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**SENSITIVITY TO ABSOLUTE AND RELATIVE PROPERTIES OF NATURAL
REWARDS AND DRUGS OF ABUSE AS A FUNCTION OF STRAIN IN RATS AND
MICE**

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ABSTRACT

Addiction is a major problem in the United States. In addition to the numerous economic and legal repercussions on society, drugs of abuse also produce devastating effects in the individual. One such consequence of continued drug use is a devaluation of the natural rewards encountered in the environment. For example, people with a history of cocaine use and even smokers who are expecting a chance to smoke in the immediate future have been found to be less sensitive to monetary reward (Goldstein et al., 2008; Wilson et al., 2008). Similarly, drug addicts tend to develop a generalized insensitivity to food and social relationships and it has been shown that addicts are more likely to weigh less, to lose their jobs, and to have their children removed from their home due to neglect (Jones et al., 1995; Santolaria-Fernandez et al., 1995; Nair et al., 1997).

A contributing factor in the devaluation of natural rewards by drugs of abuse is the principle of incentive relativity. Incentive relativity states that rewards are not evaluated in a vacuum (i.e., the value of a given reward is not determined only by its intrinsic value), but also by its relative value in comparison with other rewards. Such comparisons, however, normally occur between natural rewards found in the environment in a relatively plastic manner. When natural rewards are evaluated against artificially intense drugs of abuse, these lesser natural rewards can become devalued and are subsequently avoided.

Given that this effect of drugs of abuse on natural rewards is influenced by the comparison of rewards, individual or group differences in reward preference and/or sensitivity, both innate and relative, may play an important role in the devaluation of natural rewards observed in drug addiction. To date, however, this aspect of drug addiction has remained largely unexplored. The goal of the current thesis was to use rat and mouse strains with differential preference for rewards to evaluate the effect of differences in sensitivity to rewards in a rodent

model of drug-induced devaluation of natural rewards. Although increased reward preference was found to facilitate drug-induced suppression of a reward cue in rats in Chapter 2, and this facilitation extended to comparisons between disparate natural sweet rewards in Chapter 3, the suppressive effect of drug on reward intake was attenuated in reward-preferring mice in Chapter 4. This attenuated effect in ‘reward sensitive’ strains of mice was not due to differences in impulsivity and was shown, with the use of transgenic Δ FosB mice in Chapter 5, to be associated with an increased sensitivity to not only drug, but also to the natural reward cue. Indeed, when the natural reward cue was switched from sweet to bitter in Chapter 6, the strain effect was eliminated. Taken together, the data in this dissertation suggest that a single substrate, ultimately, can mediate sensitivity to drugs of abuse and natural rewards. Thus, while natural reward cues can predict drug availability and can, in so doing, be devalued, strong natural rewards also can blunt the rewarding impact of a drug of abuse and, thereby, responding for a drug of abuse and the ensuing development of addiction.

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LIST OF ABBREVIATIONS

AC	adenylyl cyclase
ACE	anticipatory contrast effect
ANOVA	analysis of variance of the means
CDP	chlordiazepoxide
cm	centimeter
CORT	corticosterone
CPP	conditioned place preference
CS	conditioned stimulus
CTA	conditioned taste aversion
DA	dopamine
DAT	dopamine transporter
dB	decibel
dH ₂ O	distilled water
FR	fixed ratio
GABA	γ -aminobutyric acid
GFAP	glial fibrillary acidic protein
HCl	hydrochloride
hr	hour
ip	intraperitoneal
kg	kilogram
LiCl	lithium chloride
mg	milligram

min	minute
ml	milliliter
M	molar
NaCl	sodium chloride
NAc	nucleus accumbens
PKA	protein kinase A
QHCl	quinine hydrochloride
sc	subcutaneous
sec	second
SNC	successive negative contrast
SPC	successive positive contrast
TH	tyrosine hydroxylase
US	unconditioned stimulus
VTA	ventral tegmental area

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Chapter 1

Introduction

Drug abuse and addiction are major problems in the United States. The National Institute on Drug Abuse estimates that “the total overall costs of substance abuse in the United States, including health- and crime-related costs as well as losses in productivity, exceed half a trillion dollars annually”. A total of \$185 billion is attributed to alcohol use, \$168 billion to tobacco use, and \$181 billion to the use of illicit drugs such as cocaine, ecstasy, morphine, heroin, and marijuana (<http://www.nida.nih.gov/Infofacts/understand.html>, 2009). In addition to the economic and legal repercussions on society, drugs of abuse produce devastating effects in the individual as well. Increased prevalence of cardiovascular, respiratory, and neurological complications is associated with drug use and can include disturbances in heart rhythm, heart attack, chest pain, respiratory failure, stroke, seizure, and headache (<http://www.nida.nih.gov/Infofacts/>, 2009). Drug use and addiction also shares a high comorbidity with a range of mental disorders including mood and anxiety disorders, depression, mania, panic disorders and social phobias (<http://www.drugabuse.gov/tib/comorbid.html>, 2009).

Drug addiction is a brain disease and, although initial use is voluntary, drugs of abuse alter gene expression and brain circuitry and these changes interfere with voluntary intake of the drug leading to compulsive drug craving, seeking and use (Koob et al., 1998; Robinson and Berridge, 2003; Nestler, 2004b; Kalivas and O'Brien, 2008). Ultimately, drug seeking behavior and drug-taking overrides all else and the drug addict becomes unable to refrain from these behaviors even when they result in negative consequences for themselves or for others. As such, drug addiction is characterized by a continued compulsive use of drugs in spite of adverse health or social consequences (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text

Revision, 2000). In addition, drug addicts also suffer from a devaluation of the natural rewards they encounter in the environment. For example, people with a history of cocaine use and smokers who are expecting a chance to smoke in the immediate future have been found to be less sensitive to monetary reward (Goldstein et al., 2008; Wilson et al., 2008). Similarly, drug addicts tend to develop a generalized insensitivity to food and important relationships as it has been shown that addicts are more likely to weigh less, to lose their jobs, and to have their children removed from their home due to neglect (Jones et al., 1995; Santolaria-Fernandez et al., 1995; Nair et al., 1997).

