89	0.87	0.81
(B) NTX 0.1 mg/kg						
BALB/cJ	1.36	1.22*	0.95*	0.90	1.04*$^{,+}$	1.06*
C3H/HeJ	1.14	0.86	0.73	0.75	0.89	0.86
C57BL/6J	0.45	0.44	0.46	0.51	0.51	0.51
C57BL/10J	0.51	0.55	0.51	0.50	0.56	0.65
CD-1	0.91	0.87	0.80^{+}	0.84	1.01*	0.89
DBA/2J	2.08*$^{,+,\#}$	1.21*	0.99*	0.99*$^{,+}$	1.01*	1.08*
SJL/J	1.16	0.90	0.80	0.71	0.78	0.95
SWR/J	2.04*$^{,+,\#}$	1.38*$^{,+,\#}$	1.14*$^{,+}$	1.05*$^{,+}$	1.13^{+}	1.26*$^{,+}$
129P3/J	0.66	0.65	0.72	0.90	1.01	0.89
(C) NTX 1.0 mg/kg						
BALB/cJ	1.1	0.93	0.61	0.73^{+}	0.80^{+}	0.93^{+}
C3H/HeJ	1.26	0.90	0.79	0.79^{+}	0.78	0.71
C57BL/6J	0.75	0.54	0.53	0.45	0.46	0.55
C57BL/10J	0.44	0.31	0.30	0.30	0.34	0.43
CD-1	0.60	0.56	0.56	0.64	0.70	0.76
DBA/2J	0.89	0.85	0.69	0.60	0.69	0.63

Strain	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
SJL/J	0.89	0.75	0.71	0.71	0.73	0.79
SWR/J	1.18	0.85	0.78	0.77^{+}	0.84^{+}	0.92^{+}
129P3/J	0.74	0.70	0.68	0.74^{+}	0.83^{+}	0.77
(D) NTX 5.0 mg/kg						
BALB/cJ	0.61	0.48	0.42^{+}	0.47	0.59^{+}	0.75^{+}
C3H/HeJ	0.65	0.61	0.49	0.50	0.59	0.60^{+}
C57BL/6J	0.34	0.29	0.30	0.28	0.28	0.39
C57BL/10J	0.10	0.11	0.09	0.11	0.13	0.15
CD-1	0.74	0.80	0.69^{+}	0.57	0.71^{+}	0.67^{+}
DBA/2J	0.44	0.45	0.48	0.43	0.53	0.50
SJL/J	0.88	0.74	0.69^{+}	0.60^{+}	0.56	0.56
SWR/J	1.03	0.81^{+}	0.76*$^{,+}$	0.79*$^{,+}$	0.80^{+}	0.87*$^{,+}$
129P3/J	0.58	0.58	0.63^{+}	0.70*$^{,+}$	0.67^{+}	0.61^{+}

Note. Significantly ($P < 0.05$) less inhibition than C57BL/6J(*), C57BL/10J (+) or 129P3/J (#).

Table 2. Linear regression values (ID_{50}) and fold shift of NTX-induced inhibition of sucrose intake among the inbred and outbred mouse strains across the 120 min time course.

Linear regression ID_{50}						
Strain	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
BALB/cJ[3]	6.20	4.69	4.02	4.61	5.94	8.88
C3H/HeJ[3]	8.16	7.98	4.85	4.79	6.12	6.55
C57BL/6J[2]	2.36	1.53	1.41	1.53	1.62	2.54
C57BL/10J[1]	0.75	0.40	0.49	0.54	1.05	1.62
CD-1[4]	6.69	7.97	7.01	5.37	8.00	7.97
DBA/2J[3]	4.40	4.33	5.17	4.69	4.59	4.61
SJL/J[4]	12.74	14.59	9.89	7.62	5.80	5.76
SWR/J[5]	7.96	8.28	8.75	8.80	9.45	12.07
129P3/J[2]	8.81	8.07	10.14	10.02	7.85	7.80
Sensitivity Ratio	17.0	36.5	20.7	18.5	9.0	7.5

Note. Variations in individual strain ID_{50} values across the time course may reflect the latency at which they first displayed significant dose-dependent reductions in sucrose intake following NTX: [1]: 5 min; [2]: 15 min; [3]: 30 min; [4]: 60 min; [5]: no reduction. The sensitivity ratio reflects the potency fold shift of NTX's inhibition of sucrose intake (ID_{50}) between the least sensitive strain (SJL/J (5, 15 min); SWR/J (90, 120 min);129P3/J (30, 60 min)) at each time point relative to the most sensitive strain (always C57BL/10J).

baseline sucrose intake, the B10 and B6 strains typically displayed the most potent inhibition of sucrose intake by NTX across all time points. BALB, C3H, CD-1 and DBA mice typically displayed less potent, but similar degrees of NTX- induced inhibition across all time points. In contrast, SJL, SWR and 129 strains displayed the least potency of NTX-induced inhibition of sucrose intake as a function of time. Indeed, variations in individual strain ID50 values across the time course appeared to reflect the latency at which each given strain first displayed significant dose- dependent reductions in sucrose intake following NTX: 5 (B10), 15 (B6 and 129), 30 (BALB, C3H, DBA), 60 (CD-1, SJL) min and no reduction (SWR). Thus, B10 mice displayed 17–36.5-fold greater potency than SJL mice after 5 and 15 min, 18.5–20.5-fold greater potency than 129 mice after 30 and 60 min, and 7.5–9-fold greater potency than SWR mice after 90 and 120 min. It should be noted that the rank-order of strain differences in sucrose intake following vehicle failed to correlate (r = 0.283, ns) with the rank-order of the ID50 of NTX-induced inhibition of sucrose intake after the 120 min time course, indicating that the potency of opioid effects on sucrose intake appeared unrelated the short-term consumption of sucrose in that particular strain.

Heritability estimates of naltrexone-induced inhibition of sucrose intake: Table 3 summarizes the narrow-sense heritability estimates for each NTX dose at each time point. Whereas moderate (h^2 = 0.51–0.63) heritability estimates were obtained for baseline vehicle sucrose intake across the time course, smaller though consistent (e.g., h^2 = 0.38–0.51) heritability estimates were typically obtained across the time course for the different NTX doses, although lower (h^2 = 0.18–0.31) heritability estimates were observed, particularly at the middle (1 mg/kg) NTX dose.

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Table 3. Heritability estimates for cumulative sucrose intake scores across strains for each NTX dose and each time point

Time → Condition ↓	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
Baseline/Vehicle (mg/kg)	0.51	0.42	0.47	0.58	0.59	0.63
0.01	0.39	0.42	0.45	0.51	0.50	0.43
0.1	0.39	0.42	0.38	0.46	0.43	0.54
1.0	0.18	0.19	0.21	0.31	0.38	0.40
5.0	0.45	0.51	0.49	0.40	0.40	0.42

Discussion

Marked strain differences were observed in the magnitude, time course and potency of NTX to inhibit intake of a 10% sucrose solution over a 2 h time course, indicating the presence of strong genetic variance in this pharmacological response. Given that NTX has a relatively limited (~2 h) duration of action with peak magnitude of effects observed after 1 h, the eleven different inbred and one outbred mouse strains were assessed for sucrose intake over a shorter 2 h time course. Lewis et al. (2005) found marked strain differences in a series of 24 h two-bottle preference tests across nine sucrose concentrations. Interestingly, the pattern of strain differences observed for the 10% sucrose solution in 24 h preference tests (Lewis et al., 2005) failed to correlate ($r = 0.28$, ns) with the present pattern of strain differences in 2 h sucrose intake following vehicle administration. Whereas AKR and CBA mice drank relatively little sucrose over the 2 and 24 h time courses, the A and B6 strains consumed respectively low and high amounts of sucrose after 2 and 24 h. Whereas SWR, SJL and CD-1 mice consumed relatively high amounts of sucrose after 2 and 24 h, BALB, C3H, 129 and B10 mice consumed moderate amounts of sucrose after 2 and 24 h. Finally, DBA consumed respectively high and low amounts of sucrose after 2 and 24 h.

Genetic variability in NTX-induced inhibition of sucrose intake could be clearly demonstrated in measures of magnitude of effect, sensitivity and potency of the NTX doses, and latency to produce inhibition over the time course. The B6 (~ 70–75%) and B10 (~ 80%) mouse strains displayed significant reductions in sucrose intake relative to corresponding vehicle treatment following all NTX doses. The 129 and SJL mouse strains displayed significant NTX-induced inhibition (35–40%) of sucrose intake relative to vehicle at both higher doses and at one of the two lower NTX doses. The outbred CD-1 and inbred DBA and C3H strains displayed significant NTX-induced inhibition (~ 50%) of sucrose intake relative to vehicle at only the two

higher doses, whereas the BALB strain displayed significant NTX-induced inhibition (~ 50%) only the highest dose. In contrast, SWR mice failed to display any significant reduction in sucrose intake at any time point following any of the NTX doses.

Inter-strain differences in NTX-induced inhibition of sucrose intake were apparent as well and paralleled the differences relative to vehicle treatment. B10 mice displayed significantly greater percentages of NTX-induced inhibition of sucrose intake than six inbred (BALB, C3H, DBA, SJL, SWR and 129) and the single outbred (CD-1) strains. Correspondingly, B6 mice displayed significantly greater percentages of NTX-induced inhibition of sucrose intake than four inbred (BALB, DBA, SWR and 129) and the single outbred (CD-1) strains. Interestingly, 129 mice displayed significantly greater percentages of NTX-induced inhibition of sucrose intake than two inbred (DBA, SWR) and the single outbred (CD-1) strains.

Marked strain differences in NTX-induced inhibition of sucrose intake could also be observed in terms of sensitivity and potency of the NTX doses. Again, B10 mice displayed the greatest potency of NTX-induced inhibitory effects across the time course (ID50 = 0.40–1.62 mg/kg). Correspondingly, B6 showed a comparable potency across the time course (ID50 = 1.41–2.54 mg/kg). Intermediate degrees of NTX's potency in inhibiting sucrose intake were observed across the time course in DBA (ID50 = 4.33–5.17 mg/kg), BALB (ID50 = 4.02–8.88 mg/kg), C3H (ID50 = 4.79–8.16 mg/kg) and CD-1 (ID50 = 5.37–8.00 mg/kg) strains. Although SJL and 129 mice displayed significant reductions in the magnitude of NTX-induced inhibition of sucrose intake, their potency of effects were less profound (ID50 range: SJL : 5.76–14.59 mg/kg; 129: 7.80–10.14 mg/kg) because of smaller degrees (typically 30–40%) of inhibition across the whole dose range. As expected, SWR mice displayed a low potency (ID50 = 7.96–12.07 mg/kg) of NTX-induced inhibition of sucrose intake. Such marked differences among

strains are clearly apparent given the 7.5–36.5 fold differences in the ID50 of NTX-induced inhibition of sucrose intake between the most sensitive (always B10) and least sensitive (129, SJL , SWR) strains at each time point.

The latency at which NTX initially exhibited significant effects also was subject to strain variability. The original finding in rats that naloxone primarily affected the maintenance, rather than the initiation of intake (Kirkham and Blundell, 1984; Kirkham and Blundell, 1986) was largely replicated in BALB, CD-1, C3H, DBA and SJL mice in which significant reductions in sucrose intake following different NTX doses typically began to occur 60–120 min after injection with intake after shorter 5–30 min intervals failing to differ from vehicle values. In contrast, significant reductions in sucrose intake occurred across the 120-min time course in B6, B10 and 129 mice, particularly at the higher effective doses for each strain. Yet, only at best, moderate heritability estimates were observed for the percentage of NTX inhibition over baseline intake across the time course for the 0.01 (h^2 = 0.39–0.51), 0.1 (h^2 = 0.38–0.54), 1 (h^2 = 0.18–0.40) and 5 (h^2 = 0.40–0.51) mg/kg NTX doses.

Given the fact that NTX is short-acting, and therefore must be assessed in short-term (2 h) intake tests as compared to typical 24 h intake measures, important differences in strain-specific effects upon sucrose intake must be taken into account. Importantly, correlations between short (2 h) and long (24 h) term sucrose intake failed to reveal significant relationships with each other, and strains that had high sucrose intake per se (e.g., SWR) were quite insensitive to NTX. Therefore, such data limit this finding of differential opioid modulation of sucrose intake as a function of genetic variance to these short-term intake tests that may be more indicative of hedonic and palatable consequences of sucrose relative to longer-term homeostatic

and nutritive actions. That caveat considered, these data still provide convincing evidence of marked genetic variance in sucrose intake.

Marked genetic variance has been observed for the opioid modulation of sucrose intake in mouse strains. The DA pharmacological system also plays an extensive role in food intake under a wide variety of situations, but particularly those mediated by hedonics in rodents. Given the role of DA in the modulation of sucrose intake, the next chapter evaluated genetic variance in the dopaminergic modulation of sucrose intake.

CHAPTER FOUR: GENETIC VARIANCE CONTRIBUTES TO DA RECEPTOR ANTAGONIST-INDUCED INHIBITION OF SUCROSE INTAKE IN INBRED AND OUTBRED MOUSE STRAINS

Introduction

As described in the previous chapter, there is much evidence that the opioid system is highly involved in the ingestive response to sweet solutions. The next chapter will examine what role the DA system plays in the ingestion of a sucrose solution in inbred mouse strains.

The precise role for DA in reward has been a consistent source of debate since the proposal of the "anhedonia hypothesis" over a quarter-century ago (e.g., Wise, 1982; Wise et al., 1978), and continues unabated in recent reviews (e.g., Baldo and Kelley, 2007; Barbano and Cador, 2007; Berridge, 2007; Salamone et al., 2007). In place of the anhedonia hypothesis, Berridge (Berridge, 1996; Berridge and Robinson, 1998) proposed a role for brain DA in the psychological construct, "wanting", that is, the attribution of incentive salience to reward-related stimuli. An alternative role for brain opioids was proposed in "liking", that is, the hedonic impact of reward.

Differential involvement for opioids and DA were found for reinforcement processes related to food and water intake (e.g., Agmo et al., 1993; Agmo et al., 1995). Sucrose and saccharin intake were significantly reduced by systemic pretreatment with opioid (e.g., Apfelbaum and Mandenoff, 1981; Cooper, 1983; Levine et al., 1982; Lynch and Libby, 1983) and DA D1 and D2 (e.g., Bello and Hajnal, 2006; Muscat and Willner, 1989; Tyrka et al., 1992) antagonists in rats. Using the sham-feeding procedure (Weingarten and Watson, 1982) in which ingested fluid drains out of an open gastric fistula thereby minimizing postingestive nutritive effects (Sclafani and Nissenbaum, 1985), sugar intake is also reduced following pretreatment

with opioid (Kirkham and Cooper, 1988a; Kirkham and Cooper, 1988b; Rockwood and Reid, 1982) and DA D1 and D2 (Geary and Smith, 1985; Schneider et al., 1990) antagonists. However, opioid and DA antagonists differed in their effects upon sugar-conditioned flavor preferences. Whereas the opioid antagonist, NTX failed to reduce sucrose-conditioned flavor preferences in sham-feeding rats (Yu et al., 1999), fructose-conditioned flavor preferences in real-feeding rats (Baker et al., 2004) or sucrose-conditioned flavor–nutrient preferences induced by intragastric sucrose (Azzara et al., 2000), DA D1 and/or D2 receptor antagonism significantly reduced sucrose-conditioned and fructose-conditioned flavor preferences in these conditioning paradigms (Azzara et al., 2001; Baker et al., 2003; Hsiao and Smith, 1995; Yu et al., 2000a; Yu et al., 2000b).

Through the use of inbred mouse strains, genetic variation has been observed for sucrose and saccharin intake (Bachmanov et al., 1997, 2001; Blizard et al., 1999; Capeless and Whitney, 1995; Fuller, 1974; Inoue et al., 2004; Lewis et al., 2005; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Pothion et al., 2004; Reed et al., 2004; Stockton and Whitney, 1974; Tordoff et al., 2002), accounting for 78% and 83% of the genetic variation associated with consumption of 0.1% saccharin and 3% sucrose, respectively (Ramirez and Fuller, 1976). The genetic variation in the response to saccharin and dilute

sucrose solutions is largely explained by polymorphisms in the Tas1r3 gene that encodes for the T1R3 sweet receptor (Reed et al., 2004). In studies examining pairs of strains, B6 mice displayed greater intake of five (0.005–1M) glucose and sucrose concentrations than 101Bag/R1 mice (Stockton and Whitney, 1974), of a 0.1% saccharin solution than DBA mice (Fuller, 1974), and of low sucrose concentrations than 129 mice (Bachmanov et al., 1997, 2001; Sclafani, 2006a,c; Sclafani and Glendinning, 2005; Tordoff et al., 2002). Examination of 12 mouse strains across a

range of nine sucrose concentrations revealed profound genetic variation in the sensitivity and magnitude of intake as well as alterations in corresponding chow intake (Lewis et al., 2005). Intakes of dilute (0.1%) but not concentrated (10%) sucrose correlated with Tas1r3 polymorphisms indicating that sweet taste sensitivity does not completely explain the consumption of calorically dense sugar solutions (Inoue et al., 2004). Moreover, profound genetic variance was also observed in NTX's ability to reduce intake of a 10% sucrose solution in eight inbred and one outbred mouse strains (Chapter Three). To examine potential genetic variance in the dopaminergic receptor modulation of sucrose intake, the present study tested eight inbred (BALB, C3H, B6, B10, DBA, SJL, SWR and 129) and one outbred (CD-1) mouse strains for differences in the ability of systemic administration of D1 (SCH23390) and D2 (raclopride) DA receptor antagonists to dose-dependently (50–1600 nmol/kg) and time-dependently (5–120 min) decrease intake of a 10% sucrose solution.

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This work has been published in the journal, Brain Research (Dym et al., 2009) and presented at the Society for Neuroscience meeting (Dym et al., 2007).

Experimental Procedure

Subjects: Outbred (CD-1, Charles River Laboratories, Wilmington, MA; n = 11) and eight strains of inbred BALB, C3H, B6, B10, DBA, SJL, SWR and 129 (The Jackson Laboratory, Bar

Harbor, ME) male mice (6 weeks of age) were initially acclimated to the Queens College vivarium for 1 week in group (5 per cage) housing. Ten to twelve mice of each inbred strain completed testing; variability was due to isolated deaths of animals due to unrelated factors. For housing conditions see: General Methods.

Sucrose intake procedure: All procedures were approved by the Queens College Institutional Animal Care and Use Committee. At the start of the testing procedure at approximately 3–7 h into the light cycle, chow and water were removed from the cage, and each animal was given access to approximately 8 ml of 10% sucrose in the sipper tube for 2 h (see: General Methods for detailed description of Testing Apparatus). Sucrose intake was measured as described in Chapter 3. Following determination that each mouse met the minimal 1 ml criterion sucrose intake following vehicle treatment, 2-hr sucrose intakes were measured 30-min after sc injection of the D1 (SCH23390) and D2 (raclopride) DA receptor antagonists (Sigma Chemical Co., St Louis, MO) at doses of 50, 200, 400, 800 and 1600 nmol/kg. The drugs were mixed at concentrations of 5, 20, 40, 80 and 160 nmol/ml and administered at 10 ml/kg with a minimum 72 h interval between doses. This dose range and post-injection interval were chosen on the basis of significant effects observed in rats (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a; Yu et al., 2000b). Subgroups of animals of each strain, matched for vehicle sucrose intake, received an ascending series of SCH23390 doses followed by a descending series of raclopride doses, a descending series of SCH23390 doses followed by an ascending series of raclopride doses, an ascending series of raclopride doses followed by a descending series of SCH23390 doses, and a descending series of raclopride doses followed by an ascending series of SCH23390 doses. Orders of drug or specific dose presentation failed to produce any significant effects, insuring lack of order or carry-over effects. Following all DA antagonist treatments, sucrose intake was

reassessed following a second vehicle injection. Analyses of sucrose intake under baseline and the two vehicle treatments failed to reveal any differences, and therefore, these data were pooled as a representative baseline treatment for each strain. Sample size differences among strains reflected unrelated deaths of individual animals.

Statistics: All statistical procedures are described in the General Methods. However, a sixth type of analysis assessed whether differences in the magnitude of DA D1 and D2 receptor antagonist inhibition varied as a function of strains that possessed the sweet-sensitive form of the Tas1R3 receptor (B6, B10, SJL and SWR) relative to the sweet-sub-sensitive form (BALB, C3H, DBA and 129) (Reed et al., 2004). Two-way analyses of variance were performed on the percentage of vehicle baseline intake score at 30 min for the five doses of SCH23390 and the high dose of raclopride, all of which displayed significant inhibition of sucrose intake. The 30 min intake point was chosen because overall baseline intakes of the sweet-sensitive and sweet-sub-sensitive strains were equal at this interval.

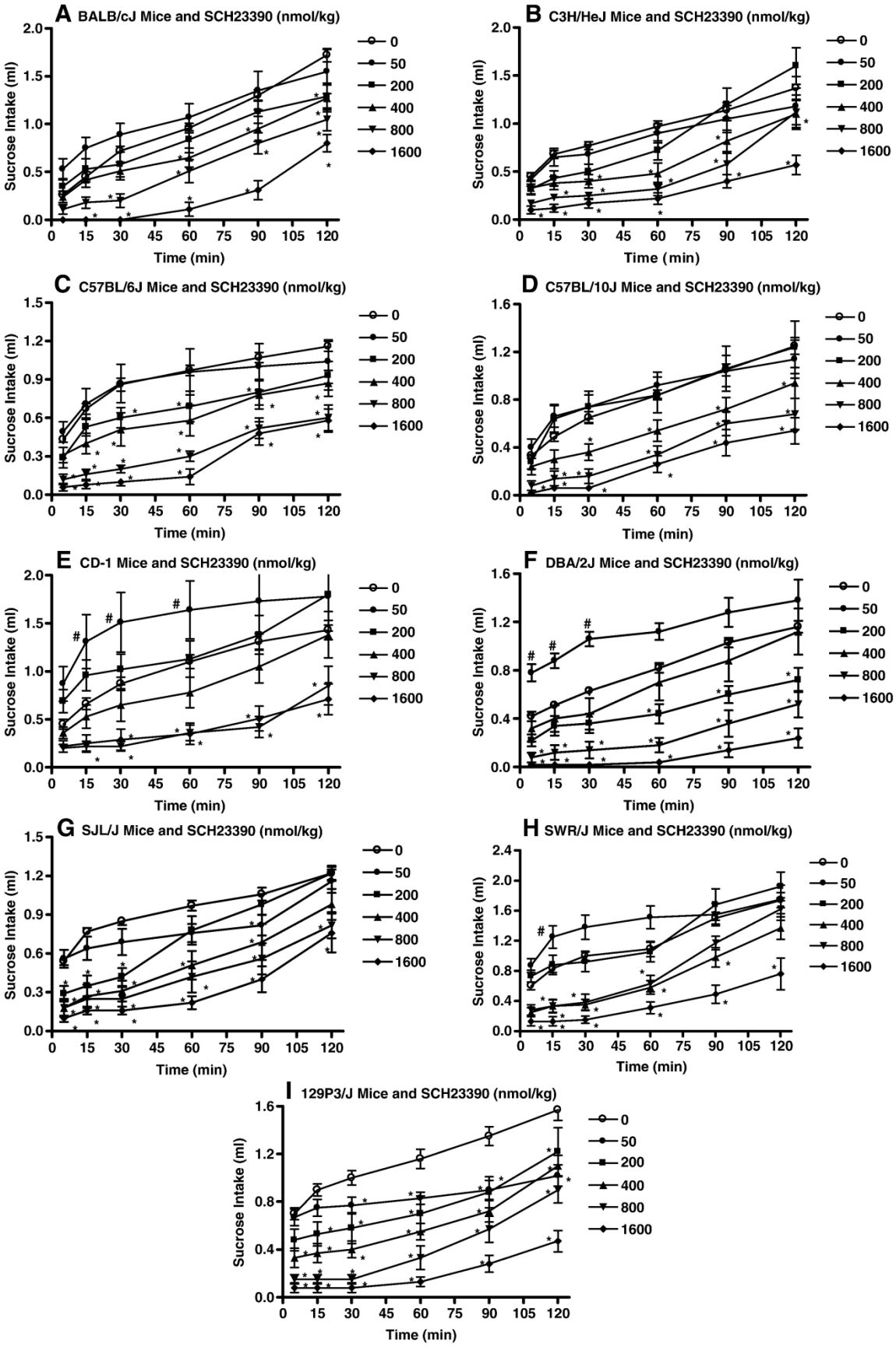
Results

Strain differences in sucrose intake following vehicle baseline injections: Evaluation of sucrose intake following vehicle baseline injections revealed significant differences among strains ($F(8,88) = 8.53$, $P < 0.0001$) and for the interaction between strains and test times ($F(40,440) = 8.83$, $P < 0.0001$). The rank-order of the cumulative 2 h baseline vehicle sucrose intake among the nine strains was: SWR (1.7 ml), BALB (1.7 ml), 129 (1.6 ml), CD1 (1.4 ml), C3H (1.4 ml), B10 (1.3 ml), SJL (1.2 ml), B6 (1.2 ml), DBA (1.2 ml). Thus, to adjust for baseline differences in sucrose intake across strains, the effects of SCH23390 and raclopride across doses and times were evaluated within each strain as well as an evaluation of the percent vehicle baseline values across strains, doses and times.

Strain differences in DA D1 antagonist (SCH23390)-induced inhibition of sucrose intake:

Overall significant differences in sucrose intake following SCH23390 were observed among the nine mouse strains ($F(8,86) = 5.54$, $P < 0.0001$), among doses ($F(5,430) = 115.76$, $P < 0.0001$), across test times ($F(5,430) = 590.04$, $P < 0.0001$), and for all two-way and three-way interactions ($P < 0.0001$). Figure 2 displays the marked strain-specific differences in the dose-dependent and time-dependent ability of SCH23390 to significantly reduce sucrose intake. The 129 mice displayed significant reductions across all five SCH23390 doses (Figure 2I), whereas the B6 and SJL mice displayed significant reductions following the four highest SCH23390 doses (Figures 2C and G). The C3H, B10 and SWR mice displayed significant reductions following the three highest SCH23390 doses (Figures 2B, D and H), whereas the BALB, DBA and the outbred CD-1 mice displayed significant reductions following only the two highest SCH23390 doses (Figures 2A, E and F). Moreover, significant enhancements of sucrose intake occurred following the lowest SCH23390 dose in CD-1 (15–60 min, Figure 2E), DBA (5–30 min, Figure 2F) and SWR (25 min, Figure 2H) mice.

Significant differences in percent vehicle baseline values of sucrose intake following SCH23390 were also observed among strains ($F(8,91) = 2.84$, $P < 0.007$), among doses ($F(4,364) = 97.55$, $P < 0.0001$), across times ($F(5,455) = 9.41$, $P < 0.0001$), and for all two-way and three-way interactions ($P < 0.0001$). Table 4 summarizes the SCH23390-induced changes in sucrose intake as the percentage of baseline vehicle intake for each strain, and indicates those instances in which one strain produced greater inhibitory effects relative to another strain. To summarize some of the major differences, 129 mice displayed significantly greater magnitudes of SCH23390-induced inhibition than BALB, CD-1 and SWR mice. SJL and B6 mice also


A BALB/cJ Mice and SCH23390 (nmol/kg)
B C3H/HeJ Mice and SCH23390 (nmol/kg)
C C57BL/6J Mice and SCH23390 (nmol/kg)
D C57BL/10J Mice and SCH23390 (nmol/kg)
E CD-1 Mice and SCH23390 (nmol/kg)
F DBA/2J Mice and SCH23390 (nmol/kg)
G SJL/J Mice and SCH23390 (nmol/kg)
H SWR/J Mice and SCH23390 (nmol/kg)
I 129P3/J Mice and SCH23390 (nmol/kg)
Sucrose Intake (ml)
Time (min)
0
50
200
400
800
1600

DYM

Figure 2. Alterations (mean, ± SEM) in sucrose intake following the five doses of the D1 DA receptor antagonist, SCH23390 in nine mouse strains. Individual analyses of variance for each strain revealed significant main dose effects and interactions between doses and times for all of the nine strains. Significant ($P < 0.05$) increases (#) and decreases (*) in sucrose intake following specific drug doses relative to corresponding vehicle intake are denoted.

Table 4. SCH23390 inhibition of sucrose (10%) intake across strains, doses and test times as a measure of percent of vehicle baseline intake.

Strain	5 min	15 min	30 min	60 min	90 min	120 min
A: SCH 50 nmol/kg						
BALB/cJ	167%[abcgh]	160%[abh]	129%	110%	108%	94%
C3H/HeJ	80%[e]	100%[e]	88%[e]	90%	100%	86%
C57BL/6J	125%[d]	100%	100%	100%	91%	83%
C57BL/10J	133%	140%	100%	113%	91%	85%
CD-1	180%[abcgh]	186%[abch]	167%[abch]	145%[ab]	131%	129%
DBA/2J	160%[abcfg]	180%[abch]	183%[abch]	138%[abc]	130%	117%
SJL/J	120%	75%	78%	80%	73%	100%
SWR/J	150%[h]	163%[ab]	140%[a]	125%[ab]	107%	106%
129P3/J	100%	89%	80%	67%	64%	63%
B: SCH 200 nmol/kg						
BALB/cJ	133%[abceg]	100%[abeh]	86%	80%	85%	76%
C3H/HeJ	60%	57%	63%	70%	109%[ae]	114%[ade]
C57BL/6J	75%[a]	71%	67%	70%	73%	75%
C57BL/10J	100%	120%[abceh]	100%[abceh]	100%	100%	92%
CD-1	140%[abcegh]	143%[abeh]	111%[abceh]	100%	108%	129%
DBA/2J	40%	60%	67%	50%	60%	58%
SJL/J	60%	50%	44%	80%	91%	100%
SWR/J	117%[abceg]	113%[abh]	90%	92%[e]	113%[ae]	112%[a]
129P3/J	71%	56%	60%	58%	64%	75%
C: SCH 400 nmol/kg						
BALB/cJ	67%[b]	80%[abf]	71%	70%	77%	76%
C3H/HeJ	60%[b]	57%	50%	50%	73%	79%
C57BL/6J	75%	57%	56%	60%	73%	75%
C57BL/10J	67%[b]	60%	57%	63%	64%	69%
CD-1	80%[b]	71%[ab]	78%	73%	85%	100%
DBA/2J	60%[b]	80%[b]	67%	88%	90%	92%

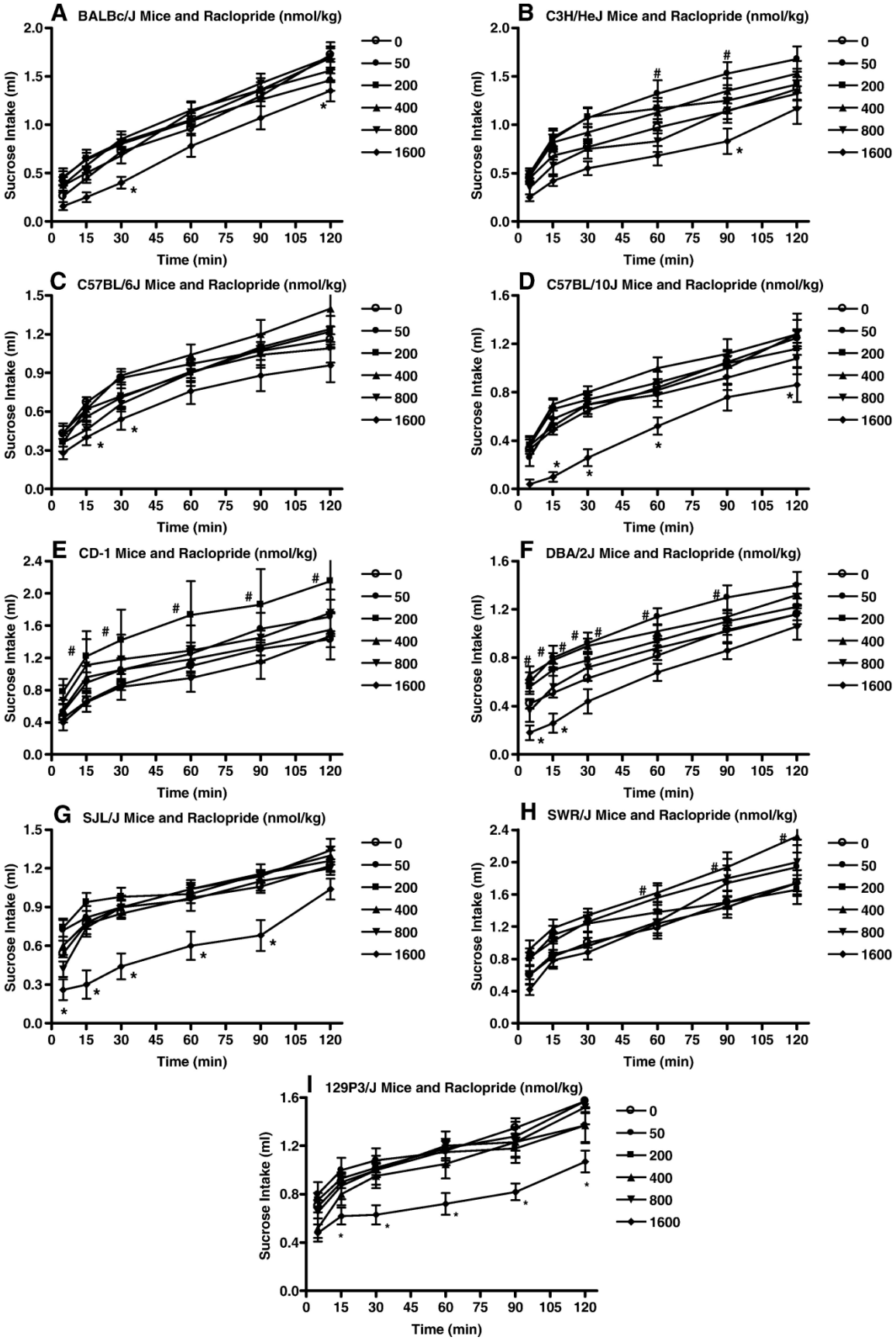
Strain	5 min	15 min	30 min	60 min	90 min	120 min
SJL/J	40%	38%	33%	50%	64%	83%
SWR/J	50%	38%	40%	50%	67%	82%
129P3/J	43%	44%	40%	50%	50%	69%
D: SCH 800 nmol/kg						
BALB/cJ	33%	40%	29%	50%	62%	65%
C3H/HeJ	40%	29%	38%	30%	55%	79%[e]
C57BL/6J	25%	29%	22%	30%	45%	50%
C57BL/10J	33%	20%	29%	38%	55%	54%
CD-1	40%	29%	22%	36%	31%	64%
DBA/2J	20%	20%	17%	25%	40%	42%
SJL/J	40%	38%	33%	40%	55%	67%
SWR/J	50%	38%	40%	50%[ace]	80%[ei]	94%[ce]
129P3/J	29%	22%	20%	25%	43%	56%
E: SCH 1600 nmol/kg						
BALB/cJ	0%	0%	0%	10%	23%	18%
C3H/HeJ	20%	14%	25%	20%	36%	43%
C57BL/6J	25%	14%	11%	10%	45%[ade]	50%[e]
C57BL/10J	0%	20%	14%	38%[e]	36%	38%
CD-1	40%[abcdefgh]	43%[acdefg]	33%[acdeg]	36%[e]	38%	50%[e]
DBA/2J	0%	0%	0%	0%	10%	17%
SJL/J	20%	25%	22%	20%	36%	67%[ae]
SWR/J	17%	13%	20%	25%	33%	47%
129P3/J	14%	11%	10%	8%	21%	31%

Note: Significantly ($P < .05$) less %Vehicle inhibition than *129P3/J*(a), *SJL/J* (b), *C57BL/6J* (c), *BALB/cJ* (d), *DBA/2J* (e), *SWR/J* (f), *C57BL/10J* (g), *C3H/HeJ* (h) or CD1 (i).

displayed significantly greater magnitudes of inhibition than BALB, CD-1 and SWR mice, but to a lesser degree than the 129 strain.

Strain differences in DA D2 antagonist (raclopride)-induced inhibition of sucrose intake: Overall significant differences in sucrose intake following raclopride were observed among strains ($F(8,84) = 5.67$, $P < 0.0001$), among doses ($F(5,420) = 20.87$, $P < 0.0001$), across times ($F(5,420) = 699.57$, $P < 0.0001$), and for all two-way and three-way interactions ($P < 0.0001$), except between strains and doses ($F(40,420) = 1.24$, n.s). Figure 3 displays the markedly lesser ability of raclopride to dose-dependently and time-dependently reduce sucrose intake in terms of the magnitude of the antagonist-induced inhibition. Seven strains (inbred BALB, C3H, B6, B10, DBA, SJL and 129: Figures 3A–D, F, I) displayed raclopride-induced reductions in sucrose intake only at the highest 1600 nmol/kg dose, and two strains (inbred SWR and outbred CD-1: Figures 3E and H) failed to display any raclopride-induced reductions in sucrose intake. Indeed, raclopride significantly increased sucrose intake in DBA mice following the 50 (15–90 min) and 400 (5–30 min) nmol/kg doses (Figure 3F), in C3H mice following the 50 (60–90 min) nmol/kg dose (Figure 3B), in SWR mice following the 400 (60–120 min) nmol/kg dose (Figure 3H), and in outbred CD-1 mice following the 200 (15–120 min) nmol/kg dose (Figure 3E). Analysis of the percent of vehicle baseline values of sucrose intake following raclopride also failed to show any genetic variability.

Strain differences in the ID50 of SCH23390-induced inhibition of sucrose intake: Table 5 summarizes the ID50 values for the ability of SCH23390 to inhibit sucrose intake across the 120 min time course. The 129 mice consistently displayed the lowest ID50 values across the first 90 min of the 2 h time course, and displayed 2–3.5-fold greater potency in SCH23390-induced inhibition of sucrose intake relative to CD-1 mice in the first hour after drug administration. In


A BALBc/J Mice and Raclopride (nmol/kg)
B C3H/HeJ Mice and Raclopride (nmol/kg)
C C57BL/6J Mice and Raclopride (nmol/kg)
D C57BL/10J Mice and Raclopride (nmol/kg)
E CD-1 Mice and Raclopride (nmol/kg)
F DBA/2J Mice and Raclopride (nmol/kg)
G SJL/J Mice and Raclopride (nmol/kg)
H SWR/J Mice and Raclopride (nmol/kg)
I 129P3/J Mice and Raclopride (nmol/kg)
Sucrose Intake (ml)
Time (min)
0
50
200
400
800
1600

DYM

Figure 3. Alterations (mean, ± SEM) in sucrose intake following the five doses of the D2 DA receptor antagonist, raclopride in nine mouse strains. Significant ($P < 0.05$) increases (#) and decreases (*) in sucrose intake following specific drug doses relative to corresponding vehicle intake are denoted.

Table 5. Linear regression values (ID_{50}) of SCH23390-induced inhibition (nmol/kg) of sucrose intake among the inbred and outbred mouse strains across the 120 min time course.

Linear regression ID_{50}						
Strain	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
BALB/cJ	967	987	642	890	875	1165
C3H/HeJ	628	733	622	763	1010	1465
C57BL/6J	672	566	649	694	1478	1629
C57BL/10J	830	888	735	998	1637	1288
CD-1	1249	1183	1043	1059	1037	1300
DBA/2J	713	896	751	681	814	1014
SJL/J	521	493	414	729	945	1873
SWR/J	1010	819	797	964	1604	1567
129P3/J	505	410	321	513	650	1072
Sensitivity ratio	2.5[a]	2.9[a]	3.2[a]	2.1[a]	2.5[b]	1.8[c]

Note: The sensitivity ratio reflects the potency fold shift of SCH23390-induced inhibition of sucrose intake (ID_{50}) between the least sensitive strain at each time point relative to the most sensitive strain.

a

CD-1 vs *129P3/J*.

b

C57BL/10J vs *129P3/J*.

c

SJL/J vs *DBA/2J*.

further agreement with the strain differences in SCH23390-induced inhibition in the percent of baseline sucrose intake, the SJL strain displayed the second highest degree of potency in inhibition of sucrose intake during the first 60 min of the time course.

Heritability estimates of SCH23390- and raclopride-induced inhibition of sucrose intake: Table 6 summarizes the narrow-sense heritability estimates for the five effective SCH23390 and one effective raclopride dose at each time point using the percent of vehicle baseline sucrose intake. Whereas moderate ($h^2 = 0.40–0.52$) heritability estimates were obtained for baseline vehicle sucrose intake across the first 30 min of the time course (data not shown), relatively meager heritability estimates were typically obtained
across the time course for the different effective SCH23390 ($h^2 = 0.05–0.32$) and raclopride ($h^2 = 0.10–0.22$) doses across test times.

Sucrose intake and T1R3 sweet taste receptor status: Four inbred mouse strains (B6, B10, SJL and SWR) had the sweet-sensitive form of the T1R3 receptor while four strains (BALB, C3H, DBA and 129) had the sweet-sub-sensitive form of the receptor (Reed et al., 2004). Evaluation of the percent vehicle sucrose intake scores at 30 min only revealed significant differences ($F(1,78) = 6.61$, $P < 0.012$) between sensitive (25.3%) and sub-sensitive (12.3%) taster strains following the 1600 nmol/kg dose of SCH23390. Differences between sensitive (90.8%) and sub-sensitive (71%) taster strains in inhibition of sucrose intake following the 200 nmol/kg dose of SCH23390 approached, but did not achieve significance ($F = 3.24$, $P = 0.076$). Differences between the two groups in inhibition of sucrose intake failed to occur following the 50 ($F = 0.28$, ns; sensitive: 111%; sub-sensitive: 104%), 400 ($F = 0.13$, ns; sensitive: 61%; sub-sensitive: 64%) and 800 ($F = 1.81$, ns; sensitive: 46.7%; sub-sensitive: 34.3%) nmol/kg doses of SCH23390, and the 1600 nmol/kg dose of raclopride ($F = 0.46$, ns; sensitive: 82.1%; sub-

Table 6. Heritability estimates for percent baseline vehicle sucrose intake scores across strains for each SCH23390 dose at each time point and for the effective raclopride dose.

Heritability scores						
Time → Condition ↓	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
SCH23390						
50 nmol/kg	.24	.25	.32	.17	.17	.15
200 nmol/kg	.22	.24	.18	.11	.19	.24
400 nmol/kg	.07	.15	.11	.06	.06	.05
800 nmol/kg	.05	.05	.06	.11	.15	.23
1600 nmol/kg	.10	.15	.22	.15	.16	.18
Raclopride						
1600 nmol/kg	.10	.22	.15	.16	.10	.12

sensitive: 75%). Thus, the sweet-sensitive strains show less suppression in sucrose intake following the highest SCH23390 dose than sweet-sub-sensitive strains.

Discussion

Marked murine strain differences were observed in the magnitude, time course and potency of the DA D1 (SCH23390), but not the D2 (raclopride), receptor antagonists to inhibit intake of a 10% sucrose solution. Whereas SCH23390 produced a heterogeneous response pattern as a function of mouse strain that differed across time course, magnitude and dose, raclopride's ability to reduce sucrose intake across strains was largely limited to effects following the 1600 nmol/kg dose for the BALB, C3H, B6, B10, DBA, SJL and 129, but not for the inbred SWR and outbred CD-1 strains. Further, heritability estimates in the ability of particular strains to display raclopride-induced inhibition of sucrose intake at the highest dose tended to be quite low ($h2 = 0.10–0.22$). Finally, strains that possessed the sweet-sensitive (B6, B10, SJL and SWR) and the sweet-sub-sensitive (BALB, C3H, DBA and 129) form of the T1R3 receptor (Reed et al., 2004) failed to differ in the degree of inhibition of sucrose intake following the highest dose of raclopride. These antagonist effects are in agreement with previously-observed significant DA antagonist-induced reductions in sucrose intake in sham-feeding rats (Geary and Smith, 1985; Hsiao and Smith, 1995; Schneider et al., 1986; Schneider et al., 1990; Yu et al., 2000a; Yu et al., 2000b) and in rat pups in independent ingestion tests (Tyrka et al., 1992), as well as in fructose intake in real-feeding rats (Baker et al., 2003). The present study also indicated that low and moderate doses of raclopride significantly increased sucrose intake in some mouse strains (C3H, DBA, SWR, and CD-1). These latter antagonist-induced facilitatory effects upon sucrose intake have also been observed previously in rats (Muscat et al., 1991; Muscat and Willner, 1989; Phillips et al., 1991). Thus, these data thereby suggest that raclopride is quite ineffective in reducing sucrose intake in mice, and that any interpretation regarding

genetic variability in D2 antagonist-induced suppression of sucrose intake is limited by the overall ineffectiveness of this response.

In contrast, genetic variability in SCH23390-induced inhibition of sucrose intake was clearly demonstrated in two independent measures in the present study: magnitude of effect, and sensitivity to lower doses of the antagonist. SCH23390 significantly reduced sucrose intake across all five doses (50–1600 nmol/kg) in 129 and SJL mice, across four doses (200–1600 nmol/kg) in B6 and BALB mice, across three doses (400–1600 nmol/kg) in SWR, C3H and B10 mice, across three different doses (200, 800, 1600 nmol/kg) in DBA mice, but only at the two highest doses (800, 1600 nmol/kg) in CD-1 mice. A similar pattern of SCH23390-induced inhibitory actions were observed in assessing antagonist ID50 potency across strains with the 129 and SJL inbred strains showing the greatest sensitivity consistently across the time course. A 2–3-fold difference was observed between the most (129) and least (CD-1) sensitive strains in the first hour following SCH23390 treatment. The strain-specific inhibition of SCH23390 sometimes occurred at different doses and time point measurement intervals of the drug. It is unclear from the present data as to whether different and distinct multiple genetic mechanisms are responsible for individual SCH23390 dose and time effects, or whether a single genetic mechanism explains all effects.

Whereas a number of these previous criteria appear to support genetic variance of the percent of vehicle intake in SCH23390-induced inhibition of sucrose intake, narrow-sense heritability estimates as a measure of genetic variance were quite meager ($h2 = 0.05–0.32$) across each of the time points and each of the SCH23390 doses that produced significant inhibition of sucrose intake. A previous study (Dym et al., 2007) examining opioid inhibition of sucrose intake indicated more consistent heritability estimates for effective NTX doses to produce

genetic variance in the inhibition of sucrose intake ($h^2 = 0.38–0.51$). Thus, whereas measures of magnitude of effect and potency of effect revealed genetic variance in SCH23390-induced inhibition of sucrose intake, this interpretation should be tempered by the meager heritability data.

It is possible that these strain-specific effects of SCH23390 could be due to other factors such as pharmacokinetic actions, actions at sites outside of the central nervous system, or actions upon activity or motor behavior. Although pharmacodynamic effects of SCH23390 acting at the D1 receptor were initially reported in rat studies (e.g., Briere et al., 1987; Hjorth and Carlsson, 1988; Reader et al., 1988; Schulz et al., 1985), the strain-specific effects of SCH23390 could alternatively be due to pharmacokinetics with SCH23390 acting as a poor D1 antagonist in some murine strains and a strong D1 antagonist in others. For instance, SCH23390 binding is significantly reduced in homozygous mice for the recessive gene weaver (Pullara and Marshall, 1989, but see Ohta et al., 1989), DA D(1A) receptor knockout mice (Miyamoto et al., 2001; Montague et al., 2001), and diabetic mice (Saitoh et al., 1998), but is increased in Purkinje Cell Degeneration mutant mice (Delis et al., 2004). In contrast, SCH23390 binding is unchanged in hypoxic mice (Arregui et al., 1994), MPTP-treated mice (Araki et al., 2001), methamphetamine-treated mice (Yoo et al., 2008), 6-hydroxydopamine and iron-deficient mice (Zhao et al., 2007), mu-opioid receptor knock-out mice (Tien et al., 2003), and cannabinoid CB1 receptor knockout mice (Houchi et al., 2005). In assessing whether inbred strains display similar or different SCH23390 binding characteristics, although restraint stress increased D1 receptor density in the nucleus accumbens of DBA mice, while it reduced D1 receptor density in the striatum of B6 mice (Cabib et al., 1998), no differences were observed in mesocorticolimbic and striatal SCH23390 binding in normal DBA and B6 mice (Erwin et al., 1993). BALB and DBA/1 mice

made aggressive by clonidine treatment displayed reduced SCH23390 binding in the limbic forebrain, an effect not observed in clonidine-treated B6 mice (Nikulina and Klimek, 1993). Kanes et al. (1993) did perhaps the most extensive strain survey in examining D1 receptor binding with [3H]SCH23390, using eight strains of which four (BALB, C3H, B6 and DBA) were evaluated in the present study. Although they failed to find any strain differences in [3H]SCH23390 uptake into the caudate or cerebellum, BALB mice displayed higher [3H]SCH23390 binding in the nucleus accumbens (19%), lateral caudate–putamen (15%) and substantia nigra, pars reticulata (27%) than B6 mice with DBA and C3H/He mice intermediate to the other strains. C3H mice displayed higher [3H]SCH23390 binding in the dorsal caudate–putamen (23%) than B6 mice with DBA and BALB mice intermediate to the other strains. Our behavioral data indicated that B6 and C3H inbred strains showed moderate sensitivity, and BALB and DBA inbred strains showed lesser sensitivity to SCH23390-inhibition of sucrose intake. [3H]SCH23390 binding data on strains (e.g., 129 and SJL) highly sensitive to SCH23390-induced inhibition of sucrose intake are not available, so it may be premature to describe these effects as D1-receptor specific, but more appropriately to ascribe these effects as SCH23390-sensitive or -subsensitive.

A second caveat is that both D1 and D2 antagonists were administered subcutaneously, and any systemic effects, particularly for SCH23390, could be due to D1 receptor blockade in relevant sites outside of the central nervous system. Relevant candidate sites displaying D1 receptor binding as measured by SCH23390 include gastric (Glavin and Hall, 1995; Nomura et al., 1995) and intestinal (Fraga et al., 2004; Marmon et al., 1993) areas. Further studies using ventricular or intracerebral administration of D1 antagonists are needed to confirm whether central sites of action are involved.

A third and final caveat is to determine whether the genetic variance in SCH23390-induced inhibition of sucrose intake is an epiphenomenon of a generalized effect on activity and/or motor behavior. Although this factor was not directly studied in this paradigm, it is important to note that the same animals in each strain were assessed for both SCH23390-induced and raclopride-induced inhibition of sucrose intake using equimolar concentrations of each drug. Whereas D1 antagonism produced both strain-specific and dose-dependent reductions in sucrose intake, raclopride only produced reductions at the highest dose. Yet D1 antagonism with SCH23390 and D2 antagonism with related antagonists to raclopride produced equal reductions in hyperlocomotion induced by caffeine, cocaine and amphetamine (Garrett and Holtzman, 1994; O'Neill and Shaw, 1999), albeit at doses higher than those employed in the present study. Therefore, it does not appear that deficits in motor activity produced the differential strain-specific and dose-dependent reductions in sucrose intake induced by SCH23390.

Genetic variability was observed in the ability of the opioid antagonist NTX to suppress sucrose intake (Chapter Three). Sucrose intake of SWR mice failed to decrease significantly following a wide range (0.01–5 mg/kg) of NTX doses. NTX's maximal magnitude of inhibitory effects was small (35–40%, (ID50 = 7.5–10 mg/kg at 60 min)) in 129 and SJL mice, moderate (~ 50%) in BALB, C3H, CD-1 and DBA mice, and profound (70–80%, (ID50 = 0.5–1.5 mg/kg at 60 min)) in B6 and B10 mice. Thus, there was a marked 37-fold difference across strains in NTX's effectiveness to reduce sucrose intake which compares with the much smaller 2–3 fold difference observed with SCH23390 in the present study. To directly assess the relationship between the respective abilities of NTX and SCH23390 to inhibit sucrose intake, we derived the ID50 value of each antagonist for each of the nine common strains tested, and the correlation between the ID50 values for each antagonist across the nine strains was calculated. There was a

weak, negative correlation between the ID50 of NTX and SCH23390 to inhibit sucrose intake 30 min following drug administration ($r = -0.32$; $r^2 = 0.11$, ns). This particular data point produced the most consistent inhibition across drugs, doses and times. Figure 4 illustrates differences in each drug's effects in the various strains. For instance, whereas the 129 and SJL were among the least responsive strains to NTX, they were among the most responsive strains to SCH23390. In contrast, the B10 and B6 strains were the most responsive strains to NTX, but were only moderately sensitive to SCH23390. The dichotomy between dopaminergic (SCH23390) and opioid (NTX) antagonist modulation of sucrose intake has been observed in other paradigms. Thus, although significant reductions in sucrose and saccharin intake have been observed in sham-feeding and real-feeding rats following DA (SCH23390: e.g., Bello and Hajnal, 2006; Geary and Smith, 1985; Muscat and Willner, 1989; Schneider et al., 1986; Schneider et al., 1990; Tyrka et al., 1992) and opioid (NTX: e.g., Apfelbaum and Mandenoff, 1981; Cooper, 1983; Kirkham and Cooper, 1988a; Kirkham and Cooper, 1988b; Levine et al., 1982; Lynch and Libby, 1983; Rockwood and Reid, 1982;) antagonists, these antagonists have different effects on the hedonic response to sweet taste (Barbano and Cador, 2007; Berridge and Robinson, 1998) and flavor conditioning effects of sugars (Azzara et al., 2000; Azzara et al., 2001; Baker et al., 2003; Baker et al., 2004; Hsiao and Smith, 1995; Yu et al., 1999; Yu et al., 2000a; Yu et al., 2000b).

Berridge (Berridge, 1996; Berridge and Robinson, 1998) proposed that involvement of brain DA in food ‘wanting’ and brain opioid systems in food hedonics (‘liking’) provides one framework to explain the differential effects of DA and opioid drugs on food-motivated behavior and reward in general. Whereas DA and especially D1 receptor antagonism decreases sucrose intake in rats (e.g., Barbano and Cador, 2007; Berridge and Robinson, 1998) and indeed all

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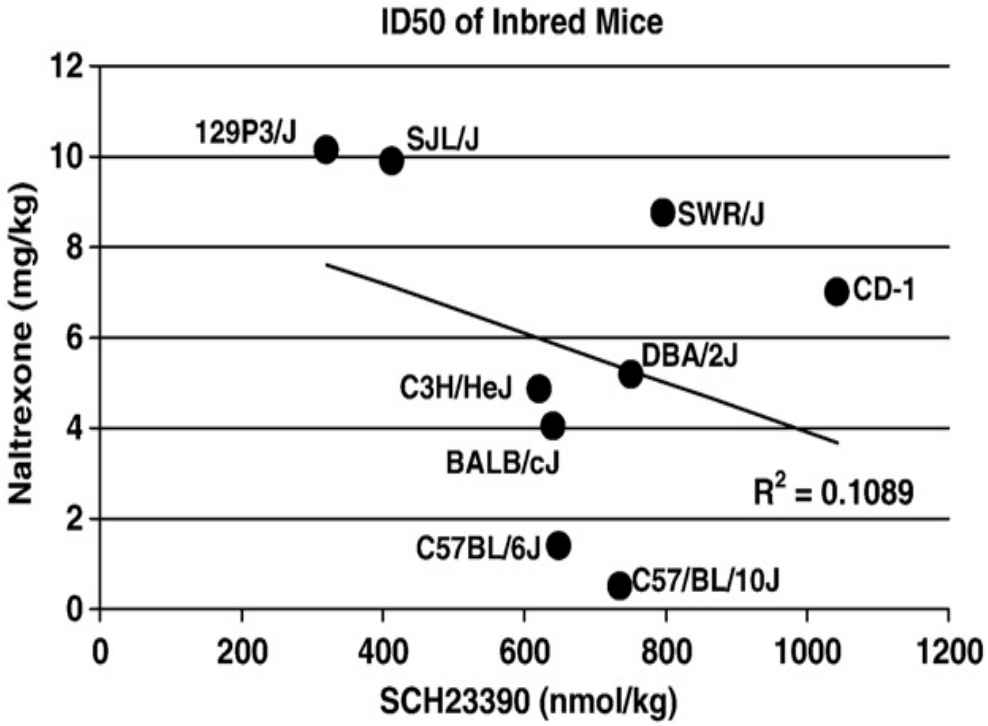

ID50 of Inbred Mice
Naltrexone (mg/kg)
SCH23390 (nmol/kg)
129P3/J
SJL/J
SWR/J
CD-1
DBA/2J
C3H/HeJ
BALB/cJ
C57BL/6J
C57/BL/10J
R^2 = 0.1089
0
2
4
6
8
10
12
0
200
400
600
800
1000
1200

DYM

Figure 4. Scatterplot and best-fit regression line of ID50 of NTX-induced inhibition (Dym et al., 2007) and SCH23390-induced inhibition of sucrose intake after 30 min across nine mouse strains. A weak, negative and non-significant correlation between the ID50 of NTX and SCH23390 to inhibit sucrose intake 30 min following drug administration ($r = -0.32$; $r^2 = 0.11$, ns) was observed.

mouse strains tested in the present study, the magnitude and potency of the effects were strain-specific. DA D1 antagonism is also particularly effective in blocking the acquisition of flavor–nutrient conditioning of simple carbohydrates (Azzara et al., 2001), the acquisition of flavor–flavor conditioning of sucrose in sham-feeding rats (Yu et al., 2000b) and of fructose in real-feeding rats (Baker et al., 2003), as well as the expression of flavor–flavor conditioning (Baker et al., 2003; Yu et al., 2000a; Yu et al., 2000b). All of these studies implicate the D1 DA receptor in reward mediated by simple carbohydrates. However, the physiological mechanism by which this mediation of reward acts is still unclear. The present and previous (Chapter Three) study demonstrating differences in the pattern and effectiveness of dopaminergic and opioid antagonism of sucrose intake across different inbred mouse strains may provide yet another form of converging evidence for separation of these two pharmacological systems, although our knowledge of the ability of a wide variety of mouse strains to display these specific attributes of food seeking is presently limited to the eight inbred strains evaluated. Other studies of the 129 and B6 inbred strains provide some intriguing information relevant to this issue. These two strains differ in their sweet taste sensitivity with B6 mice possessing the sweet-sensitive form of the Tas1R3 receptor, and 129 possessing the sweet-sub-sensitive form of the receptor (Reed et al., 2004). Consistent with this finding, 129 mice underconsumed a variety of natural and artificial sweeteners compared to B6 mice (Bachmanov et al., 2001; Reed et al., 2004). However, after experience with concentrated sugar solutions, the difference in the sucrose preferences of the two strains disappeared, an effect attributed to the postingestive conditioning actions of the sugar (Sclafani, 2006b). Indeed 129 mice showed a flavor conditioning response to intragastric sucrose infusions that was as strong, if not more so, as that displayed by B6 mice when the sweetness of the cue flavors were equated for the two strains (Sclafani and Glendinning, 2005).

Furthermore, sucrose-experienced 129 mice licked more on a progressive ratio schedule for sucrose rewards than did B6 mice (Sclafani, 2006a). The respective strong and weak sensitivity of 129 mice to SCH23390- and NTX-induced inhibition of sucrose intake may reflect their greater reliance on the postingestive conditioning of sweet rewards than the activity of their subsensitive sweet taste receptors. Supporting this interpretation are the findings that SCH23390, but not NTX, blocks the postingestive conditioning actions of sugars (Azzara et al., 2000; Azzara et al., 2001). Further limited support for this concept is the present finding that the highest dose of SCH23390 produced significantly greater inhibition of sucrose in the sweet-sub-sensitive strains (BALB, C3H, DBA and 129) relative to the sweet-sensitive strains (B6, B10, SJL and SWR). Therefore, such data suggest that inbred genetic differences among mouse strains may provide a viable model for differentiating among the multiple processes involved in food reward.

While the previous two studies examined the role that the dopaminergic and opioid system play in sweet intake, little was known about how these pharmacological systems affect intake of other nutrients in inbred mice. The next experiment (Chapter 5) examined genetic variance and the role of opioids and DA in the intake of another nutrient - fat.

CHAPTER FIVE: GENETIC VARIANCE CONTRIBUTES TO DA AND OPIOID RECEPTOR ANTAGONIST-INDUCED INHIBITION OF INTRALIPID (FAT) INTAKE IN INBRED AND OUTBRED MOUSE STRAINS

Introduction

In Chapters 3 and 4, the role that the opioid and dopaminergic systems play in the intake of sucrose was examined. However, genetic variance has been observed for many aspects of ingestive behavior in inbred mouse strains (see review: Reed et al., 1997), including the intake of dietary fat (see review: West and York, 1998). Thus, the current chapter examined to what extent genetic variance in the intake of a fat solution would be observed following manipulation of the DA and opioid systems.

Mouse strain differences have been observed in the general intake of complete high-fat diets, fat selection in nutrient self-selection paradigms, or intake of fat emulsions (e.g., Alexander et al., 2006; Bachmanov et al., 2001; Glendinning et al., 2008; Sclafani, 2007; Sclafani and Glendinning, 2005; Smith et al., 2000; Smith et al., 2001; Smith-Richards et al., 1999; Smith-Richards et al., 2002). Specifically, AKR inbred mice consumed moderate amounts of a high-fat diet that promoted weight gain and obesity, whereas carbohydrate-preferring SWR mice consumed large amounts of the same diet that was not accompanied by weight gain (Smith et al., 2000; Smith et al., 2001). The different patterns of fat intake and compensatory weight changes observed in AKR and SWR strains persisted across different types of fat sources and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999). Indeed, whereas fat consumption and accumulation of ependymal fat were correlated in AKR and B6 mice, fat consumption in SWR and CAST/Ei strains was inversely correlated with accumulation of ependymal fat (Smith et al., 2000; Smith et al., 2001). Diet-sensitive AKR and DBA strains

consumed greater amounts of fat, displayed more adiposity, and displayed elevated levels of leptin and insulin, whereas B6 mice showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). In contrast, obesity-resistant SWR and A mice that consumed more fat than carbohydrate displayed lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin, and/or reduced arcuate NPY levels (Leibowitz et al., 2005). Finally, chromosomal mapping analyses revealed a series of genetic loci (mob 1–4), located on chromosomes 9 and 15, that appeared to explain some of these genetic variations for fat and obesity (e.g., Bachmanov et al., 2001; Smith-Richards et al., 2002).

Our laboratory has also investigated genetic variance in fat preference and intake (Lewis et al., 2007) using a liquid fat source in the form of Intralipid, a stable soybean oil emulsion, which is highly attractive and avidly consumed by rodents (e.g., Higgs and Cooper, 1998). Eleven inbred and one outbred mouse strains, including some (A, AKR, B6, DBA, SWR) described in the earlier studies, were given 24-h two-bottle tests with Intralipid vs. water over a wide range of lipid concentrations (0.0001–5%). We found significant increases in Intralipid intake in BALB mice at the seven highest concentrations, in SWR, AKR, B6 and DBA mice at the four highest concentrations, in CD-1, B10 and SJL mice at the three highest concentrations, and in A, C3H, CBA and 129 mice at the two highest concentrations, thereby demonstrating a wide degree of genetic variance in the detection sensitivity to consume a fat solution.

Among the multiple pharmacological substrates governing intake of fat in outbred rats are the opioid and dopaminergic receptor systems. Fat intake is significantly reduced in rats by general and selective mu and kappa opioid receptor antagonists (Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid

et al., 2007; Sahr et al., 2008). Chronic mu, mu-1, delta-1, and delta-2 opioid antagonists also significantly decreased weight and intake of a fat source in rats during development of dietary obesity (Cole et al., 1995). Correspondingly, administration of the mu-selective opioid agonist, DAMGO into the nucleus accumbens stimulates high-fat intake in rats (Zhang et al., 1998). Fat intake is also significantly reduced by systemic treatment with DA antagonists in rats as well (Baker et al., 2001; Davis et al., 2006; Rao et al., 2008; Weatherford et al., 1988; Weatherford et al., 1990).

The aforementioned data therefore indicate that control of a critical macronutrient, fat, is influenced by DA and opioid receptor systems and by genetic factors. Whether genetic variance plays a role in the opioid and dopaminergic modulation of fat intake is unknown, and is the purpose of this study. Thus, the present study examined whether genetic variance exists in the dose-dependent ability of general opioid (NTX) or DA D1-like receptor (SCH23390) antagonism to alter Intralipid intake in a short-term (2 h) test developed previously for the examination of genetic variance in the pharmacology of sucrose intake (Chapter Three and Chapter Four). A 5% Intralipid concentration was selected because it generated substantial intakes in our prior 24-h study (Lewis et al., 2007). We focused on the D1-like antagonist, SCH23390, rather than the D2-like antagonist, raclopride, since the latter drug was relatively ineffective in reducing sucrose intake and/or displaying genetic variance in sucrose intake (Chapter Four). As in Chapter Three and Chapter Four, mouse strains were initially assessed for their ability to display short-term (2 h) Intralipid intake at or above a criterion baseline criterion intake (> 1 ml) to allow for the reliable observation of short-acting antagonist-induced reductions. Seven inbred (BALB, B6, B10, DBA, SJL, SWR, 129) and one outbred (CD-1) mouse strains met this criterion of short-term Intralipid intake, and were used in the pharmacological analyses, while other strains

showing genetic variance in fat intake (AKR, A) failed to meet this criterion, and were therefore not included.

This work has been published in the journal, Brain Research (Dym et al., 2010) and presented at the Society for Neuroscience meeting (Dym et al., 2008).

Experimental Procedure

Subjects: Outbred (CD-1, Charles River Laboratories, Wilmington, MA; n = 11) and seven inbred BALB, B6, B10, DBA, SJL, SWR and 129 (Jackson Laboratories, Bar Harbor, ME; n = 10–12) male mice (6 weeks of age) were initially acclimated to the Queens College vivarium for 1 week in group housing (5 per cage). The animals were then housed individually in plastic cages (30 × 20 × 15 cm) with stainless steel tops and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22 °C. All animals were provided with chow (Lab Diet Mouse Chow 5015) and water ad libitum throughout the experiment, except when experimental testing was conducted. It should be noted that the fat content of the mouse chow is higher than standard rodent chow (e.g., 26% by energy) which may influence fat preference (Reed and Friedman, 1990). However, the same chow diet was used in our prior studies of sucrose and fat intake (Lewis et al., 2005; Lewis et al., 2007). All procedures were approved by the Queens College Institutional Animal Care and Use Committee.

Intralipid intake procedure: At 3–7 h into the light cycle, chow and water were removed from the cage, and each animal was given 2-h access to 5% Intralipid. The Intralipid was prepared by diluting 20% Intralipid (Baxter Healthcare Corporation, Deerfield, Illinois) with water and was presented in a 10-ml plastic syringe (8 ml Intralipid solution and 2 ml air to allow robust licking behavior) fitted with a stainless steel sipper tube (see: General Methods for further detail on testing apparatus). Intakes were measured to the nearest 0.2 ml at 5, 15, 30, 60, 90, and 120 min

and then food and water were returned. Each animal was exposed to one Intralipid session per day until a criterion minimum of 1 ml was consumed over three consecutive exposures; three to five sessions were typically needed to reach this criterion in tests conducted approximately three times each week; the eight strains listed above met this criterion. A ninth strain (C3H) that was included in prior studies of opioid and dopaminergic antagonist actions on sucrose intake (Chapter Three and Chapter Four), failed to reach criterion and was excluded. This criterion was employed to avoid "floor effects" of antagonist treatment. Following this initial training period, the mice received an intraperitoneal (ip) vehicle injection (0.3 ml distilled water/30 g body weight, 10 ml/kg) and Intralipid intake was measured over 2 h. Intralipid tests were then conducted following subcutaneous (sc) injection of the D1 DA receptor antagonist (SCH23390, Sigma Chemical Co., St Louis, MO) at doses of 50, 200, 400, 800 and 1600 nmol/kg and ip injection of the opioid receptor antagonist (naltrexone hydrochloride, Sigma Chemical Co.) at doses of 0.01, 0.1, 1.0 and 5.0 mg/kg. These dose ranges were identical to those used in strain surveys of D1 and opioid antagonist effects upon sucrose intake (Chapter Three and Chapter Four). Subgroups of animals of each strain, matched for vehicle Intralipid intake, were tested with one of four drug regimens: ascending series of SCH23390 doses followed by a descending series of NTX doses, descending series of SCH23390 doses followed by an ascending series of NTX doses, an ascending series of NTX doses followed by a descending series of SCH23390 doses, or descending series of NTX doses followed by an ascending series of SCH23390 doses. A minimum of 72 h elapsed between injections to minimize carry-over effects as performed in previous pharmacological studies on sucrose intake (Chapter Three and Chapter Four). Indeed, orders of drug or specific dose presentation failed to produce any significant effects, insuring lack of order or carry-over effects. Following all antagonist treatments, Intralipid intake was

reassessed following a second vehicle injection. Analyses of Intralipid intake under the two vehicle treatments failed to reveal any differences, and therefore, these data were averaged and served as the vehicle baseline. Sample size differences among strains reflect deaths of individual animals or a failure to reach criterion intake for a given animal.

Results

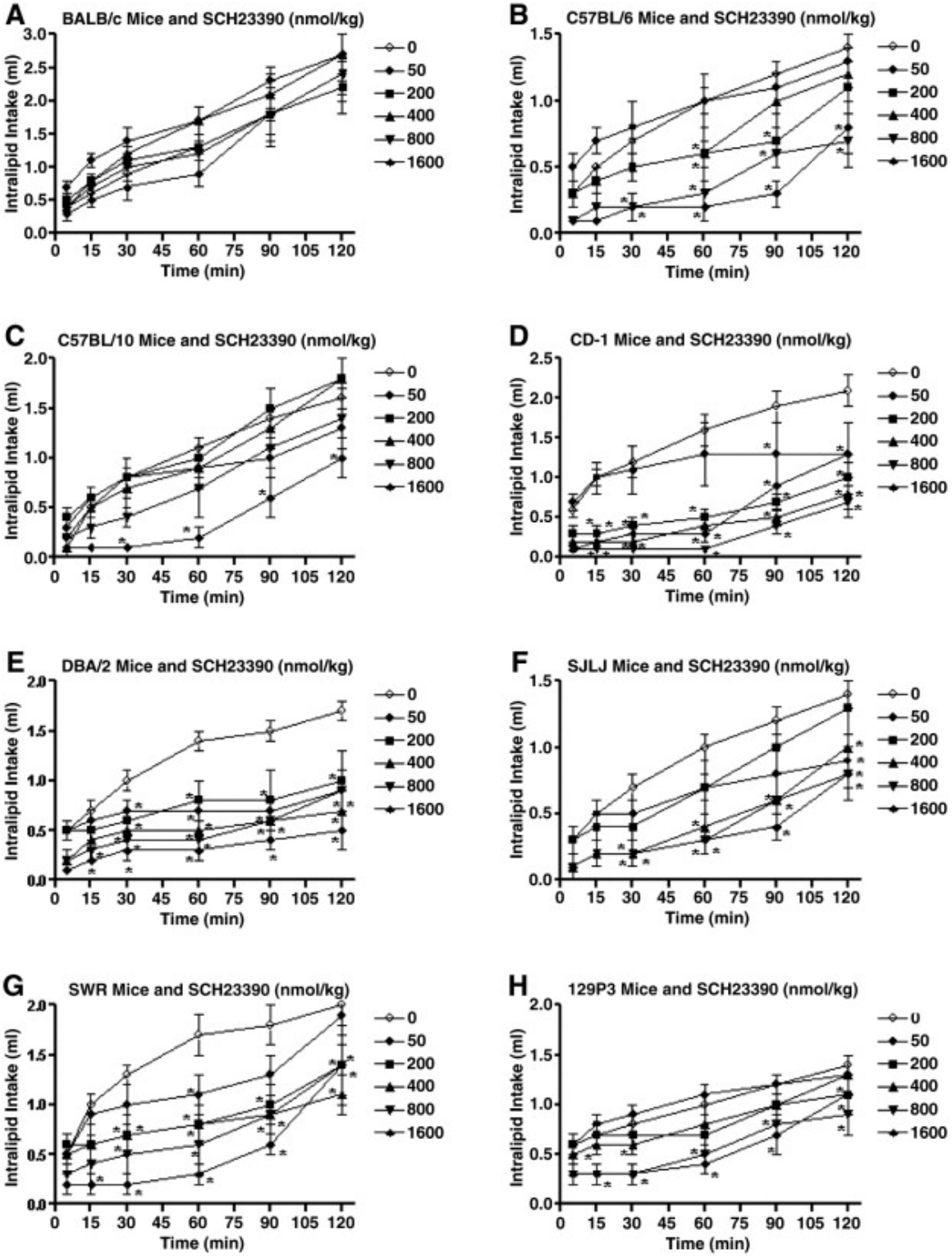
Strain differences in Intralipid intake following vehicle baseline injections: Evaluation of Intralipid intake following vehicle baseline injections revealed significant differences among strains ($F(7,70) = 7.45$, $P < 0.0001$) and for the interaction between strains and test times ($F(35,350) = 5.68$, $P < 0.0001$). The rank-order of the cumulative 2-h baseline vehicle Intralipid intake among the eight strains was: BALB (2.2 ml), CD-1 (2.1 ml), SWR (2.0 ml), DBA (1.7 ml), B10 (1.6 ml), SJL (1.4 ml), 129 (1.4 ml) and B6 (1.4 ml). To account for these baseline differences in Intralipid intake, the drug effects were evaluated both on absolute Intralipid intakes and on intakes as a percent of baseline levels.

Strain differences in SCH23390-induced inhibition of Intralipid intake: Overall significant differences in Intralipid intake following SCH23390 were observed among the eight mouse strains ($F(7,69) = 6.40$, $P < 0.0001$), among doses ($F(5,65) = 51.13$, $P < 0.0001$), across test times ($F(5,65) = 71.36$, $P < 0.0001$), and for all two-way and three-way interactions (P's < 0.019–0.0001). Figure 5 displays the marked strain-specific differences in the dose-dependent and time-dependent ability of SCH23390 to significantly reduce Intralipid intake. CD-1, DBA and SWR strains displayed significant reductions across all five SCH23390 doses (Figures 5D, E, G), whereas SJL and B6 strains displayed significant reductions following four SCH23390 doses (Figures 5B, F). 129 and B10 strains displayed significant reductions following three and one

SCH23390 doses respectively (Figures 5C, H). In contrast, BALB mice failed to display SCH23390-induced reductions in Intralipid intake at any of the tested doses (Figure 5A).

Significant differences in percent baseline values of Intralipid intake following SCH23390 were also observed among strains ($F(7,69) = 9.26$, $P < 0.0001$), among doses ($F(4,66) = 28.20$, $P < 0.0001$), across times ($F(5,65) = 26.81$, $P < 0.0001$), for the strain and time and dose and time interactions (P's < 0.037–0.0001), but not for the interactions between strains and doses or among strains, doses and times. SCH23390 did not alter the percentage of Intralipid intake in BALB mice at 50, 200 and 400 nmol/kg doses, and only transiently reduced percent intake by 15% and 35% at the 800 and 1600 nmol/kg doses, respectively. All other strains displayed marked 66–90% inhibition following the 1600 nmol/kg dose, 51–82% inhibition following the 800-nmol/kg dose, and correspondingly dose-dependent lower percentages of inhibition at the three lower SCH23390 doses. The following significant interstrain differences were observed. BALB mice displayed significantly less inhibition than DBA, SWR, SJL, B6 and CD-1 mice following the 50-, 400-, 800- and 1600-nmol/kg SCH23390 doses, effects that typically persisted across the entire time course. B10 mice displayed significantly less inhibition than DBA mice following the 200, 400 and 800 nmol/kg doses only after 5 min, than SWR mice following the 50-, 200- and 800-nmol/kg doses only after 5 min, than SJL mice following the 50-, 200- and 800-nmol/kg doses only after
5 min and the 400 nmol/kg dose after 5–60 min, and then CD-1 mice following the 200 (5–30 min), 400 (5–120 min) and 800 (5–15, 60–120 min) nmol/kg doses.

Strain differences in NTX-induced inhibition of Intralipid intake: Significant differences in Intralipid intake following NTX were observed among the strains ($F(7,68) = 6.07$, $P < 0.0001$), among doses ($F(4,65) = 38.52$, $P < 0.0001$), across times ($F(5,64) =$

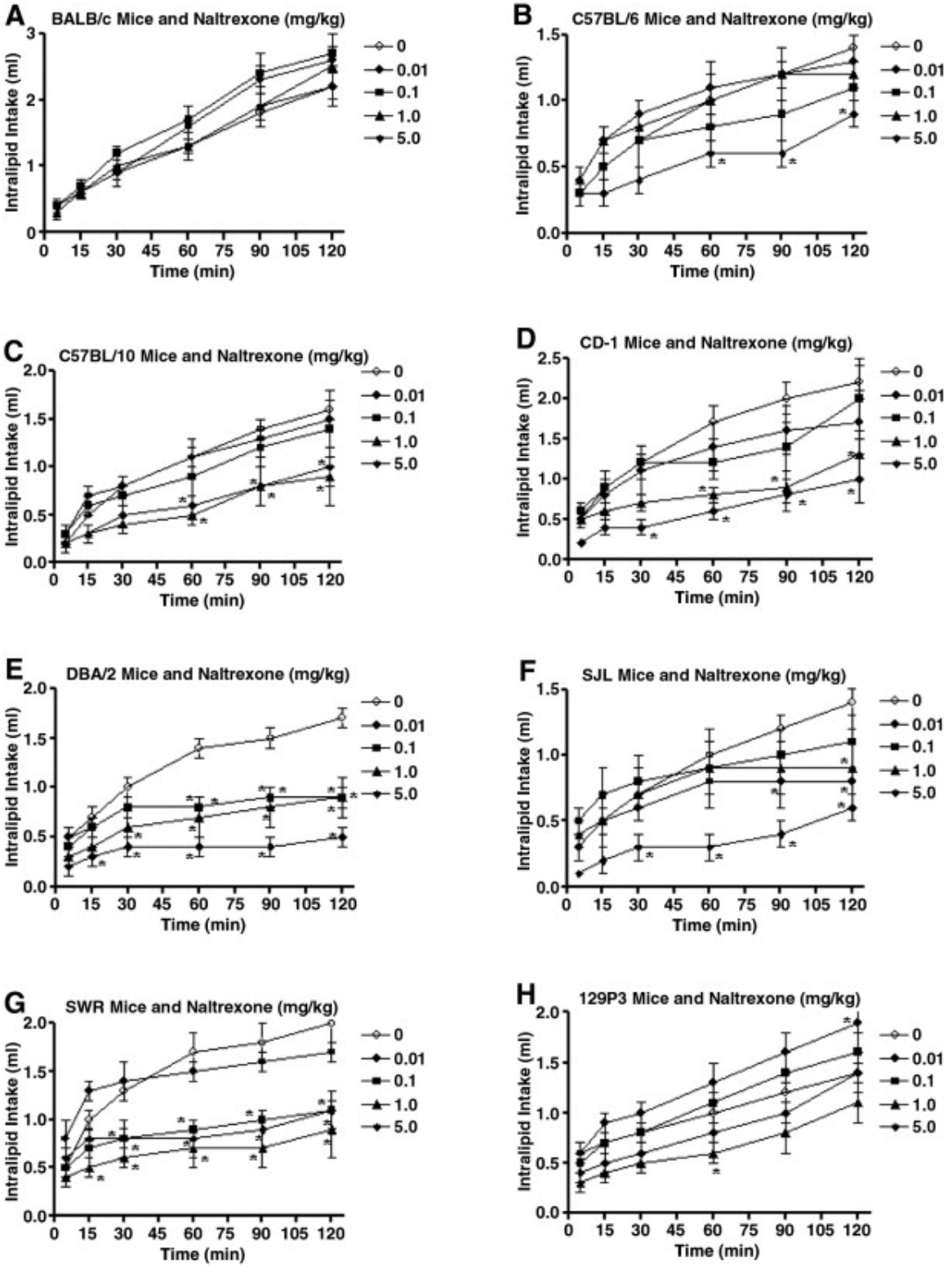

A
BALB/c Mice and SCH23390 (nmol/kg)
B
C57BL/6 Mice and SCH23390 (nmol/kg)
C
C57BL/10 Mice and SCH23390 (nmol/kg)
D
CD-1 Mice and SCH23390 (nmol/kg)
E
DBA/2 Mice and SCH23390 (nmol/kg)
F
SJLJ Mice and SCH23390 (nmol/kg)
G
SWR Mice and SCH23390 (nmol/kg)
H
129P3 Mice and SCH23390 (nmol/kg)
Intralipid Intake (ml)
Time (min)
0
50
200
400
800
1600

DYM

Figure 5. Alterations (mean ± SEM) in intralipid intake following five doses of the D1-like DA receptor antagonist, SCH23390 in eight mouse strains. Individual analyses of variance revealed significant main dose effects and interactions between doses and times for all, but the BALB/cJ mouse strains. Significant ($P < 0.05$) decreases (*) in intralipid intake following specific drug doses at particular time points relative to corresponding vehicle intake are denoted.

92.89, P < 0.0001), and for all two-way (P's < 0.0001), but not the three-way interactions. Figure 6 displays the marked strain-specific differences in the dose-dependent and time-dependent ability of NTX to significantly reduce Intralipid intake. Whereas DBA mice displayed significant reductions across all four NTX doses (Figure 6D), SJL and SWR mice displayed significant reductions following three NTX doses (Figures 6F, G). The B10 and CD-1 strains displayed significant reductions following the two highest NTX doses respectively (Figures 6C, D), whereas the B6 and 129 strains displayed significant reductions only following the highest NTX dose (Figures 6B, H). In contrast, BALB mice failed to display NTX-induced reductions in Intralipid intake at any of the tested doses (Figure 6A).

Significant differences in percent baseline values of Intralipid intake following NTX were also observed among strains ($F(7,68) = 3.60$, $P < 0.002$), among doses ($F(3,66) = 18.89$, $P < 0.0001$), across times ($F(5,64) = 13.65$, $P < 0.0001$), and only for the interaction between strains and times ($F(35,340) = 1.59$, $P < 0.022$), but not for the other two-way or three-way interactions. NTX-induced changes in the percentage of Intralipid intake relative to vehicle was not altered in BALB mice following the 0.01, 0.1 and 5 mg/kg doses, and was reduced by only 12% following the 1 mg/kg dose. The lowest 0.01 mg/kg NTX dose reduced the percentage of Intralipid intake relative to vehicle by 21–41% in only the CD-1, DBA, and SJL strains. However, all strains other than the BALB strain displayed dose-dependent inhibition relative to vehicle of 18–44% following the 0.1 mg/kg dose, 23–59% following the 1 mg/kg dose, and 28–71% following the 5 mg/kg dose. The following significant interstrain differences were observed. BALB mice displayed significantly less inhibition than DBA and SWR mice following the three highest NTX doses, and than SJL, B6, 129, B10 and CD-1 mice following the two highest NTX doses, effects that typically persisted from 30 to 120 min. B10 mice displayed


A
BALB/c Mice and Naltrexone (mg/kg)
B
C57BL/6 Mice and Naltrexone (mg/kg)
C
C57BL/10 Mice and Naltrexone (mg/kg)
D
CD-1 Mice and Naltrexone (mg/kg)
E
DBA/2 Mice and Naltrexone (mg/kg)
F
SJL Mice and Naltrexone (mg/kg)
G
SWR Mice and Naltrexone (mg/kg)
H
129P3 Mice and Naltrexone (mg/kg)
Intralipid Intake (ml)
Time (min)
0
0.01
0.1
1.0
5.0

DYM

Figure 6. Alterations (mean ± SEM) in intralipid intake following four doses of the opioid receptor antagonist, NTX in eight mouse strains. Individual analyses of variance revealed significant main dose effects and interactions between doses and times for all, but the BALB/cJ mouse strains. Significant ($P < 0.05$) decreases (*) in intralipid intake following specific drug doses at particular time points relative to corresponding vehicle intake are denoted.

significantly less inhibition than DBA mice following the all NTX doses only after 5 min, than SWR mice following the 1 mg/kg dose for up to 30 min, than SJL mice following the 0.01, 0.1 and 5 mg/kg NTX doses only after 5 min, than CD-1 mice following the 0.01 and 5 mg/kg NTX doses only after 5 min, and than 129 mice following the 0.1 and 1 mg/kg NTX doses only after 5 min. B6 mice displayed significantly less inhibition than DBA, SWR, CD-1, 129 and B10 mice for up to 30 min following the 1 mg/kg NTX dose.

Strain differences in the ID50 of SCH23390- and NTX-induced inhibition of Intralipid intake: Table 7 summarizes the ID50 values for the respective abilities of SCH23390 and NTX to inhibit Intralipid intake 60 min following antagonist treatments; these data are consistent with peak inhibition for each time point across the 120 min time course (data not shown) for each strain and each antagonist. DBA mice displayed the lowest ID50 values for SCH23390-induced (145 nmol/kg) and NTX-induced (1.5 mg/kg) inhibition of Intralipid intake. In contrast, BALB mice displayed the highest ID50 values for SCH23390-induced (1928 nmol/kg) and NTX-induced (14 mg/kg) inhibition of Intralipid intake, resulting in respective 13.3-fold and 9.3-fold differences between the most-sensitive (DBA) and least (BALB) sensitive strains in D1-like antagonist-induced and general opioid antagonist-induced inhibition of Intralipid intake. There was a strong, significant and positive correlation ($r = 0.91$, $P < 0.01$) between the respective abilities of SCH23390 and NTX to inhibit Intralipid intake across strains.

In addition to the current study, our laboratory performed parallel studies examining the role of genetic variance in the dose-dependent effects of NTX and SCH23390 in inhibiting sucrose intake (Chapter Three and Chapter Four). To examine whether there were any relationships between the inhibitory effects of NTX or SCH23390 upon sucrose intake with that of Intralipid intake, Table 7 also provides the ID50 values for the previously-determined

Table 7. Linear regression values (ID_{50}) of SCH23390 (SCH: nmol/kg)- and NTX (NTX: mg/kg)-induced inhibition of Intralipid (FAT; 5%) and sucrose[a,b] (SUC: 10%) intakes among the inbred and outbred mouse strains at 60 min.

Strain	**SCH FAT**	**SCH SUC[b]**	**SCH Ratio**	**NTX FAT**	**NTX SUC[a]**	**NTX Ratio**
BALB/cJ	1928	890	2.2	14.0	4.6	3.0
C57BL/6J	598	694	0.9	4.4	1.5	2.9
C57BL/10J	1112	998	1.1	4.3	0.5	8.6
CD-1	174	1059	0.2	2.6	5.4	0.5
DBA/2J	145	681	0.2	1.5	4.7	0.3
SJL/J	487	729	0.7	3.5	7.6	0.5
SWR/J	203	964	0.2	4.0	8.8	0.5
129P3J	1016	513	2.0	8.4	10.0	0.8
Sensitivity Ratio[c]	13.3[d]	2.1[e]		9.3[f]	20[g]	

a
Data derived from Dym et al., 2007.

b
Data derived from Dym et al., 2009.

c
The sensitivity ratio refers to the potency shift of antagonist-induced inhibition of intake (ID_{50}) between the least sensitive strain on a particular antagonist and intake measure relative to the most sensitive strain.

d
SCH FAT: *BALB/cJ* vs. *DBA/2J*.

e
SCH SUC: CD-1 vs. *129P3J*.

f
NTX FAT: *BALB/cJ* vs. *DBA/2J*.

g
NTX SUC: *129P3J* vs. *C57BL/10J*.

(Chapter Three and Chapter Four) respective abilities of SCH23390 and NTX to inhibit sucrose intake 60 min following antagonist treatments. For SCH23390, three of the strains (CD-1, DBA and SWR) exhibited far greater inhibition of Intralipid intake relative to sucrose intake. In contrast, two strains (BALB and 129) exhibited far greater D1-like antagonist-induced inhibition of sucrose intake relative to Intralipid intake, whereas the remaining three strains (B6, B10 and SJL) produced fairly comparable degrees of inhibition following D1-like antagonism. This mixed relationship was confirmed by the lack of a correlation ($r = -0.03$, ns) of SCH23390-induced inhibition of Intralipid and sucrose intake across the tested strains. For NTX, four of the strains (CD-1, DBA, SJL and SWR) exhibited far greater inhibition of Intralipid intake relative to sucrose intake. In contrast, three strains (BALB, B6 and B10) exhibited far greater opioid antagonist-induced inhibition of sucrose intake relative to Intralipid intake, whereas the remaining strain (129) produced fairly comparable degrees of inhibition following opioid antagonism. This mixed relationship was confirmed by the weak correlation ($r = 0.11$, ns) of NTX-induced inhibition of Intralipid and sucrose intake across the tested strains. We (Dym et al., 2009) previously reported that there was a weak correlation ($r = -0.33$, ns) between the respective abilities of SCH23390 and NTX to inhibit sucrose intake across the tested strains.

Heritability estimates of SCH23390- and NTX-induced inhibition of Intralipid intake:

Table 8 summarizes the narrow-sense heritability estimates for each effective SCH23390 and NTX dose at each time point. Whereas relatively consistent heritability estimates ($h2 = 0.31–0.48$) were obtained for baseline vehicle Intralipid intake across the entire 120 min time course (data not shown), a more time-dependent relationship for heritability estimates were obtained across the time course for the different SCH23390 and NTX doses. Thus, for SCH23390, heritability estimates were quite modest (e.g., h2 =

DYM

Table 8. Heritability estimates for percent baseline vehicle intake scores across strains for each SCH23390 and NTX dose at each time point.

Time → Condition ↓	**5 min**	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
SCH23390						
50 nmol/kg	0.23	0.20	0.22	0.20	0.30	0.32
200 nmol/kg	0.18	0.18	0.24	0.20	0.27	0.22
400 nmol/kg	0.31	0.31	0.32	0.37	0.37	0.41
800 nmol/kg	0.12	0.19	0.30	0.28	0.30	0.42
1600 nmol/kg	0.20	0.25	0.26	0.23	0.26	0.25
Naltrexone						
0.01 mg/kg	0.20	0.28	0.22	0.23	0.35	0.37
0.1 mg/kg	0.10	0.08	0.13	0.22	0.36	0.33
1.0 mg/kg	0.09	0.13	0.17	0.20	0.25	0.30
5.0 mg/kg	0.35	0.38	0.34	0.40	0.46	0.42

0.12) in the first 5 min, and typically increased over time up to the 120 min time point (e.g., h2 = 0.42). This appeared to be associated with the onset of significant SCH23390-induced inhibition of Intralipid intake noted across strains as early as 15 min (DBA, SWR, 129, CD-1) and 30 min (SJL, B6, B10), but not in BALB mice (Figure 5). Similarly, with NTX, heritability estimates were again modest (e.g., h2 = 0.08–0.10) in the first 15 min and time-dependently increased at the 120 time point (e.g., h2 = 0.30 0.42). These effects also appeared to be associated with the onset of significant NTX-induced inhibition of Intralipid intake noted across strains as early as 15 min (DBA, SWR), 30 min (CD-1, SJL) and 60 min (B6, B10, 129), but not in BALB mice (Figure 6). Such delays in the onset of NTX-induced inhibition of Intralipid intake is consistent with evidence that opioid antagonism is more associated with the inhibition of the maintenance, rather than the initiation of palatable intake (e.g., Frisina and Sclafani, 2002; Kirkham and Cooper, 1988a; Kirkham and Cooper, 1988b; Rockwood and Reid, 1982).

Discussion

Control of a critical macronutrient, fat, is mediated by at least two well-studied factors: a) DA and opioid receptor signaling and b) genetic variance. The present study demonstrated that the effectiveness of DA D1-like and general opioid antagonists to reduce Intralipid intake was subject to genetic variance. Thus, SCH23390 produced differential and significant strain-dependent reductions in Intralipid intake at five (DBA, SWR, CD-1), four (SJL, B6), three (129) and one (B10) of the doses tested, and failed to affect Intralipid intake in BALB mice. Evaluation of the potency of SCH23390 to produce a 50% inhibition of Intralipid intake revealed a 13.3-fold difference between the most (DBA) and least sensitive (BALB) mouse strains. Further, NTX produced differential and significant strain-dependent reductions in Intralipid intake at four (DBA), three (SWR, SJL), two (CD-1, B10) and one (B6, 129) of the doses tested, and also failed to affect Intralipid intake in BALB mice. Correspondingly, there was a 9.3-fold difference in the potency of NTX to produce a 50% inhibition of Intralipid intake in the most (DBA) and least (BALB) sensitive strains studied. Interestingly, there was a highly-significant correlation ($r = 0.91$) in the strain-dependent abilities of SCH23390 and NTX to induce suppressive effects on Intralipid intake across the eight strains. The presence of profound genetic variance in the ability of DA D1 and general opioid antagonists to inhibit fat intake is discussed in terms of: (a) the importance of genetic variance in differentially mediating the dopaminergic and opioid pharmacological substrates underlying fat and sugar intake and (b) their relationship to previous studies demonstrating genetic variance in fat intake and the concept of allelic variation in fat intake.

Genetic variance and the pharmacological substrates mediating Intralipid and sucrose intake: The present study was part of our systematic comparison of the role of genetic variance

in sugar (sucrose) and fat (Intralipid) intake, and the role of DA and opioid receptor systems in sugar and fat preferences. In comparing genetic variance in 24-h intakes across different concentrations of sucrose (Lewis et al., 2005) and Intralipid (Lewis et al., 2007), twelve mouse strains displayed differences in sucrose (0.0001–20%) intake with the greatest amount of sucrose consumption in SWR, CD-1, SJL, A, B6, and B10 strains, intermediate intake in BALB and 129 strains, and significantly lower intake in AKR, CBA, C3H and DBA strains. The same 12 mouse strains also displayed differences in Intralipid (0.00001–5%) intake such that significant increases in Intralipid intake were observed in BALB mice at the seven highest concentrations, in SWR, AKR, B6 and DBA mice at the four highest concentrations, in CD-1, B10 and SJL mice at the three highest concentrations, and in A, C3H/He, CBA and 129 mice at the two highest concentrations (Lewis et al., 2007). Indeed, a positive strong correlation ($r = 0.87$) was observed among the 12 strains in the magnitude of sucrose and Intralipid intake, suggesting an underlying close strain-specific relationship between sugar and fat intake.

As described in the previous chapters, Dym et al. (2007), examined the role of genetic variance in general opioid as well as DA D1-like and D2-like (Dym et al., 2009) antagonism of sucrose intake. NTX significantly and dose-dependently inhibited sucrose (10%) intake with the greatest sensitivity in B10 and B6 strains, intermediate sensitivity in BALB, C3H, CD-1 and DBA mice, and the least sensitivity in 129, SWR and SJL strains, revealing a 37-fold difference in the ID50 of the most and least responsive strains to NTX-induced suppression of sucrose intake (Dym et al., 2007). Raclopride was ineffective in producing strain-dependent effects, and only significantly reduced sucrose intake at the highest test dose in seven of the nine strains. In contrast, SCH23390 significantly reduced sucrose intake at all five doses in 129 and SJL mice, at four doses in B6 and BALB mice, at three doses in DBA, SWR, C3H and B10 mice, and at the

two highest doses in CD-1 mice, revealing a 3-fold difference in the drug effect between the most and least responsive strains (Dym et al., 2009). A weak and non-significant relationship ($r = -0.32$) was observed between the potency of general opioid and D1-like antagonists to produce a 50% inhibition of sucrose intake across the nine mouse strains.

Given the strong positive correlation among strains between the 24-h intakes of sucrose and Intralipid (Lewis et al., 2005; Lewis et al., 2007), we hypothesized that genetic variance in the pharmacological sensitivity to DA D1-like and general opioid antagonism in inhibiting Intralipid intake would be similar to the observed genetic variance in DA D1-like (Dym et al., 2009) and opioid (Dym et al., 2007) antagonist-induced inhibition of sucrose intake. However, direct comparison of the potencies of SCH23390 and NTX to inhibit sucrose and Intralipid intakes failed to reveal similar drug effects. In particular, there were no correlations of SCH23390-induced inhibition of Intralipid and sucrose intake ($r = -0.03$) or NTX-induced inhibition of Intralipid and sucrose intake ($r = 0.11$) across the tested strains. Further, our previous studies (Dym et al., 2007; Dym et al., 2009) revealed a weak negative correlation ($r = -0.33$, ns) between the inhibitory effects of SCH23390 and NTX on sucrose intake across strains. Further within-strain comparisons of D1-like antagonist effects on the intake of sugar and fat revealed that whereas CD-1, DBA and SWR strains exhibited far greater SCH23390-induced inhibition of Intralipid intake relative to sucrose intake, BALB and 129 strains exhibited far greater D1 antagonist-induced inhibition of sucrose intake relative to Intralipid intake. Moreover, whereas CD-1, DBA, SJL and SWR strains exhibited far greater NTX-induced inhibition of Intralipid intake relative to sucrose intake, BALB, B6 and B10 strains exhibited far greater opioid antagonist-induced inhibition of sucrose intake relative to Intralipid intake. These data

indicate heterogeneous patterns of sucrose and fat intake responses across strains to D1 and opioid antagonism, and a lack of an orderly relationship for rank-ordered genetic variance.

Genetic variance and allelic variation of fat intake: The present study revealed a strong relationship between the inhibitory actions of DA D1-like and general opioid antagonists on Intralipid intake. In this regard, DBA mice displayed the most potent inhibition of Intralipid intake following both SCH23390 and NTX. CD-1 mice displayed the second-most potent inhibition of Intralipid intake following both SCH23390 and NTX; these were followed by comparable levels of inhibition noted for SWR and SJL mice. In contrast, BALB mice failed to display any significant inhibition following any dose of either SCH23390 or NTX. Interestingly, changes in heritability estimates obtained across the time course for the different SCH23390 and NTX doses appeared to be associated with the onset of significant SCH23390-induced inhibition of Intralipid intake noted across strains as early as 15–30 min as well as the delayed onset of significant NTX-induced inhibition of Intralipid intake.

Three strains (SWR, DBA and B6) examined in the present study have considerable previous information concerning their responsiveness to fat. Although generally considered a carbohydrate-preferring strain, SWR mice consumed large amounts of a fat diet that was not accompanied by weight gain, and was inversely correlated with accumulation of ependymal fat (Smith et al., 2000; Smith et al., 2001). These effects persisted in SWR mice irrespective of the make-up of the fat source and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999). Potential mechanisms by which SWR mice consumed more fat than carbohydrate, yet failed to gain weight, were lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin, and/or reduced arcuate NPY levels (Leibowitz et al., 2005). DBA strains consumed greater amounts of fat, displayed more

adiposity, and displayed elevated levels of leptin and insulin (Alexander et al., 2006). B6 mice self-selected the highest proportion of fat in macronutrient diet selection with accumulation of ependymal fat correlating with fat consumption (Smith et al., 2000; Smith et al., 2001). Further, the B6 strain showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). Thus, DBA and B6 strains act like AKR mice in their avidity for fat that correlated with weight gain, whereas SWR mice consumed fat, but failed to show weight gain. The dopaminergic and opioid pharmacological controls of Intralipid intake do not show this dichotomous relationship among these strains. Whereas DBA mice exhibit the greatest sensitivity to DA D1-like and opioid antagonism of Intralipid intake, SWR mice are nearly as sensitive to SCH23390-induced inhibition of Intralipid intake as the DBA strain and almost 3-fold more sensitive relative to the B6 strain. For opioid antagonism, B6 and SWR mice display comparable sensitivity that is 2.5- to 3-fold less sensitive than DBA mice. Two of the three other strains previously showing genetic variance in fat intake (AKR, A) failed to meet the criterion of a minimum consumption of at least 1 ml of Intralipid in the short-term (2 h) intake paradigm, and were therefore not included in the pharmacological analysis. In sum, it does not appear that dopaminergic or opioid signaling contribute to these previously described strain-dependent dichotomies between fat intake and weight accumulation.

Another area of allelic variation, especially when considering opioid control of Intralipid intake as a function of mouse strain, is baseline differences in opioid receptor mRNA and endogenous opioids. The B6 and DBA strains have been examined because of their respective preference and avoidance responses for alcohol that are under opioid control. DBA mice have higher contents of kappa opioid receptors, pro-dynorphin mRNA and dynorphin peptides in the limbic system relative to B6 mice (Jamensky and Gianoulakis, 1997; Korostynski et al., 2006;

Winkler and Spanagel, 1998). Further, alcohol-preferring B6 and alcohol-avoiding BALB mice also show differential kappa opioid receptor splicing and gene expression, whereas DBA differ from both of these strains in yet another aspect of kappa receptor gene expression (Saito et al., 2003). In contrast, CXBK mice have decreased delta opioid receptor binding than their BALB progenitors (Kest et al., 1998), but similar mu and delta binding relative to outbred Swiss-Webster mice (Duttaroy et al., 1999; Duttaroy and Yoburn, 2000). It should be noted that the present study employed NTX, a general opioid antagonist with actions at mu, delta and kappa opioid binding sites. Future studies using selective mu, delta and kappa opioid receptor subtype antagonists might amplify this genetic variation further.

The present findings can also be considered in light of emerging quatitative trait loci (QTL) analyses of fat intake and its consequences. An initial study (Smith-Richards et al., 2002) examined a F2 population derived from fat-preferring (B6) and carbohydrate-preferring (CAST/Ei) strains that were given a choice of either a fat/protein or carbohydrate/protein diet. QTL analyses revealed three significant loci for fat intake on chromosomes 8 (Mnif1), 18 (Mnif2) and X (Mnif3). A thrifty gene on chromosome 14 defined by caloric efficiency was a dominant-acting genetic component of high-fat diet-induced obesity in a F2 intercross of B6 and 129S6/SvEv mice (Almind and Kahn, 2004). Finally, using a F2 intercross, QTL analyses of fat depots in mouse lines divergently selected for food intake revealed that chromosomes 4 and 19 were associated with both white and brown adipose tissue depots, whereas chromosome 9 was associated with only white adipose tissue depots (Rance et al., 2007). It would be of future interest to link underlying pharmacological control of fat intake with QTL analyses of highly-sensitive (e.g., SWR, DBA) and less-sensitive (BALB) strains.

Roles of genetic variance and pharmacological substrates in fat and sugar intake pertaining to food reward: The present and previous (Dym et al., 2007; Dym et al., 2009) findings are of more general interest because DA and opioid receptors are thought to mediate different aspects of food reward (Berridge, 1996; Barbano and Cador, 2007). In particular, Berridge (1996) initially proposed that several psychological constructs can be supported by neurobiological evidence, such that DA receptors mediate food "wanting" whereas opioid receptors mediate food "liking" (Berridge, 2009). An implicit assumption is that such mechanisms are centrally-mediated. However, the present study employed a systemic route of drug administration, raising the possibility that the pharmacological effects of treatments may occur outside the central nervous system. The fact that SCH23390-induced and NTX-induced suppressive effects on Intralipid intake were highly correlated ($r = 0.91$) in the eight strains, would appear to suggests a close relationship between the food liking and wanting systems among the mouse strains tested, at least for a liquid fat source. Of more interest is the possibility that the differential effects of NTX and SCH23390 on sucrose and Intralipid intakes are related to neurotransmitter-specific effects on nutrient intake and preference. Several macronutrient diet selection studies suggest that brain opioid receptors are specifically involved in fat preference, although other studies suggest that opioids modulate the intake of preferred foods irrespective of nutrient content (Glass et al. 1999). There is less information available on the possible nutrient specificity of DA drug effects. An important consideration in evaluating claims of neurotransmitter-nutrient specificity, are findings that drug effects may be influenced by the specific nutrient type used (e.g., sugar vs. starch, corn oil vs. lard; Glass et al., 1999). The physical form of nutrients (e.g., liquid vs. solid) may also influence drug effects since it can have profound effects on food preference and consumption (Lucas et al., 1989; Sclafani, 1987). Therefore, it is possible that the present results

obtained with sucrose and Intralipid solutions would not be replicated in mice offered solid diets or with different carbohydrate and lipid sources. These considerations aside, it is clear that intake of solutions rich in sugar and fat are differentially inhibited by DA D1-like and general opioid antagonists in different inbred strains of mice, thereby demonstrating an interaction between genetic variance and pharmacological mechanisms of action.

Much information has been gained by comparing general intake of sucrose and fat solutions following administration of opioid and dopaminergic receptor antagonists to several mouse strains. However, intake of palatable nutrients is affected by more than pharmacology and genetics. The environment - in the form of learned preferences - also factors in as a tremendous influence on nutrient intake. The next chapter will examine the role that learning plays in preference of a palatable substance and will also combine the previous chapters' investigation of genetic variance and pharmacology.

CHAPTER SIX: DA AND OPIOID RECEPTOR SIGNALING DIFFERENTIALLY MODULATE THE ACQUISITION AND EXPRESSION OF SUCROSE-CONDITIONED FLAVOR PREFERENCES IN BALB AND SWR MICE

Introduction

In addition to innate factors intimately implicated in the intake of foods and solutions containing sucrose, animals use both flavor (taste, odor and texture) and post-ingestive cues to guide their selection of sucrose solutions (Capaldi, 1996; Sclafani, 1995). The current chapter investigated the genetic variance, pharmacology and learning by observing the ability of SCH23390 and NTX to alter the expression and acquisition of sucrose-CFP in 2 inbred mouse strains.

In flavor-flavor conditioning, learned associations are made between the various flavor elements in the foods, whereas in flavor-nutrient conditioning, learned associations are made between the flavor cues and their post-ingestive consequences (Sclafani, 1995). Overconsumption of sucrose and its resultant potent reward value can be driven by innate factors as well as by learned flavor-flavor and flavor-nutrient conditioned flavor preferences (CFP). Both DA and opioid neurotransmitter systems have been extensively studied in rats to examine their modulation of these three different aspects of sucrose intake.

DA has been implicated in sucrose's reward value primarily because it activates mesolimbic DA circuits that are involved in the mediation of natural as well as drug rewards in the nucleus accumbens (e.g., Cheng and Feenstra, 2006; Genn et al., 2004; Hajnal et al., 2003). DA receptor antagonism suppresses the intake of sucrose solutions in rats (e.g., Geary and Smith, 1985; Muscat and Willner, 1989; Xenakis and Sclafani, 1981), presumably due to reductions in the hedonic value (e.g., Schneider, 1989; Smith, 1995) or incentive salience (e.g., Berridge and

Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone et al., 1997) of sweet taste. DA antagonists also alter the ability of sucrose solutions to reinforce CFP. Rats reduced their preference for a flavored 10% sucrose solution paired with an injection of the D2 antagonist, raclopride relative to a differently-flavored sucrose solution paired with a vehicle injection (Hsiao and Smith, 1995). Acquisition of flavor-nutrient conditioning, investigated in rats trained to drink differently flavored saccharin solutions paired with intragastric (IG) infusions of sucrose and water respectively, was blocked by systemic pretreatment with DA D1 (SCH23390), but not D2 (raclopride) receptor antagonism; expression of flavor-nutrient CFP was minimally affected (Azzara et al., 2001). Further, direct DA D1 antagonist administration into the nucleus accumbens shell, amygdala, lateral hypothalamus or medial prefrontal cortex of rats similarly blocked the acquisition, and to a lesser degree, the expression of IG glucose-conditioned flavor-nutrient preferences in rats (Touzani et al., 2008, 2009a. 2009b, 2010). Expression, but not acquisition of flavor-flavor learning, investigated in sham-feeding rats consuming flavored 16% sucrose and 0.2% saccharin solutions, was blocked by systemic DA D1 and D2 receptor antagonism (Yu et al., 2000a, 2000b). Flavor-flavor conditioning was also investigated by training rats to “real-feed” similar amounts of flavored fructose and less-preferred saccharin solutions (Baker et al., 2003) because fructose has minimal post-oral flavor conditioning effects (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). Systemic DA D1, and to a lesser degree, D2 antagonism blocked acquisition of a fructose-CFP in rats, whereas both antagonists dose-dependently eliminated the expression of a fructose-CFP (Baker et al., 2003). Direct administration of DA D1 and D2 antagonists into either the nucleus accumbens or amygdala significantly reduced the expression of fructose-CFP (Bernal et al., 2008, 2009). Hastened extinction of the acquisition of fructose-CFP occurred following DA D1 and D2 antagonists into

the nucleus accumbens shell (Bernal et al., 2008) and following DA D1 antagonists into the amygdala (Bernal et al., 2009).

The endogenous opioid peptide system has also been implicated in the reward value of sweet taste (see reviews: Bodnar, 2004; Cooper, 2007; Levine, 2006). Rodent studies demonstrate that opioid receptor agonists selectively increased, whereas antagonists selectively reduced, the intake of preferred foods and fluids when administered systemically or directly into the brain. General opioid antagonism of the hedonic, or more broadly the reward evaluation of sweet substances (a) suppresses intake of sweet solutions more than plain water (e.g., Cooper, 1983; Le Magnen et al., 1980; Sclafani et al., 1982); (b) blocks the portion of feeding that appears driven by sweet taste in food-restricted animals (e.g., Levine et al., 1995); (c) reduces sucrose's positive hedonic qualities in a taste reactivity paradigm (e.g., Levine et al., 1995; Parker et al., 1992); (d) reduces sucrose intake in sham-feeding tests which minimize post-oral effects (e.g., Kirkham and Cooper, 1988; Rockwood and Reid, 1982), but (e) does not reduce sweet taste discrimination (O'Hare et al., 1994).

Although an early study implicated the opioid system in sugar-CFP in rats (Mehiel, 1996), our laboratory failed to demonstrate that systemic administration of the opioid receptor antagonist NTX altered the acquisition or expression of flavor preferences conditioned by either the sweet taste of sucrose in sham-feeding rats or fructose in real-feeding rats (flavor-flavor conditioning: Baker et al., 2004; Yu et al., 1999) or the post-oral nutritive effects of sucrose in rats (flavor-nutrient conditioning: Azzara et al., 2000). Moreover, infusions of NTX into either the core or shell regions of the nucleus accumbens failed to alter the expression of CFP conditioned by the sweet taste of fructose or the post-ingestive consequences of IG glucose

(Bernal et al., 2010). These findings would appear to conflict with the general idea that central opioid signaling modulates the avidity for preferred foods and fluids (see review: Cooper, 2007).

Genetic variance has been implicated in the innate consumption of sucrose intake in inbred mouse strains (Bachmanov et al., 1997; Blizard et al., 1999; Capeless and Whitney, 1995; Lewis et al., 2005; Lush, 1989; Pothian et al., 2004; Stockton and Whitney, 1974). A strain analysis (Lewis et al., 2005) revealed that inbred SWR, A and B6, B10 and SJL strains consumed both the greatest amount and the greatest percentage of kilocalories consumed as sucrose. Whereas inbred AKR, C3H and DBA strains consumed the smallest amount of sucrose and percentage of kilocalories consumed as sucrose, inbred 129, CBA and BALB mice consumed intermediate levels of sucrose.

Our laboratory (Pinhas et al., 2011) also systematically evaluated inbred mouse strains for the magnitude and persistence of sucrose-CFP. While eight of the tested inbred strains displayed sucrose-CFP, SWR, BALB, C3H and DBA mice displayed the greatest magnitude of effects (86-94%) that persisted over the six days of testing needed to perform pharmacological analyses, the SWR and BALB strains displayed the greatest amount of intake during sucrose-CFP testing integral for the analyses of antagonist effects.

There is evidence indicating that SWR and BALB strains of mice differ in the opioid control of sucrose intake. We recently reported that systemic treatment with an opioid receptor antagonist, naltrexone (NTX) significantly reduced 1-h sucrose intake to a lesser degree in SWR mice than in BALB mice (Dym et al., 2007). Furthermore, other data indicate that opioid-mediated reward processes may be compromised in SWR mice. In particular, SWR mice display significantly less morphine self-administration behavior as compared to 14 other inbred strains (Belknap et al., 1993) and display less morphineinduced conditioned place preferences (Gieryk

et al., 2010; Solecki et al., 2009). In contrast, we found that SWR and BALB mice were similar in their suppression of sucrose intake in response to the D1 DA receptor antagonist SCH23390 (SCH) (Dym et al., 2009). Yet, BALB mice differed from 10 other inbred strains in their failure to self-administer cocaine, suggesting a sub-sensitivity in the DA reward system (Thomsen and Caine, 2011). However, SWR mice were not included in this study so it is not clear that BALB and SWR mice differ in this respect

In the present study, therefore, we investigated the effects of SCH and NTX on sucrose-CFP in SWR and BALB mice. To this end, the effects of SCH (50–800 nmol/kg) and NTX (1–5 mg/kg) on the expression of a previously conditioned CS+ preference was evaluated by administering the drugs just prior to the two-bottle CS+ vs. CS− choice tests. Drug effects on the acquisition (learning) of this preference were evaluated by treating the animals with SCH (50 nmol/kg) or NTX (1 mg/kg) throughout the one-bottle training, and subsequently conducting the two-bottle choice tests in the absence of drug treatments. In light of our previous studies on NTX- and SCH-induced suppression of sucrose intake in BALB and SWR mice (Dym et al., 2007, 2009), we predicted that NTX would be more effective in impairing sucrose-CFP in BALB than in SWR mice, but that SCH would be equally effective in attenuating sucrose-CFP in the two strains.

This work has been presented at the Society for Neuroscience meeting (Dym et al., 2009).

Experimental Procedure

Subjects: Inbred BALB and SWR male mice (Jackson Laboratories, Bar Harbor, ME, 6 weeks of age) were initially acclimated to the Queens College vivarium for one week in group (5 per cage) housing. The animals were then housed individually in plastic cages (30 x 20 x 15 cm)

with stainless steel tops throughout the entire study, and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22oC. All animals were provided with chow (Lab Diet Mouse Chow 5015) and water ad libitum throughout the experiment, except when experimental testing was conducted.

Test solutions: The training solutions consisted of a 16% sucrose (Domino) or a 0.05% saccharin (Sigma-Aldrich Laboratories, St. Louis, MO) solution; the solutions were flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft Foods, White Plains, NY). Half of the mice in each strain had the cherry flavor added to the sucrose solution, and the grape flavor added to the saccharin solution; the flavors were reversed for the remaining mice of each strain. In the two-bottle preference tests, the cherry and grape flavors were each presented in a 0.2% saccharin solution in a paradigm identical to that used previously (Pinhas et al., 2008). The sucrose-paired flavor is referred to as the CS+ and the saccharin-paired flavor as the CS- because 16% sucrose is preferred to 0.05% saccharin in rats (e.g., Sclafani and Nissenbaum, 1985). CS+/Suc refers to the flavored sucrose solution used in training, and CS+/s refers to the same flavor presented in saccharin-only during choice testing. The CS-/Sac refers to the flavored saccharin solution used in training and testing, and CS-/s refers to the same flavor presented in saccharin-only during choice testing. All testing took place in each mouse's home cage during the mid-light phase of the light:dark cycle. Two weeks before testing began, the mice were placed on a food restriction schedule that maintained their body weights at 85-90% of their ad libitum level. The mice were initially trained to drink unflavored 0.2% saccharin solution from a retrofitted testing stainless steel sipper tube connected to a 10 ml plastic syringe (Dym et al., 2009). The sipper tube was firmly secured to the stainless steel top of the cage by a taut metal spring (100 mm) with clips at each end that affixed to the cage top so that the gradations and

meniscus were easily visible allowing accurate measurement (+0.1 ml gradations) of the solution (8 ml). This training procedure was repeated daily until all mice sampled the sipper tubes with short (< 1 min) latency, typically within three days. The limited food rations were given 1 h after each training and testing session.

Expression procedure: Thirty-one BALB and 35 SWR inbred mice received ten one-bottle training sessions (60 min/day) with 8 ml of the CS+/Suc solution presented on odd-numbered days, and 8 ml of the CS-/Sac solution presented on even-numbered days. On days 9 and 10, all mice had access to a second sipper tube containing water. This familiarized the mice to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. The position of the CS and water sipper tubes varied across the 2-bottle training and subsequent testing days using a left-right-right-left pattern in half of the mice of each strain, and a reversed pattern in the other half of each strain. Solution intakes during training were measured by weighing (0.1 g) the sipper tubes before and after the 60-min sessions.

Following training, the mice were given six two-bottle choice test sessions (60 min/day) with access to the CS+/s and CS-/s solutions; consistent sucrose-CFP was observed over this interval regimen in these strains previously (Pinhas et al., 2011). Thirty min prior to the first pair of two-bottle test sessions, all mice of all strains were given either a subcutaneous (sc) or intraperitoneal (ip) vehicle (0.9% normal saline) injection as controls for the SCH23390 and NTX groups respectively. Thirteen BALB and 11 SWR mice received pairs of sc 200 and 800 nmol/kg doses of the D1 antagonist, SCH23390 (Sigma Chemical Co.) with half tested with an ascending dose order and the remainder with a descending dose order. Another ten BALB and 9 SWR mice received a pair of sc 50 nmol/kg SCH23390 doses. The drugs were mixed at concentrations of 5, 20 and 80 nmol/ml and administered at a concentration of 10 ml/kg; this

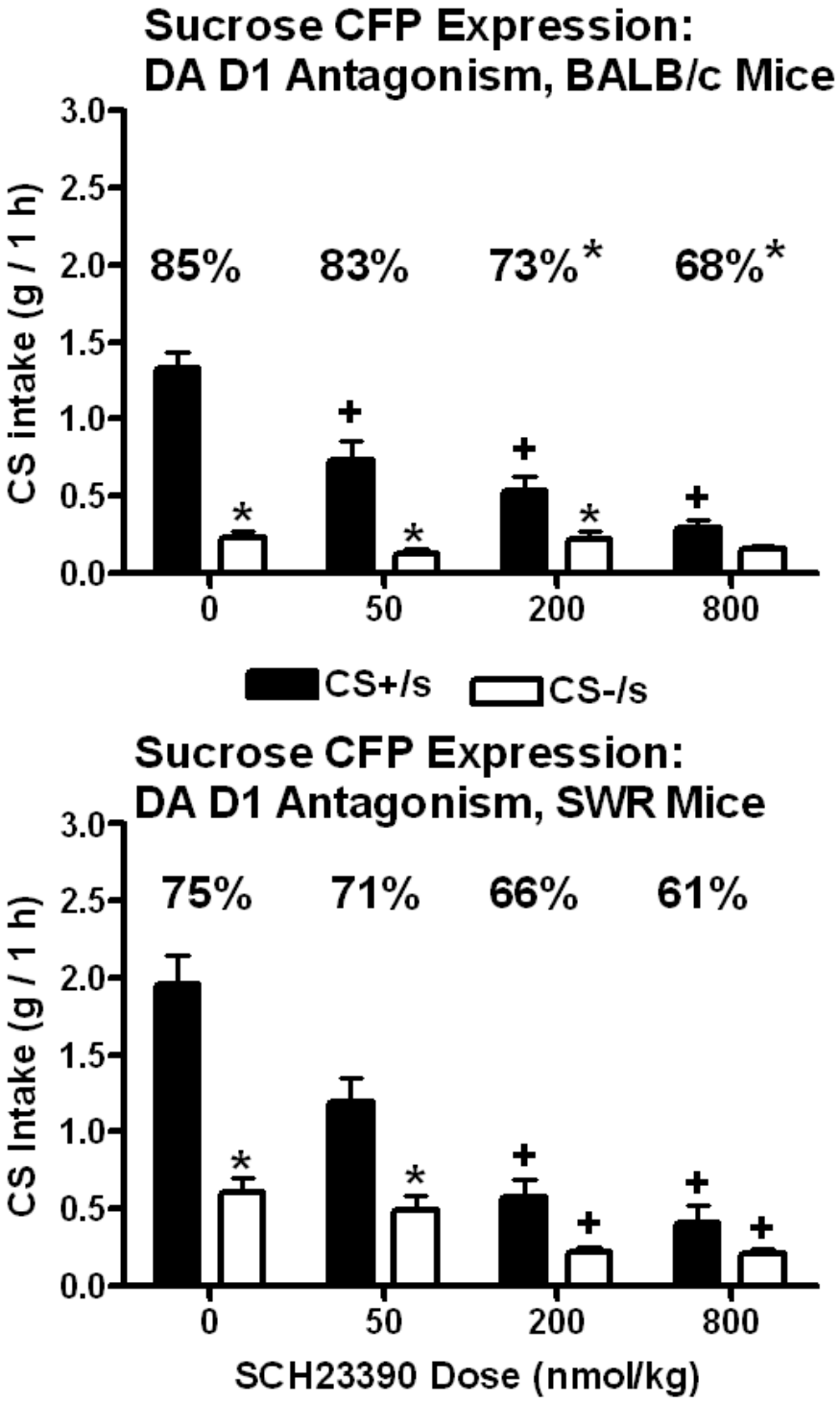
dose range and post-injection interval were chosen on the basis of significant CFP effects observed in rats (Baker et al., 2003; Yu et al., 2000a, 2000b), and effects upon sucrose intake in these mouse strains (Dym et al., 2009). Eight BALB and 15 SWR mice received pairs of ip 1 and 5 mg/kg doses of the general opioid antagonist, NTX (Sigma Chemical Co) with half tested with an ascending dose order and the remainder with a descending dose order. The drugs were mixed at concentrations of 0.1 and 0.5 mg/ml and administered at a concentration of 10 ml/kg; this dose range and post-injection interval were chosen on the basis of evaluated CFP effects in rats (Baker et al., 2004; Yu et al., 1999), and evaluation of sucrose intake in these mouse strains (Dym et al., 2007). Solution intakes during testing were measured by weighing (0.1 g) the sipper tubes before and after the 60-min sessions.

Acquisition procedure: Twenty-one BALB and 21 SWR mice received 10 one-bottle training sessions (60 min/day) with the CS+/Suc solution presented on odd-numbered sessions, and the CS-/Sac solution presented on even-numbered sessions. Thirty min prior to each of the ten 1-bottle training sessions, eight BALB and eight SWR mice received either sc (n=4) or ip (n=4) vehicle injections. Accordingly, six BALB and six SWR mice received a sc injection of SCH23390 (50 nmol/kg), and seven BALB and seven SWR mice received an ip injection of NTX (0.1 mg/kg). Following training, all mice of both strains were given four daily 2-bottle choice sessions (60 min/day) with access to the CS+/s and CS-/s solutions; no drugs were administered prior to these sessions. The positions of the CS+/s and CS-/s solutions were counterbalanced across testing sessions, and the results were analyzed as mean 60-min intakes during successive pairs of sessions (referred to as Tests 1 and 2).

Results

DA D1 antagonist effects on the expression of sucrose-CFP in BALB and SWR mice: In BALB mice, the mean one-bottle training intake of the CS+/Suc solution (1.85 ml) significantly ($F(1,20)= 145.18$, $p<0.0001$) exceeded that of the CS-/Sac solution (0.89 ml). In the two-bottle preference tests, overall, BALB mice consumed significantly more CS+/s than CS-/s ($F(1,55)= 88.68$, $p<0.0001$) with a significant main effect of drug dose ($F(3,55)= 17.74$, $p<0.0001$) and a significant CS x Dose interaction ($F(3,55)= 18.07$, $p<0.0001$). CS+/s intakes significantly exceeded CS-/s intakes at the 0 (vehicle), 50 and 200, but not the 800, nmol/kg doses of SCH23390 (Figure 7, upper panel). BALB mice consumed significantly less CS+/s at all SCH23390 doses as compared to vehicle; CS-/s intakes failed to differ as a function of SCH23390 dose (Figure 7, upper panel). Correspondingly, total saccharin intake significantly declined following the 50 (0.43 ml), 200 (0.37 ml) and 800 (0.22 ml) nmol/kg SCH23390 doses relative to vehicle (0.78 ml). Significant differences in the percent CS+/s intakes were observed ($F(3,55)= 4.95$, $p<0.004$), and the preference at the 800 (68%), but not 50 (83%) or 200 (73%) nmol/kg SCH23390 doses were significantly lower than the preference (85%) following vehicle (Figure 7, upper panel).

In SWR mice, the mean one-bottle training intake of the CS+/Suc solution (1.71 ml) was significantly ($F(1,19)= 12.44$, $p<0.002$) lower than that of the CS-/Sac solution (2.27 ml). In the two-bottle preference tests, overall, the SWR mice consumed significantly more CS+/s than CS-/s ($F(1,47)= 33.33$, $p<0.0001$) with a significant main effect of drug dose ($F(3,47)= 23.94$, $p<0.0001$) and a significant CS x Dose interaction ($F(3,47)= 6.82$, $p<0.0007$). CS+/s intakes significantly exceeded CS-/s intakes at the 0 (vehicle) and 50, but not at the 200 or 800 nmol/kg doses of SCH23390 (Figure 7, lower panel). SWR mice consumed significantly less CS+/s at all SCH23390 doses as compared to vehicle; CS-/s

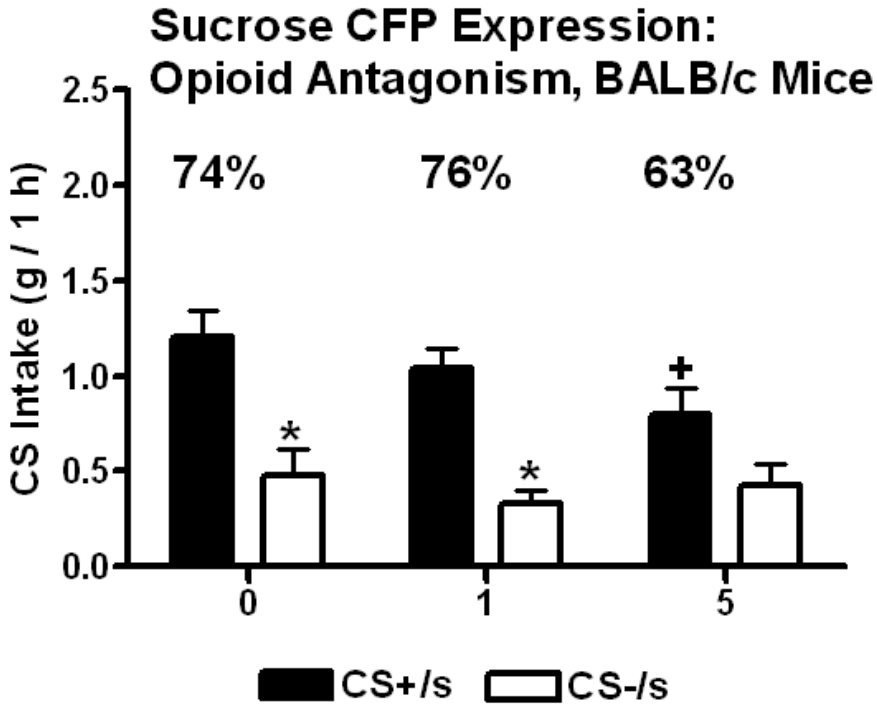

Sucrose CFP Expression:
DA D1 Antagonism, BALB/c Mice
CS intake (g / 1 h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
85%
83%
73%*
68%*
0
50
200
800
CS+/s
CS-/s
Sucrose CFP Expression:
DA D1 Antagonism, SWR Mice
CS Intake (g / 1 h)
75%
71%
66%
61%
SCH23390 Dose (nmol/kg)

DYM

Figure 7. (Expression Procedure). Intakes (mean +SEM, g/60 min) of CS+/s and CS-/s solutions in two-bottle tests in BALB/cJ (upper panel) and SWR (lower panel) inbred mice receiving systemic injections of the DA D1-like antagonist, SCH23390 at doses of 0, 50, 200 and 800 nmol/kg 30 min prior to testing. Significant differences in this and the next figure are denoted between CS+/s and CS-/s intake within an injection condition (*) and between CS+/s intake following a drug dose relative to the vehicle treatment (+). The percentages of CS+/s intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (*) noted.

intake significantly declined at the two higher SCH23390 doses (Figure 7, lower panel). Correspondingly, total saccharin intake significantly declined following the 50 (0.84 ml), 200 (0.40 ml) and 800 (0.31 ml) nmol/kg SCH23390 doses relative to vehicle (1.28 ml). However, significant differences in the percent CS+/s intakes failed to be observed ($F(3,47)= 1.68$, ns) among the 0 (vehicle: 75%), 50 (71%), 200 (66%) and 800 (61%) SCH23390 doses in SWR mice (Figure 7, lower panel).

Opioid antagonist effects on the expression of sucrose-CFP in BALB and SWR mice: In BALB mice, the mean one-bottle training intake of the CS+/Suc solution (1.76 ml) significantly ($F(1,7)= 70.51$, $p<0.0001$) exceeded that of the CS-/Sac solution (0.78 ml). In the two-bottle preference tests, overall, BALB mice consumed significantly more CS+/s than CS-/s ($F(1,21)= 30.77$, $p<0.0001$), but significant differences failed to occur among drug doses ($F(2,21)= 1.90$, ns) or for the CS x Dose interaction ($F(2,21)= 1.11$, ns). CS+/s intakes significantly exceeded CS-/s intakes at the 0 (vehicle) and 1, but not at the 5 mg/kg doses of NTX (Figure 8, upper panel). BALB mice consumed significantly less CS+/s at the highest NTX dose as compared to vehicle; CS-/s intakes failed to differ as a function of NTX dose (Figure 8, upper panel). Total saccharin intake failed to change across the 0 (0.84 ml), 1 (0.68 ml) and 5 (0.61 ml) mg/kg doses of NTX. Significant differences in the percent CS+/s intakes failed to occur ($F(2,21)= 1.16$, ns) across the vehicle (74%), 1 (76%) and 5 (63%) mg/kg NTX doses (Figure 8, upper panel). In SWR mice, the mean one-bottle training intake of the CS+/Suc solution (1.55 ml) was similar ($F(1,14)= 1.08$, ns) to that of the CS-/Sac solution (1.40 ml). In the two-bottle preference tests, overall, the SWR mice consumed significantly more CS+/s than CS-/s ($F(1,42)= 40.59$, $p<0.0001$), with a significant main effect of drug dose ($F(2,42)= 4.42$, $p<0.018$) and a significant CS x Dose interaction ($F(2,42)= 5.47$, $p<0.008$). CS+/s intakes significantly exceeded CS-/s


Sucrose CFP Expression:
Opioid Antagonism, BALB/c Mice
74%
76%
63%
CS Intake (g / 1 h)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
5
CS+/s
CS-/s

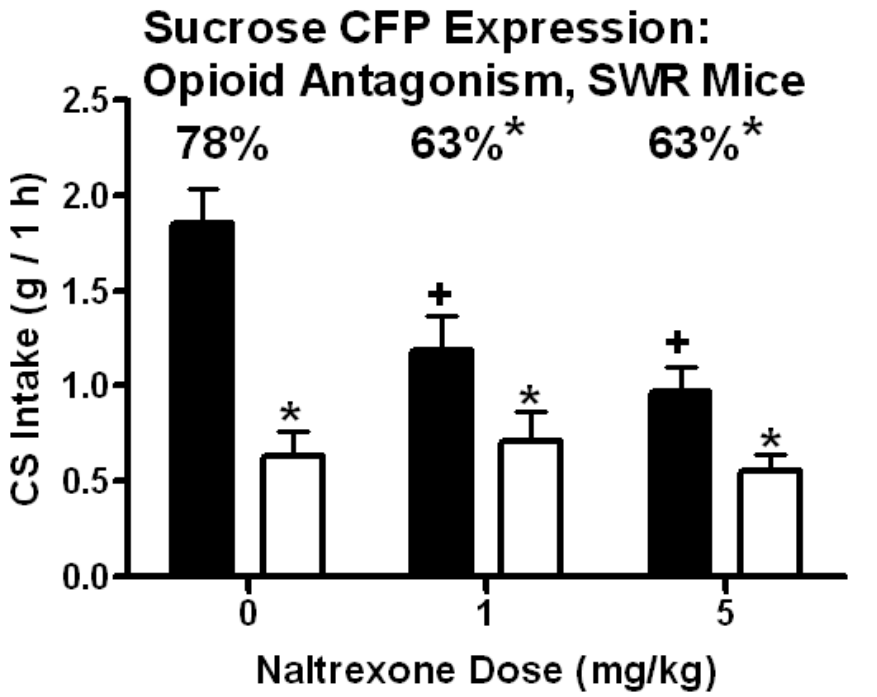
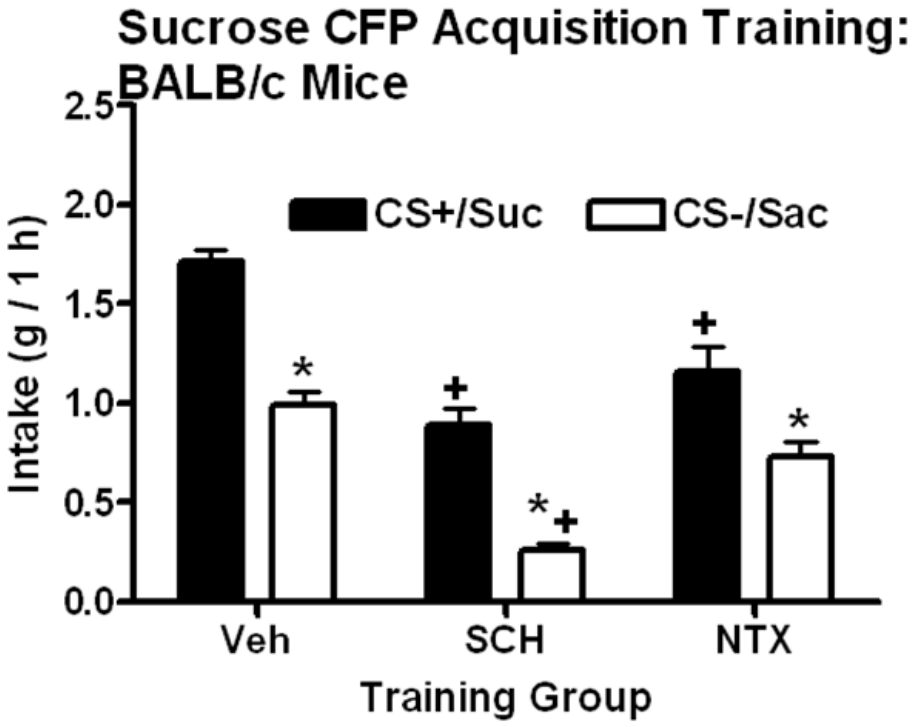

Sucrose CFP Expression:
Opioid Antagonism, SWR Mice
78%
63%*
63%*
CS Intake (g / 1 h)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
5
Naltrexone Dose (mg/kg)

Figure 8. (Expression Procedure). Intakes (mean +SEM, g/60 min) of CS+/s and CS-/s solutions in two-bottle tests in BALB/cJ (upper panel) and SWR/J (lower panel) inbred mice receiving systemic injections of the general opioid antagonist, NTX at doses of 0, 1 and 5 mg/kg 30 min prior to testing.

intakes at the 0 (vehicle), 1 and 5 mg/kg doses of NTX (Figure 8, lower panel). SWR mice consumed significantly less CS+/s at the 1 and 5 mg/kg NTX dose as compared to vehicle; CS-/s intake failed to differ across NTX doses (Figure 8, lower panel). Correspondingly, total saccharin intake significantly declined following the 1 (0.95 ml) and 5 0 (0.77 ml) mg/kg NTX doses relative to vehicle (1.24 ml). Significant differences in the percent CS+/s intakes occurred ($F(2,42)= 3.96$, $p<0.027$) with significant reductions following the 1 (63%) and 5 (63%) mg/kg NTX doses relative to vehicle (78%) in SWR mice (Figure 8, lower panel).

DA D1 and opioid antagonist effects on the acquisition of sucrose-CFP in BALB mice: In the one-bottle training intakes, overall CS+/Suc intake (1.29 ml) significantly ($F(1,18)= 133.44$, $p<0.0001$) exceeded CS-/Sac intake (0.69 ml). The three groups displayed significant differences ($F(2,18)= 39.02$, $p<0.0001$), but the group x condition interaction failed to differ ($F(2,18)= 2.78$, ns). Total intake was significantly lower in BALB mice receiving SCH23390 (0.58 ml) or NTX- (0.94 ml) relative to vehicle (1.35 ml) during training. CS+/Suc intake was significantly greater than CS-/s intake in BALB mice receiving vehicle, SCH23390 and NTX during training (Figure 9, upper panel). Vehicle-trained BALB mice had significantly greater CS+/Suc intake than their SCH23390- and NTX-trained counterparts, and significantly greater CS-/s intake than SCH23390-trained mice (Figure 9, upper panel).

In the two-bottle preference tests, overall, the BALB mice consumed significantly more CS+/s than CS-/s ($F(1,18)= 43.20$, $p<0.0001$), but significant differences failed to vary as a function of group ($F(2,18)= 0.05$, ns), test pair ($F(1,18)= 0.06$, ns) or any 2-way or 3-way interaction. CS+/s intakes significantly exceeded CS-/s intakes for both test pairs in the vehicle-trained and SCH23390-trained groups (Figure 9, lower panel). In the NTX-trained group, CS+/s and CS-/s intake failed to differ from each other for the first test pair, but CS+/s intake


Sucrose CFP Acquisition Training:
BALB/c Mice
Intake (g / 1 h)
2.5
2.0
1.5
1.0
0.5
0.0
CS+/Suc
CS-/Sac
Veh
SCH
NTX
Training Group

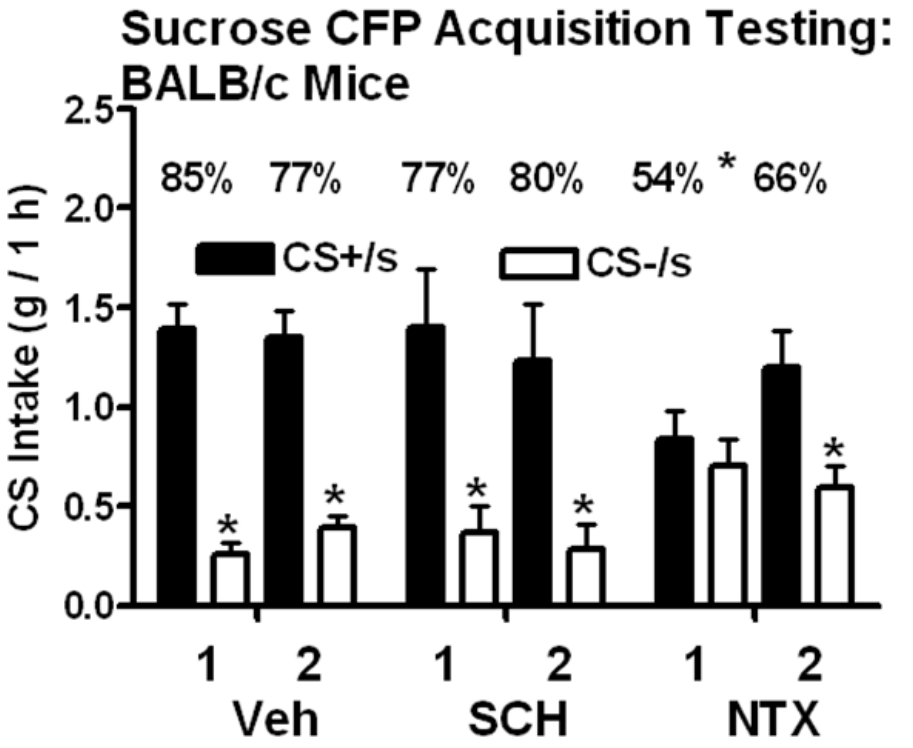
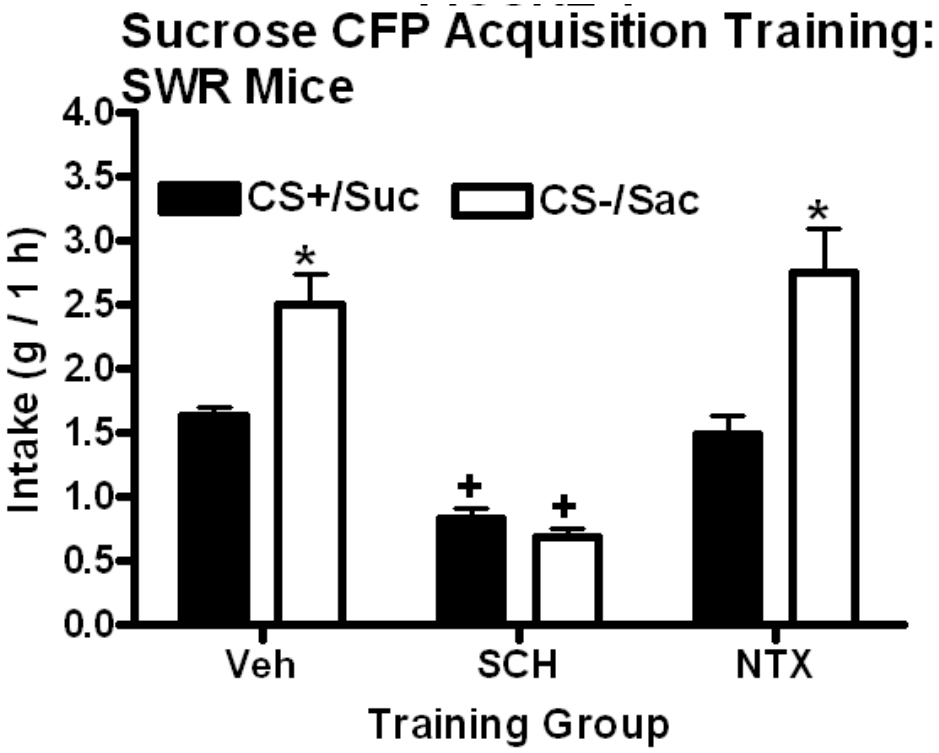

Sucrose CFP Acquisition Testing:
BALB/c Mice
CS Intake (g / 1 h)
2.5
2.0
1.5
1.0
0.5
0.0
85% 77% 77% 80% 54% * 66%
CS+/s
CS-/s
1 2 1 2 1 2
Veh
SCH
NTX

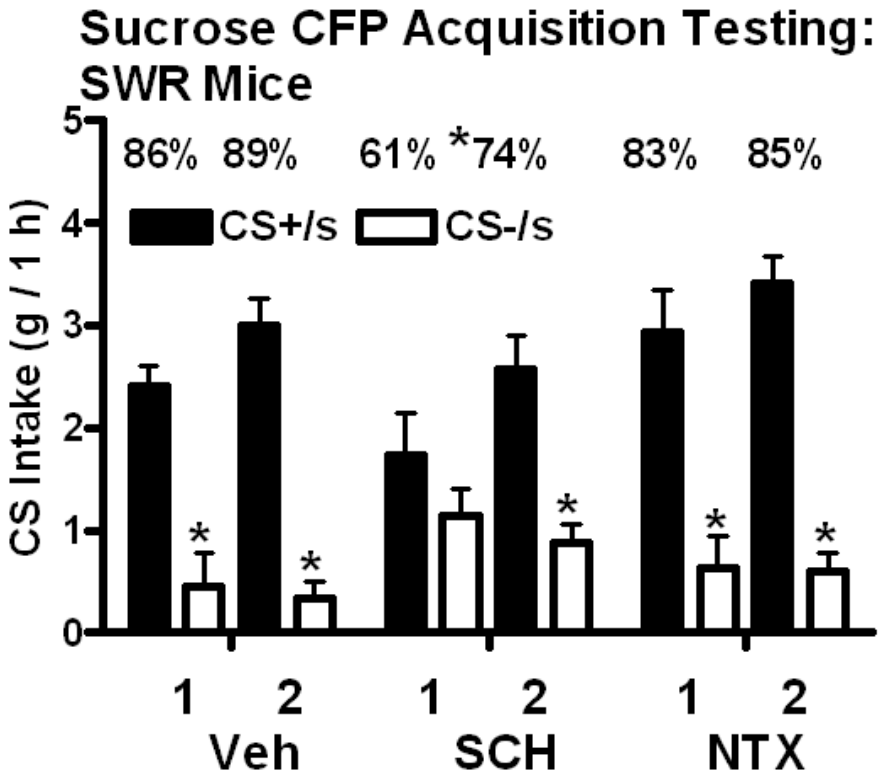
Figure 9. (Acquisition Procedure). Upper Panel: Training intakes (mean +SEM, g/60 min) of CS+/Suc and CS-/Sac solutions in BALB/cJ inbred mice pretreated 30 min earlier with vehicle (Veh), SCH23390 at a dose of 50 nmol/kg (SCH) or NTX at a dose of 0.1 mg/kg (NTX). Significant differences in this and the next figure are denoted between CS+/Suc and CS-/Sac intakes within each group (*) as are significant differences in CS+/Suc or CS+/Sac intakes following SCH or NTX relative to corresponding Veh (+). Lower Panel: Testing intakes (mean +SEM, g/60 min) of CS+/s and CS-/s solutions during two-bottle Tests 1 and 2 in inbred BALB/cJ mice receiving Veh, SCH or NTX during training. Numbers atop bars represent the mean percent intakes of CS+/s (%CS+). Significant differences in this and the next figure are denoted between CS+/s and CS-/s intake and for %CS+ intake within each test (*).

significantly exceeded CS-/s intake in the second test pair (Figure 9, lower panel). Significant differences in the percent CS+/s intakes were observed among groups ($F(2,18)= 4.05$, $p<0.035$), but not between test pairs ($F(1,18)= 0.23$, ns) or for the interaction between groups and test pairs ($F(2,18)= 1.55$, ns). Overall, NTX-trained (60%), but not SCH23390-trained (79%), BALB mice displayed significantly lower %CS preferences than vehicle-trained BALB mice (81%). The significantly lower %CS preference occurred only in the first test pair in NTX-trained (54%) relative to vehicle-trained (85%) BALB mice (Figure 9, lower panel).

DA D1 and opioid antagonist effects on the acquisition of sucrose-CFP in SWR mice: In the one-bottle training intakes, overall CS+/Suc intake (1,35 ml) was significantly ($F(1,18)= 31.15$, $p<0.0001$) lower than CS-/Sac intake (2.07 ml) with significant effects also observed among the three groups ($F(2,18)= 21.19$, $p<0.0001$) and for the group x condition interaction ($F(2,18)= 11.72$, $p<0.0001$). Total intake was significantly lower in SWR mice receiving SCH23390 (0.76 ml), but not NTX (2.12 ml) trained relative to vehicle (2.07 ml) during training. CS-/Sac intake was significantly greater than CS+/Suc intake in SWR mice receiving vehicle and NTX, but not SCH23390 during training (Figure 10, upper panel). Vehicle-trained SWR mice had significantly greater CS+/Suc and CS-/Sac intakes than their SCH23390-, but not NTX-trained counterparts (Figure 10, upper panel).

In the two-bottle preference tests, overall, SWR mice consumed significantly more CS+/s than CS-/s ($F(1,18)= 126.72$, $p<0.0001$) with significant differences also observed across test pairs ($F(1,18)= 8.21$, $p<0.01$) and for the group x condition interaction ($F(2,18)= 5.62$, $p<0.013$), but not among groups ($F(2,18)= 2.34$, ns). CS+/s intakes significantly exceeded CS-/s intakes for both test pairs in the vehicle-trained and NTX-trained groups (Figure 10, lower panel). In the SCH23390-trained group, CS+/s and CS-/s intake failed to differ from each other for the first test


Sucrose CFP Acquisition Training:
SWR Mice
Intake (g / 1 h)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CS+/Suc
CS-/Sac
*
*
+
+
*
Veh
SCH
NTX
Training Group


Sucrose CFP Acquisition Testing:
SWR Mice
CS Intake (g / 1 h)
5
4
3
2
1
0
86%
89%
61%
*74%
83%
85%
CS+/s
CS-/s
*
*
*
*
*
*
1
2
1
2
1
2
Veh
SCH
NTX

DYM

Figure 10. (Acquisition Procedure). Upper Panel: Training intakes (mean +SEM, g/60 min) of CS+/Suc and CS-/Sac solutions in SWR/J inbred mice pretreated 30 min earlier with vehicle (Veh), SCH23390 at a dose of 50 nmol/kg (SCH) or NTX at a dose of 0.1 mg/kg (NTX). Lower Panel: Testing intakes (mean +SEM, g/60 min) of CS+/s and CS-/s solutions during two-bottle Tests 1 and 2 in inbred SWR/J mice receiving Veh, SCH or NTX during training. Numbers atop bars represent the mean percent intakes of CS+/s (%CS+). The Y-axis has been modified to accommodate the increased intake of this strain in this paradigm.

pair, but CS+/s intake significantly exceeded CS-/s intake in the second test pair (Figure 10, lower panel). Significant differences in the percent CS+/s intakes were observed among groups ($F(2,18)= 4.90$, $p<0.02$), but not between test pairs ($F(1,18)= 2.09$, ns) or for the interaction between groups and test pairs ($F(2,18)= 0.66$, ns). Overall, SCH23390-trained (68%), but not NTX-trained (83%) SWR mice displayed significantly lower %CS preferences than vehicle-trained SWR mice (88%). The significantly lower %CS preference occurred only in the first test pair in SCH23390-trained (61%) relative to vehicle-trained (86%) SWR mice (Figure 10, lower panel).

Discussion

In this study, we explored the role of DA D1-like receptor and opioid receptor signaling in BALB and SWR strains of mice in flavor preference learning induced by the ingestion of sucrose. These strains were selected based on their similar robust sucrose-CFP in a survey of nine strains (Pinhas et al., 2012) and their differential NTX induced suppression of sucrose intake (Dym et al., 2007). The results revealed that antagonism of D1-like receptors impaired the acquisition of sucrose-CFP in SWR, but not in BALB mice. In contrast, antagonism of opioid receptors disrupted the acquisition of sucrose-CFP in BALB, but not in SWR mice. The expression of sucrose-CFP was reduced by SCH and NTX in both strains. These findings demonstrate that DA and opioid reward systems can independently contribute to the establishment of sucrose-CFP in different inbred mouse strains. Consistent with prior results (Pinhas et al., 2012), the BALB and SWR mice learned preferences for the sucrose-paired CS+ flavor that were of similar magnitude (82% and 79%, respectively). The strains were also comparable in their training intakes of the CS+ solution, but differed in that the BALB mice consumed significantly less CS− than CS+ solutions, whereas the SWR mice consumed as much or more CS−than CS+during training. We previously observed similar training intake differences in BALB and SWR strains (Pinhas et al., 2012). We speculated that the higher intake of the saccharin sweetened CS− by SWR mice was due to them having the sensitive form of the sweet taste receptor which increases saccharin preference and intake relative to that observed in mice with the sweet sub-sensitive receptor including BALB mice (Reed et al., 2004). SWR mice also drink more sucrose than BALB mice in 24-h tests (Lewis et al., 2005), but interestingly not in the 1-h training sessions in the present and previous (Pinhas et al., 2012) studies.

DA D1 signaling and modulation of sucrose-CFP learning: When treated with SCH prior to the two-bottle choice tests (expression experiment), both strains reduced their CS intakes and CS+ preferences. The strains differed only in that the SWR mice failed to prefer the CS+ at both 200 and 800 nmol/kg doses, whereas the BALB mice lost their preference only at the 800 nmol/kg dose. In the acquisition experiment, SCH treatment during training prevented the SWR mice from displaying a significant CS+ preference in the first test (61% in SCH mice vs. 86% in Veh mice), but a significant, albeit attenuated CS+ preference emerged in the second test (74% vs. 89% in Veh mice). The initial absence and subsequent recovery of the sucrose-CFP observed in the SWR SCH group were unexpected. One would expect if anything, that the CS+ preference would decline with repeated testing due to "extinction" given that the CS+ flavor was no longer paired with sucrose. Note, however, that the SWR Veh mice tended to increase their CS+ intake and preference in Test 2 and we previously observed persistent CS+ preferences over three tests in SWR mice (Pinhas et al., 2012). One possible explanation for the low Test 1 CS+ preference displayed by the SWR SCH mice was a drug state dependency effect that dissipated in Test 2. According to this view, which requires further testing, SWR SCH mice may display a stronger CS+ preference if they were treated with SCH prior to Test 1. It should also be noted that the suppressed training intakes of the SWR SCH may have contributed to their reduced CS+ preference displayed in Tests 1 and 2. In contrast to SWR mice, SCH failed to reduce the acquisition of the CS+ preference in the BALB mice relative to BALB vehicle controls in Test 1 (77% vs. 85%) and Test 2 (80% vs. 77%). It is possible that higher SCH doses administered during training would have blocked CS+ preference learning in the BALB mice. However, higher training SCH doses may reduce training intakes to low levels that precluded learning.

This is suggested by the low CS intakes produced by the 200 and 800 nmol/kg SCH doses in the expression study.

Taken together, the findings indicate that SCH impairs the expression and acquisition of a sucrose-CFP more in SWR mice than in BALB mice. The pattern of these results differs from SCH-induced reductions in sucrose intake in these strains. Our laboratory (Dym et al., 2009) previously found that SCH produced similar dose-dependent reductions in 1-h sucrose intake in the two strains following the 200 (BALB: 17% inhibition; SWR: 6% inhibition; t=0.50, ns) and 800 (BALB: 74%; SWR: 61% inhibition; t=0.91, ns) nmol/kg doses employed in the present study. Thus, the strain differences in the SCH effect on sucrose-CFP are not readily explained by the drug effects on sucrose intake per se, but may be due specifically to drug effects on preference learning. However, in a parallel study (Dym et al., 2010) examining DA D1 antagonist effects on fat (Intralipid) intake, SCH reduced intralipid intake to a far greater degree in SWR mice than in BALB mice following the 200 (BALB: 26% increase; SWR: 44% inhibition; t=2.80, pb0.022) and 800 (BALB: 10% increase; SWR: 68% inhibition; t=3.16, pb0.009) nmol/kg doses. Therefore, it will be of interest to compare SCH drug effects in SWR and BALB mice using other preference learning paradigms such as fat-CFP.

In prior rat experiments, we investigated the effect of SCH on flavor conditioning using different training paradigms to separate the conditioning effects of sweet taste vs. post-ingestive nutrient actions. Overall systemic SCH administration at high doses prior to preference testing reduced the expression of the CS+ preference in rats trained with real-fed fructose, sham-fed sucrose, or IG sucrose infusion (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). This is consistent with the reduced expression of the sucrose-conditioned CS+ preference produced by SCH treatment in the BALB and SWR mice. In addition, systemic SCH treatment

during training attenuated or blocked the acquisition of sugar-CFP in rats trained with these three different paradigms (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000b). These effects are consistent with the failure of the SWR SCH mice to display a significant CS+ preference in Test 1 but conflict with the recovery of a CS+preference in Test 2 and the persistent CS+ preference displayed by BALB SCH mice. As mentioned above, a higher SCH training dose may more profoundly impair sucrose conditioning in mice. Alternatively, the different training procedures used in the rat and mouse experiments may contribute to the discrepant results. In particular, whereas the mice in the present study were exposed to both the sweet taste and positive post-ingestive actions of sugars, the rats in the prior studies experienced only sweet taste or post-ingestive sugar rewards.

As reviewed elsewhere (Sclafani et al., 2011), there is extensive evidence that central DA circuits are involved in food reward processing. Of particular relevance to sugar conditioning studies are reports that the sweet taste as well as the post-ingestive actions of sugar promote DA release in the nucleus accumbens (NAc) (Hajnal et al., 2004; Ren et al., 2010). Furthermore, local SCH microinjections into the NAc during training blocked or attenuated sugar-conditioned preferences in rats (Bernal et al., 2008; Touzani et al., 2008) and similar effects were observed in the amygdala and medial prefrontal cortex regions which receive projections of the DA reward system (Bernal et al., 2009; Malkusz et al., 2012; Touzani et al., 2009, 2010). Thus, differences in the response to D1-like receptor antagonism in BALB and SWR mice may be related to differences in the sensitivity to SCH or a defect (affinity, number) in the D1-like receptors in the BALB mice. To the best of our knowledge, there are no published studies comparing the D1 receptor ligand binding in these two strains, which warrant further investigation.

Opioid signaling and modulation of sucrose-CFP learning: In the expression experiments, when BALB mice were treated with NTX prior to preference testing, the high dose significantly reduced CS+ intake to a level which no longer significantly exceeded CS− intake, and non-significantly reduced the preference from 74% to 63%. SWR mice were more influenced by NTX treatment; their total CS intake and percent CS+ preference in the expression study were significantly reduced following both NTX doses compared to vehicle treatment. On the other hand, in the acquisition experiment, BALB mice were more impaired than SWR mice by NTX treatment during training. The BALB NTX mice failed to prefer the CS+ (54%) in Test 1 although they displayed a significant although reduced preference in Test 2 (66% vs. 77% in Veh mice). In contrast, NTX failed to impair the acquisition of the sucrose-CFP in the SWR mice as revealed by their robust CS+ preferences in both tests compared to the Veh mice (83% vs. 86% and 85% vs. 89% in Tests 1 and 2, respectively). It is possible that a higher NTX training dose may impair the acquisition of a sucrose-CFP in SWR mice. However, higher training NTX doses may reduce training intakes to low levels that precluded learning. As with the results obtained with the SWR SCH mice, the recovery of a significant CS+ preference by the BALB NTX mice in Test 2 was unexpected. In this case, the BALB Veh showed a slight reduction not an increase in CS+ preference from Test 1 to Test 2. Further work is needed to determine if the low Test 1 CS+ preference displayed by the BALB NTX mice was a drug state dependency effect that dissipated in Test 2.

We have previously observed that NTX significantly reduced 1-h sucrose intake to a greater degree in BALB mice than in SWR mice (Dym et al., 2007), an effect consistent with the NTX effects on CS training intakes observed in the present study. SWR mice appear to show attenuated opioid-mediated responses to rewards. These mice displayed significantly less

morphine self-administration behavior as compared to 14 other inbred strains (Belknap et al., 1993) and were impaired in morphine-induced conditioned place preferences (Gieryk et al., 2010; Solecki et al., 2009). These findings along with our results suggest that SWR mice have a hyposensitive opioid reward system, perhaps due to reductions in the number and/or affinity of opiate receptors in the brain areas involved in processing reward and reward-related learning.

In contrast to the present results, our earlier studies with Sprague–Dawley rats (outbred strain) revealed little or no effect of NTX treatment on the expression or acquisition of sugar-CFP. This was true for rats trained with real-fed fructose, sham-fed sucrose, or IG sucrose infusions (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). Given the extensive literature implicating central opioid receptors in sweet taste reward (see reviews: Bodnar, 2004; Cooper, 2007; Levine et al., 1995, 2003), it was surprising that NTX treatment had virtually no effect on sugar-CFP in Sprague–Dawley rats. The possibility that other strains of rats might be sensitive to the antagonistic effect of opiate receptors on CFP remains an open question. A recent study in rats reported that NTX treatment did not differentially alter sucrose motivation in rats selectively bred to drink high or low amounts of saccharin but drug effects on sucrose-CFP were not examined (Gosnell et al., 2010). As noted above, the different training procedures used in the rat and mouse experiments may contribute to the discrepant results obtained with NTX.

In summary, the present findings demonstrate a double-dissociation in the effects of DA and opioid receptor antagonism on the acquisition of sucrose-CFP in two-inbred mouse strains; SWR and BALB. The two strains were selected for study because they displayed comparable strong sucrose-CFP despite differing in their sweet taste sensitivity. As in rats, SCH treatment attenuated the expression of the sucrose-CFP in both strains but suppressed preference learning in only the SWR mice. Unlike rats, however, the acquisition of sucrose-CFP was impaired by

NTX in BALB mice, which recommends this strain for further study of opioid mediation of food-related learning. These findings indicate clearly that both the DA and opioidergic reward systems can equally and independently contribute to the establishment of sucrose-CFP in rodents and that caution must be exerted in interpreting reward-related learning findings in rat studies using the Sprague–Dawley strain.

CHAPTER SEVEN: General Discussion

The studies comprising this dissertation utilized inbred mouse strains to examine genetic variance in dopaminergic and opioid modulation of overall sweet (sucrose) (Chapters 3 and 4; Dym et al., 2007, 2009) and fat (Intralipid) intake (Chapter 5; Dym et al., 2010), as well as acquisition and expression of conditioned flavor preference of sucrose (Chapter 6). The following section will discuss how the results from the above studies relate to the expected hypotheses of each Specific Aim. This will be followed by a general discussion of the following topics: A) SWR and BALB mouse strains; B) The TAS1R3 gene and differential outcome between "sweet-sensitive" and "sweet- sub-sensitive" inbred mouse strains; C) Future directions of research; and D) Implications for genetic variance of pharmacology of palatable intake and CFP

IA. NTX and DA D1 and D2 receptor antagonists differentially reduce sucrose intake in inbred mouse strains (Specific Aims 1 and 2). Although opioid antagonists produce marked reductions in intake of sweet solutions (e.g., see review: Bodnar, 2004), and although DA D1 and D2 receptor antagonists suppress intake of sweet solutions (Geary and Smith, 1985; Muscat and Wilner, 1989; Schneider et al., 1986a, 1986b, 1988, 1989; Smith and Schneider, 1988; Wise and Rompre, 1989; Xenakis and Sclafani, 1981), few studies have collectively examined both genetic and pharmacological influences on sweet intake. Thus, the goal of **First Specific Aim** was to examine whether the general opioids antagonist, NTX, would dose-dependently decrease intake of sweet tasting sucrose in eight inbred- and one outbred- mouse strains. The corresponding goal of the **Second Specific Aim** was to examine sucrose intake following administration of the D1 antagonist, SCH23390, and the D2 antagonist, raclopride, to the same mouse strains.

The hypothesis (**Specific Aims 1B, 2A**) that genetic variance in overall sucrose intake would be observed across strains was *confirmed.* Whereas the B6 and SJL strains consumed

respectively low amounts of sucrose, the outbred CD-1 strain as well as inbred, C3H, and B10 strains consumed moderate amounts of sucrose, whereas the SWR, BALB and 129 mouse strains consumed relatively high amounts of sucrose over 2 h. Sucrose intake for the DBA mice varied with high consumption in one study and low consumption in the other. QTL analysis of inbred mouse has revealed that both the saccharin (Sac) preference locus and the sweet taste receptor gene, Tas1R3, play a role in detection of sweet taste (Blizard et al., 1999; Fuller, 1974; Inoue et al., 2004b). Differences in expression of the Tas1R3 gene has been proposed as an explanation for genetic variance in sucrose intake in inbred mouse strains (Reed et al., 2004). Its influence on strain differences of sweet solution intake will be discussed further detail in Section II.

The hypothesis (**Specific Aim 1A**) that NTX would dose-dependently reduce sucrose intake in all strains was *not confirmed*. Clear differences in NTX's ability to dose-dependently reduce sucrose intake was apparent among strains. Indeed some strains failed to display reduced intake following NTX administration, *confirming* the hypothesis that genetic variance would be observed in the dose-dependent inhibition of sucrose intake following NTX administration (**Specific Aim 1C)**. NTX significantly and dose-dependently inhibited sucrose intake with the greatest sensitivity in B10 and B6 strains, intermediate sensitivity in BALB, C3H, CD-1 and DBA mice, and the least sensitivity in the 129 and SJL strains. In contrast to all these other strains, SWR mice failed to display any significant reduction in sucrose intake at any time point following any of the NTX doses. These data support the idea that genetic influences strongly contribute to strain differences in the opioid modulation of intake of sucrose. The unique extreme response of the SWR strain in this study as well as other studies examining opioid modulation of behaviors (Kest et al., 2002a; Kest et al., 2002b) makes it an ideal candidate for future study and warrants further discussion in Section II.

The hypothesis that SCH23390 would dose-dependently reduce sucrose intake in all trains was *not confirmed* (**Specific Aim 2B**), as this dose-dependent response was subject to genetic variance following SCH23390 administration (**Specific Aim 2E)**. SCH23390 significantly reduced sucrose intake across all five doses in 129 and SJL mice, across four doses in B6 and BALB mice, across three doses in SWR, C3H and B10 mice, across three different doses in DBA mice, but only at the two highest doses in CD-1 mice. A similar pattern of SCH23390-induced inhibitory actions were observed in assessing antagonist ID50 potency across strains. Interestingly, when examining the responses of specific strains, it appeared that strains that possess the sweet-sensitive form of the Tas1R3 receptor (B6, B10 , SJL and SWR) were less responsive to SCH23390 than strains possessing the sweet-sub-sensitive form of the receptor (BALB, C3H, DBA and 129) (Reed et al., 2004). Implications for this discrepancy will be discussed in Section II.

The hypothesis that raclopride would dose dependently reduce sucrose intake in all strains was *not confirmed* (**Specific Aim 2C**). Furthermore, the hypothesis that genetic variance would be observed in the dose dependent inhibition of sucrose intake following raclopride was only *partially confirmed* **(Specific Aim 2D)**. Raclopride's ability to reduce sucrose intake was limited only to the 1600 nmol/kg dose for the inbred BALB, C3H, B6, B10, DBA, SJL and 129 strains, and not at all for the inbred SWR and outbred CD-1 strains. Moreover, heritability estimates for raclopride-induced inhibition of sucrose intake at the highest dose were low. This result was somewhat surprising given that DA D2 receptor antagonists have been shown to affect rat intake of sweet solutions in real feeding and learning paradigms (Schneider et al., 1986a, Xenakis and Sclafani, 1981). DA D2, but not D1 receptor antagonism suppressed operant responding for corn oil in mice (Yoneda et al., 2007), whereas D1, but not D2 receptor

antagonism attenuated place preference conditioning by corn oil intake (Imaizumi et al., 2000). Both systemic and central administration of D2 receptor antagonists in flavor-flavor conditioned preference studies reduce its expression (Baker et al., 2003; Bernal et al., 2008, 2009; Hsiao and Smith, 1995; Malkusz et al., 2008; Pritchett and Hajnal, 2011; Sclafani et al., 2011; Yu et al., 2000a, 2000b), while DA D1, but not D2 receptor antagonists reduce CS+ expression in a flavor-nutrient paradigm (Azzara et al., 2001). In examining lick microstructure in rats, DA D1 receptors have been implicated in providing incentive salience (Berridge, 2007) or reward-associated cues, while DA D2 receptors were described as providing the "reboost" – or continuous update of the reward cue – to prevent extinction of a given behavior (D'Aquila, 2010).

This raises the possibility that the DA D2 receptor does play a role in acceptance and preference of palatable intake; however, its effect may be apparent only under certain conditions. In general, drug effects may be influenced by the specific nutrient utilized in a given study (e.g., sugar vs. starch, corn oil vs. lard; Glass et al., 1999). Thus, while the DA system has been implicated in fat consumption (Baker, Osman, & Bodnar, 2001; Davis et al., 2006; Rao et al, 2008; Weatherford et al. 1988; Weatherford et al., 1990), the D2 DA receptor may factor in more strongly when acting together with the nutrient fat as opposed to sugar. In acceptance of dietary fat, SCH23390 failed to alter high-fat intake, while raclopride reduced intake following several doses over 2 hours (Baker et al., 2001) as well as in rats exposed to a daily fat emulsion (Rao et al., 2008). A high fat diet also interacts with overall sucrose intake, with rats on a high fat/low carbohydrate diet dose-dependently reducing sucrose intake following raclopride administration as compared to rats in equal fat/carbohydrate and standard chow groups (Pritchett and Hajnal,

2011). Taken together, future studies are needed to reconcile these conflicting results and fully understand how DA D2 receptors influence the intake of palatable substances.

IB. NTX and D1 DA antagonists differentially affect Intralipid intake in several inbred mouse strains (Specific Aim 3). In examining intake of palatable substances, genetic variation has also been found for consumption of fat (Alexander et al., 2006; Bachmanov et al., 2001; 2002b; Leibowitz et al., 2005; Lewis et al., 2007; Reed et al., 2003; Smith et al., 1999; 2000; West et al., 1992). Furthermore, both the opioid and dopaminergic systems have been implicated in modulating fat intake (Gosnell et al., 1990a; Schneider et. al, 1988; Schneider et al., 1989; Smith, 1995, Weatherford et al., 1988; Weatherford et al., 1990; Wise and Rompre, 1989; Zhang et al., 1998). Therefore, this dissertation continued to investigate genetic variance of palatable nutrient consumption by measuring intake of a liquid fat solution (Intralipid) following administration of SCH23390 and NTX to seven inbred and one outbred mouse strain (**Specific Aim 3)**.

The hypothesis that genetic variance in Intralipid intake would be apparent across strains was *confirmed* (**Specific Aim 3A**). Rank-order of the cumulative 2-h baseline vehicle Intralipid intake among the eight strains was: BALB, CD-1, SWR, DBA, B10, SJL, 129 and B6. Given the strong positive correlation among strains between the 24-h intakes of sucrose and Intralipid (Lewis et al., 2005; Lewis et al., 2007), it was expected that similar pharmacologically-mediated results would be found for attenuating sucrose and Intralipid intake. However, there were no correlations of SCH23390-induced inhibition of Intralipid and sucrose intake or NTX-induced inhibition of Intralipid and sucrose intake across the tested strains, suggesting differential pharmacological mechanisms for intake of fat and sweet tasting stimuli.

The hypothesis that NTX would dose dependently reduce Intralipid intake across strains was *not confirmed* (**Specific Aim 3B**). Depending on the strain, differences in NTX's ability to attenuate Intralipid intake was found, *confirming* the hypothesis that genetic variance would be observed in the dose dependent response to NTX and Intralipid intake (**Specific Aim 3D).** In examining each strain, NTX produced reduced Intralipid intake at four (DBA), three (SWR, SJL), two (CD-1, B10) and one (B6, 129) of the doses tested, and also failed to affect Intralipid intake in BALB mice. As hypothesized, genetic variance was also observed in the dose dependent response to SCH23390 and Intralipid intake (**Specific Aim 3C**). Following SCH23390 administration, DBA, SWR, and CD-1 strains reduced intake at all five doses, SJL and B6 at four doses, 129 at three doses, B10 at one dose, and BALB at none of the doses tested. A high significant correlation was found in the strain-dependent abilities of SCH23390 and NTX to induce suppressive effects on Intralipid intake, but not sucrose intake, and no correlations were found for NTX or SCH23390 to attenuate fat and sucrose intake, suggesting that multiple pharmacological mechanisms are responsible for the variable genetic responses in fat and sugar intake.

IC. <u>NTX and SCH23390 affect the acquisition and expression of sucrose-CFP in the SWR and BALB inbred mouse strains (Specific Aim 4).</u> Whereas Specific Aims 1-3 examined genetic variation in overall intake of palatable substances, they do not account for experiential factors which can greatly influence sugar intake and preference in inbred mouse strains (Sclafani, 2006a). The complex relationship of DA and opioid involvement in food reward processes and genetic variance was extended in the present dissertation to its relationship with CFP. Although DA and opioid mediation of CFP has been examined extensively in rats (see reviews: Sclafani et al., 2011; Touzani et al., 2010), no systematic analysis of DA and opioid control of sucrose-CFP

in inbred mice has been performed. Thus, **Specific Aim 4** examined whether systemic DA D1 receptor antagonism with SCH23390 or opioid antagonism with NTX alter acquisition and expression of a sucrose-CFP in BALB and SWR inbred mouse strains.

The hypothesis that similar to rats, SCH23390 would reduce the **acquisition** of a sucrose-CFP in both strains of mice, was *partially confirmed* (**Specific Aim 4A)**. Strain differences were apparent in SCH23390-mediated sucrose-CFP acquisition (**Specific Aim 4C).** While systemic administration of a 50 nmol/kg dose of SCH23390 during training produced significant reductions in one-bottle intakes of the CS+/Suc and CS-/Sac solutions in both BALB and SWR strains, in two-bottle choice tests, BALB mice displayed significantly greater CS+/s intake over CS-/s intake, and comparable percent CS+ intakes relative to their preference under vehicle. Conversely, SWR mice failed to display significant differences between CS+/s and CS-/s intakes in the first (but not second) pair of two-bottle preference tests, and displayed significantly lower percent CS+ intakes (61%) relative to vehicle (86%). Thus, the involvement of DA D1 receptor signaling on the initial acquisition of a sucrose-CFP appears to be strain-specific and thereby subject to genetic variance. These data contrast rat conditioned flavor-flavor and flavor-nutrient conditioned preferences in which DA D1 and occasionally DA D2 receptor antagonists prevent or hasten the extinction of a CFP by blocking training during the acquisition phase (Azzara et al., 2001; Baker et al., 2003; Bernal et al., 2008; Bernal et al., 2009; Touzani et al., 2008, 2009a. 2009b, 2010), demonstrating that DA control over acquisition of a sucrose-CFP is species and strain-specific.

The hypothesis that SCH23390 would dose-dependently reduce the **expression** of a sucrose-CFP in both strains of mice, as seen in rats, was *confirmed* (**Specific Aim 4B)**, with little genetic variance seen between SWR and BALB mouse strains (**Specific Aim 4D)**. SWR mice

failed to display significant increases of CS+/s intake over CS-/s intake following the 400 and 800 nmol/kg SCH23390 doses and BALB mice failed to display significant increases of CS+/s intake over CS- intake following the 800 nmol/kg SCH23390 dose. In comparing percentage of sucrose-CFP, both BALB and SWR strains displayed dose-dependent reductions. Thus, the two inbred strains showed comparable DA D1 antagonist-induced reductions in the expression of sucrose-CFP and is unsurprising given that DA antagonists block expression of CFP when centrally and systemically administered in flavor-flavor and flavor-nutrient conditioning paradigms in rats (Azzara et al., 2001; Baker et al., 2003; Bernal et al., 2008, 2009; Yu et al., 2000a, 2000b; Touzani et al., 2008, 2009a. 2009b, 2010).

The hypothesis that similar to rats, NTX would fail to reduce the **acquisition** of a sucrose-CFP in both strains of mice, was *partially confirmed* (**Specific Aim 4E)**. Strain differences were apparent in NTX-mediated sucrose-CFP acquisition (**Specific Aim 4F).** Interestingly, in contrast to rat studies which demonstrated no effect of NTX on acquisition of orosensory sucrose-CFP in sham-feeding rats, fructose-CFP (Baker et al., 2004; Yu et al., 1999) and nutrient-flavor sucrose-CFP in rats (Azzara et al., 2000), the present study displayed strain-specific NTX attenuation of sucrose-CFP during acquisition. NTX (1 mg/kg dose) administration during training produced significant reductions in the one-bottle intake of the CS+/Suc, but not CS-/Sac solution in BALB, but not SWR strains relative to vehicle treatment. In subsequent two-bottle choice tests, NTX-treated BALB mice failed to display significant differences between CS+/s and CS-/s intakes in the first pair (but not second pair) of 2-bottle preference tests, while SWR mice displayed significantly greater CS+/s intake over CS-/s intake during preference tests and comparable percent CS+ intakes (83-85%) relative to their vehicle-trained counterparts. The divergent responses found during acquisition, whereas SWR, but not

BALB mice displayed initial impairment in the acquisition of sucrose-CFP following DA D1 antagonism and BALB, but not SWR mice displayed an initial impairment in the acquisition of sucrose-CFP following opioid antagonism suggests that pharmacological modulation of sucrose-CFP is species specific as well as subject to genetic variance and requires further study.

The hypothesis that NTX would fail to dose-dependently reduce the **expression** of a sucrose-CFP in both strains of mice, as seen in rats, was *not confirmed* (**Specific Aim 4H)**, with little genetic variance seen between SWR and BALB mouse strains (**Specific Aim 4G)**. Despite the data that NTX failed to affect flavor-flavor (Baker et al., 2004; Yu et al., 1999) and flavor-nutrient (Azzara et al., 2000) conditioned processes in rats, NTX administration during the current study exerted significant reductions in both strains of inbred mice tested. BALB mice failed to display significant increases of CS+/s intake over CS- intake following the 5 mg/kg NTX dose, and reduced the percentage of sucrose-CFP from 74% to 63%. Although SWR mice displayed significant increases of CS+/s intake over CS-/s intake following vehicle and both NTX doses, the percentage of sucrose-CFP significantly declined from 78% in vehicle treatment to 63% following the 1 and 5 mg/kg NTX doses. These data continue to stress an important species-specific difference in the ability of opioid receptors to reduce sucrose-CFP.

IIA. <u>SWR and BALB mouse strains.</u> As described in section IC, the SWR and BALB strains were selected for use in this dissertation's sucrose-CFP pharmacology study due to their powerful and persistent flavor preferences (Pinhas et al., 2012). Pinhas et al. (2012) examined sucrose-CFP in eight inbred and one outbred mouse strains. All strains displayed sucrose-CFP, while SWR, BALB, C3H and DBA mice displayed the greatest magnitude of effects that persisted over the six days of testing, with the SWR and BALB strains displaying the greatest amount of intake during sucrose-CFP testing. This made these two strains the ideal candidates

for pharmacological testing. However, in addition to their ability maintain a strong sucrose-CFP preference, the SWR and BALB mice are unique strains as seen in their responses within various behavioral and pharmacological studies and warrants further discussion.

The BALB strain is among the top 2-3 most widely used inbred strains in a variety of disciplines (www.jax.org/jaxmice). They have good breeding performance and a long reproductive life span. This strain is particularly known for its development of tumors including reticular, lung, and renal tumors. Its characteristic behaviors include high intrastrain aggression, high spontaneous locomotor activity, low avoidance conditionability and low shock avoidance learning in males (www.jax.org/jaxmice). The BALB strain is relatively resistant to the development of artheriosclerosis (White et al., 2007) and exhibits a low alcohol preference (Saito et al., 2003). The studies performed in our laboratory, and specifically for this dissertation, demonstrate several different phenotypic expressions of BALB mice in terms of ingestive behavior. When free-feeding, BALB mice drink moderate amounts of sucrose after 2 h (Dym et al., 2007) and 24 h periods (Lewis et al., 2005). In examining pharmacology and nutrient interactions, BALB mice were moderately responsive to NTX, and SCH23390 attenuated intake of 10% sucrose (Dym et al., 2007, 2009). It is in the pharmacological substrates of fat intake in which the BALB mice demonstrate their most unique genetic or allelic variation. When examining the effect of opioid and dopaminergic manipulation on Intralipid intake, only the BALB strain failed to display any reduction in Intralipid intake even at very high doses of SCH23390 or NTX (Dym et al. 2010). One possible reason for this may be the avid fat appetite of BALB mice as indicated by their high short-term (2 h) vehicle baseline intake (Dym et al., 2010) as well as their strong 24 h preference for Intralipid over water at seven different concentrations (0.001–5%) (Lewis et al., 2007). In contrast, strains (DBA, SWR, B6) sensitive

to DA and opioid antagonism showed increased Intralipid intake over water intake only at the four highest concentrations (0.5–5%) (Lewis et al., 2007). South and Huang (2006) examined five strains for preferences between high-fat/low-carbohydrate and low-fat/high-carbohydrate diets and found that B6 mice consumed 72% of their calories as high fat, whereas BALB mice failed to display a preference. BALB mice showed a 34% higher expression level of agouti-related peptide, but not pro-opiomelanocortin than B6 mice, suggesting important strain differences in this feeding-related peptide**.** Thus, because of this strain's unique response to fat, the BALB strain appears to be an ideal candidate to utilize in future studies examining pharmacological influence on dietary fat intake.

This laboratory also examined the BALB strain within several CFP paradigms. The BALB mice were found to display strong and lasting sucrose-CFP (e.g. Pinhas et al., 2012, Chapter 4-vehicle conditions). In determining DA and opioid influence on expression of a sucrose-CFP, the BALB mice displayed significantly reduced CS+ intake following SCH23390 (800 nmol/kg), dose and demonstrated dose-dependent reductions in CFP. Following administration of NTX (5mg/kg), the BALB mice failed to display significant increases of CS+/s intake over CS- intake, and reduced their percentage of sucrose-CFP from 74% to 63%. During the acquisition phase of this sucrose-CFP paradigm, SCH23390 treated (50 nmol/kg) BALB mice displayed comparable CS+/s intake and preference during 2 bottle-test to the vehicle treated group. In contrast, when NTX (0.1 mg/kg dose) was administered during acquisition training, BALB mice produced significant reductions in the one-bottle intake of the CS+/Suc, but not CS-/Sac solution and failed to display significant differences between CS+/s and CS-/s intakes during two-bottle testing. BALB's reduced sucrose intake and CFP during acquisition training as a result of NTX administration was surprising given that this result is not commonly

replicated in rats (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). This leads to the possibility that the BALB strain has distinctive genotypic functioning to learned sucrose-CFP. Together with its unique response to fat intake, it would be interesting to examine the BALB within a pharmacologically-manipulated fat-CFP paradigm to gain more understanding of how these systems operate and will be further discussed in Section III.

The SWR inbred mouse strain is widely in research as a general purpose strain. The SWR mice are high responders to exogenous hormones, have good resistance to lysis following microinjection, and are genetically well-defined. They are characterized behaviorally as having low intra-strain aggression, though when being handled, they are also described as jumpy, and unlike most other inbred strains, aggressive toward people. This strain displays a high incidence of diabetes insipidus and high systolic blood pressure (www.jax.org/jaxmice). In terms of ingestive behaviors, the SWR mice appear to be the only inbred strain carrying the *Soa*^{a} (Taster) allele, which is characterized by the avoidance of sucrose octaacetate solutions at low concentrations (0.00001M) (Lush, 1982). Our laboratory has found several additional phenotypic expressions of SWR mice for ingestive behavior. SWR mice consumed the largest amount of sucrose and moderate amounts of fat (Intralipid) relative to water in 24 h two-bottle preference tests in comparison to 12 other mouse strains (Lewis et al., 2005, 2007). They consumed the largest amount of a sucrose solution and high amounts of a liquid fat emulsion over 2 h (Dym et al., 2007, 2010). Interestingly, despite their high fat consumption, SWR mice fail to gain weight and accumulation of ependymal fat has been inversely correlated with this behavior (Smith et al., 2000; Smith et al., 2001). These effects persisted in SWR mice irrespective of the make-up of the fat source and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999).

During pharmacological manipulation of ingestive processes, SWR mice were sensitive to SCH23390 administration leading to significant reductions in Intralipid intake (Dym et al., 2010). SWR mice displayed moderately reduced sucrose intake to SCH23390-induced inhibitory actions (Dym et al., 2009). NTX administration resulted in moderate attenuation of Intralipid intake in the SWR mice (Dym et al., 2009). NTX administration followed by measurement of sucrose intake, however, resulted in no attenuation at any dose and any point in the 120 min time course (Dym et al., 2007). This insensitivity to NTX-induced inhibition of sucrose intake also stands in stark contrast to the extreme sensitivity displayed by SWR mice using other opiate-dependent measures. Thus, analysis of genetic variability revealed that SWR mice display the largest numbers of jumping responses by naloxone-precipitated withdrawal in mice made acutely dependent on morphine, chronically dependent on morphine, or made morphine-dependent through continuous infusions (Kest et al., 2002b). SWR mice also display among the most marked potency shifts in morphine analgesia following acute treatment as well as in tolerance development (Kest et al., 2002a). In assessing potential relationships between opiate-induced responses in the present study and these other measures, significant correlations failed to be observed between the ID50 of NTX-induced inhibition of sucrose intake at 60 min and naloxone-induced acute morphine dependence ($r = 0.18$, ns) as well as naloxone-induced chronic infusion morphine dependence ($r = -0.11$, ns) (Kest et al., 2002b). In contrast, a significant inverse correlation ($r = -0.68$, $P < 0.05$) was observed between the ID50 of NTX-induced inhibition of sucrose intake and the potency shift in morphine tolerance (Kest et al., 2002a). Taken together, these data dissociate opiate-mediated dependence-tolerance responses and opiate-mediated ingestive responses, as well as provide support for the contention that different brain areas mediate opiate mechanisms related to tolerance and dependence (e.g.,

periaqueductal gray, nucleus raphe magnus (e.g., Mizutani et al., 2005; Morgan et al., 2005a; Morgan et al., 2005b) on the one hand, and opioid mediation of palatable intake (e.g., nucleus accumbens, hypothalamic paraventricular nucleus (e.g., see review: Bodnar, 2004) on the other hand.

The SWR strain was also examined within a CFP paradigm. Like the BALB mice, SWR mice were found to display strong sucrose-CFP (e.g. Pinhas et al., 2012, Chapter 4-vehicle conditions). In examining CFP expression, SWR failed to display significant increases of CS+/s intake over CS-/s intake following the 400 and 800 nmol/kg SCH23390 doses and percentage of sucrose-CFP significantly declined following the 1 and 5 mg/kg NTX doses. Systemic administration of a 50 nmol/kg dose of SCH23390 during acquisition training produced significant reductions in one-bottle intakes of the CS+/Suc and CS-/Sac solutions in the SWR strains, and reduced CFP in two-bottle choice tests. Conversely, NTX treated mice displayed significantly greater CS+/s intake over CS-/s intake during one-bottle training and two-bottle preference tests. That SWR mice failed to show opioid-sensitive effects upon the acquisition of sucrose-CFP is not surprising given the failure of rats to show similar opioid-sensitive acquisition effects upon orosensory-mediated (Yu et al., 1999) or post-ingestive-mediated (Azzara et al., 2000) sucrose-CFP, and given the inability of NTX to block sucrose intake in SWR mice as described above (Dym et al., 2007).

In addition to the BALB and SWR mouse strains displaying unique behavioral responses to dietary- and pharmacologically-mediated fat and sugar (respectively) and their contrasting responses to SCH23390 and NTX during acquisition of a sucrose-CFP, they also differ based on their expression of the Tas1R3 gene which will be described in more detail in the next section.

IIB. <u>The Tas1R3 gene and differential outcome between "sweet-sensitive" and "sweet- sub-sensitive" inbred mouse strains.</u> One of the purposes in identifying extreme responding strains, such as in the previous section, is for those strains to ultimately serve as progenitors for QTL analysis and subsequently, for the identification of trait-relevant genes. For example, four studies (Blizard et al., 1999; Fuller, 1974; Inoue et al., 2004b; Lush, 1989) utilized QTL techniques to evaluate saccharin intake by crossing sensitive responding B6 mice with subs-sensitive DBA or 129 mice. These revealed that both the saccharin (Sac) preference locus and the sweet taste receptor gene, Tas1R3, were localized to distal chromosome 4. The Sac locus and Tas1R3 gene encode for the T1R3 receptor protein, which with the T1R2 and T1R3 proteins combine to function as a sweet receptor for a diverse range of sweet tasting substances (Inoue et al., 2004b; Nelson and Hoon, 2001). Sequence variants of the Tas1R3 taste receptor gene, and their relevance to saccharin intake, were demonstrated by phenotyping these polymorphisms in 30 inbred strains and comparing their allelic frequencies for these variants with their saccharin preference. This study ultimately concluded that the mouse Sac locus is identical to the Tas1R3 gene (Reed et al., 2004). Additionally, by comparing phenotypic response with Tas1R3 polymorphisms, Reed et al. (2004) identified specific strains which were genetically predisposed as highly sensitive to sweet stimuli, known as *sweet-sensitive*, and strains that were less sensitive to sweet stimuli or *sweet-sub-sensitive*. The sweet-sensitive strains include the B6, B10, SJL and SWR and the sweet-sub-sensitive strains include the BALB, C3H, DBA and 129 strains.

In examining general intake of sweet solutions within our laboratory, significant correlations were observed between these polymorphisms and moderate (0.01%: r=0.83; 0.1%: r=0.91; 2.5%: r=0.86), but not higher (5-20%) sucrose concentrations (Lewis, 2005). This finding, as well as limited correlations at the higher concentrations with a previous survey of

saccharin intake (Lush, 1989) supports the assertion that the regulation of sucrose intake is under polygenic control, with distinct genetic mechanisms underlying the intake of solutions with high and low sweetener concentrations. It is plausible therefore that the Tas1R3 gene is responsible for strain variance in sucrose intakes at the lower concentrations, where taste is a primary cue. At higher sucrose concentrations, there is a greater involvement of post-oral factors, which are not likely related to polymorphisms of the T1R3 taste receptor, yielding less consistent correlations as described above. This was seen in naïve 129 mice, which have the sub-sensitive sweet receptor, and displayed weaker preferences for dilute sucrose solutions than do sweet sensitive B6 mice, but after experience with concentrated sugar solutions, the sugar preferences of the two strains were indistinguishable (Sclafani, 2006a). The current dissertation utilized a 10% sucrose solution in Chapters Three and Four. Over a 2 h time course, the B6 and SJL strains consumed respectively low amounts of sucrose, the CD-1, C3H, and B10 strains moderate amounts, and the SWR, BALB and 129 mouse strains consumed relatively high amounts of sucrose. Additionally, other recent data also show that the sweet taste sensitivity of inbred mouse strains does not predict their short-term licking response to concentrated sugar solutions (Glendinning et al., 2005). Thus, the utilization of a high sucrose concentration - and thus greater post-oral effect - may explain the lack of differential sucrose intake between sweet-sensitive and sweet-sub-sensitive strains.

The data in Chapter Three demonstrate the importance in assessing genetic variance as well as opioid modulation of sucrose intake. Consistent with the proposals of sensitive B6 and sub-sensitive 129 mice defined by their patterns of consumption of sucrose across paradigms (Bachmanov et al., 2001; Sclafani, 2006a; Sclafani, 2006b; and Sclafani and Glendinning, 2005), treatment with NTX showed that closely-related B10 and B6 strains displayed far greater

sensitivity and magnitude of NTX-induced inhibition of sucrose intake than 129 mice. These differences are not trivial given the fact that B6, 129 or some inbred combination of the two strains are commonly used as "background" strains to develop knockout mice. Therefore, considerable differences in interpretation can emerge in terms of which "wild type" comparison should be used relative to the knockout animal (Bothe et al, 2004). However, as mentioned in section IIA, the SWR mice, also belonging to the sub-sensitive subset displayed no attenuation of sucrose, necessitating further study of NTX influence on inbred mouse strains.

While B10 and B6 strains displayed greater sensitivity of NTX-induced inhibition of sucrose intake than 129 mice, a contradictory relationship was seen with SCH23390 treatment prior to sucrose intake. Strains that possess the sweet-sensitive form of the Tas1R3 receptor (B6, B10, SJL and SWR) were less responsive to SCH23390 than strains possessing the sweet-sub-sensitive form of the receptor (BALB, C3H, DBA and 129) (Dym et al., 2009). Significant differences were seen following the 1600 nmol/kg dose for the sensitive (25.3%) and sub-sensitive (12.3%) taster strains, and approaching significance following the 200 nmol/kg dose for the sensitive (90.8%) and sub-sensitive (71%) taster strains. Thus, the sweet-sensitive strains appear to display less suppression in sucrose intake following SCH23390 than sweet-sub-sensitive strains. Interestingly, when examining sucrose-CFP in the sweet-sensitive SWR and sub-sensitive BALB strains, the opposite effect occurred. During the acquisition phase of the experiment, SCH23390 treated SWR mice failed to display significant differences between CS+/s and CS-/s test, while NTX treated BALB mice failed to display significant differences between CS+/s and CS-/s during the 2-bottle preference tests. Consistent with this finding, sucrose-CFP did not vary as a function of sweet taster status in the inbred strains, while fructose-CFP varied as a function of taster status but not in the expected direction, with the sub-sensitive

mice displayed stronger fructose-CFP (Pinhas et al., 2012). Taken together, current findings demonstrate that sweet taste sensitivity does not necessarily predict overall intake of sweet solution and sugar-reinforced behavior in mice. Intake of palatable substances is complex and requires additional research to further elucidate the behaviors involved.

IIIA. Future Directions.

In the general discussion of Section II, we have identified several extreme responding strains for pharmacologically-mediated sucrose and fat intake as well as sucrose-CFP. Ultimately, in the future, this phenotypic information may be used to perform QTL analysis. QTL analyses are used to localize chromosomal regions, and ultimately genes, critically involved in such differences. While QTL analysis has been used to localize and ultimately identify genes responsible for sucrose (Blizard et al., 1999; Fuller, 1974; Inoue et al., 2004b; Lush, 1989) and fat (Reed et al., 2003; Smith-Richards et al., 2002) consumption, this dissertation has demonstrated that these genes are not solely responsible for consumption behaviors of palatable substances. QTL analyses based on other divergent responses identified in our series of studies such as the dopaminergic and opioid mediated intake of fat and sucrose and sucrose-CFP, can provide further insight into feeding disorders like obesity and diabetes.

Possible future studies also stemming from this dissertation can examine CFP in more detail. Surprising differences between rat and mouse behavior were found as described in the Discussion section in Chapter Six. In general, while rats are wholly insensitive to opioid manipulated sucrose-CFP (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999FQTL), mice displayed differential strain response. Additional studies can examine other aspects of CFP in inbred mouse strains such as flavor-nutrient conditioning, conditioning with alternative nutrients such as Intralipid, and other pharmacological drugs. For instance, while raclopride failed to

display any significant sucrose intake attenuation (Dym et al, 2009), perhaps in a CFP paradigm, significant responses would be observed.

IIIB. Implications.

As indicated in the outset of this dissertation, obesity is recognized as growing public health problem in the United States (http://www.cdc.gov/healthyyouth /obesity/facts.htm; http://www.cdc.gov/ obesity/data/trends.html). The etiology of obesity is complex but there is general agreement that the overabundance of palatable, energy dense foods is an important contributing factor. The studies compiled in this dissertation help to elucidate the neurotransmitter systems and genetic modulation involved in food intake and learned preferences of palatable food. The understanding of the basic mechanisms involved in the general food intake and the development of food preferences in animals may be directly translatable and thereby provide insights into the clinical treatment of overeating and obesity.

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