A contributing factor in this devaluation of natural rewards by drugs of abuse is the principle of incentive relativity. Incentive relativity states that rewards are not evaluated in a vacuum and that the value of any given reward is determined not only by its intrinsic, absolute properties, but by its relative comparison with other rewards experienced in the past and present and with those expected in the future. The evolution of this system of incentive relativity has allowed humans and animals to more discretely judge the value of a particular reward given a certain situation or set of circumstances. However, such comparisons and resultant alterations in behavior (termed contrast effects) normally occur between natural rewards found in the environment. When natural rewards are evaluated against artificially intense drugs of abuse, these rewards can become devalued and subsequently avoided.

As the effects of drugs of abuse on natural rewards are influenced by the comparison of rewards, differences in reward preference, both innate and relative, may play an important role in the devaluation of natural rewards observed in drug addiction. To date, however, this aspect of drug addiction has remained largely unexplored. The devaluation of natural rewards by drugs of abuse, as well as changes in behavior due to comparisons between natural rewards, has been successfully modeled in the laboratory, most commonly in rodents. Contrast paradigms, such as simultaneous, successive, and anticipatory contrast, model behaviors observed in the comparison

between natural rewards and are often defined by changes in intake of a gustatory reward when paired with another gustatory reward of significantly higher or lower value. Similarly, the reward comparison paradigm models behavior that results from the comparison of a natural reward and a drug of abuse. In the reward comparison paradigm, intake of a gustatory reward is significantly decreased when paired with a drug of abuse. These models provide an avenue for the evaluation of differential reward preference and its effect on comparisons between natural rewards and on the devaluation of natural rewards by drugs of abuse. Therefore, in addition to a review of contrast effects and reward comparison in the context of drug addiction, the aim of this dissertation is to evaluate these paradigms in strains of rats and mice that differ in their relative preference for rewards with the goal of better elucidating the devaluation of natural rewards observed in drug addiction.

Contrast Effects

In the laboratory, there are three animal models of contrast: simultaneous, successive, and anticipatory. Simultaneous contrast involves the comparison of two natural rewards which repeatedly alternate and are presented over a short amount of time. Successive contrast involves the retrograde comparison of two natural rewards where rats determine the value of a particular reward based on the memory of a previously received, greater reward. Finally, anticipatory contrast involves the anterograde comparison of two paired natural rewards where rats determine the value of a particular reward based on the anticipation of access to a greater reward.

Simultaneous Contrast

Contrast occurs as intake of one reward either increases or decreases based on the perceived hedonic value of the alternating reward. With simultaneous contrast effects the two disparate rewards are presented closely in time. For example, Flaherty and Largent (1975) allowed rats to have 6 minutes access a day to two different concentrations of sucrose. For the first minute, all rats received access to a bottle of 4% sucrose; for the second minute, half of the rats received access (in a different bottle) to more of the 4% sucrose while the other half received 32% sucrose. This procedure then repeated twice for a total of 6 minutes access time. First bottle intake of 4% sucrose was lower in rats that received 32% sucrose in the second bottle compared with those that received 4% sucrose. This is referred to as a negative simultaneous contrast effect. In addition, rats increased intake of the 32% sucrose solution if it was alternated with access to the 4% sucrose solution compared to rats that received only the 32% sucrose solution. This phenomenon is referred to as a positive simultaneous contrast effect. Thus, simultaneous contrast is an example of changes in perceived reward value that occur due to comparisons made in the present as opposed to anterograde (anticipatory contrast) or retrograde (successive contrast) processes. Studies have shown that the brainstem alone can support this more simple reward comparison process (Grigson et al., 1997), suggesting that it is a short-term memory dependent phenomenon.

Successive Contrast

Successive negative contrast (SNC) involves the retrograde comparison of a lesser reward with the memory of a greater reward and is often used as a model for the perceived loss of reward. Successive positive contrast (SPC) involves the comparison of a greater reward with the

memory of a lesser reward. Successive positive contrast is often used to model a perceived gain in reward. While both SNC and SPC have been demonstrated in the laboratory, SNC has been shown to be more robust and more readily demonstrated. Therefore, the negative form of successive contrast will be a focus of this overview.

In the standard SNC paradigm, an unshifted group of rats is given 5 minutes daily access to a 0.1 M sucrose solution and a shifted group is given 5 minutes daily access to a preferred 1.0 M sucrose solution. After 10 days of access to these preshift solutions, all rats receive 5 minutes daily access to the weaker sucrose solution across a 4 day post-shift period. A SNC effect occurs when rats shifted from the high to the low reward consume significantly less of the low reward than the unshifted controls on the first post-shift day (i.e., day 11). Recovery from contrast occurs as intake returns to the level of the unshifted controls over the 4 day post-shift period.

Behavioral, pharmacological, and lesion data have been used to develop a multistage model of negative contrast has been proposed (Flaherty, 1999) which incorporates exploratory and emotional interpretations of this phenomenon. This model states that a rat transitions through several processes in response to the loss of reward in the SNC paradigm. These stages can be divided into cognitive and emotional components. The cognitive component consists of the development of a pre-shift concentration representation; detection of the post-shift concentration; evaluation of the lesser concentration compared to the memory of the previously received tastant; a search for the missing reward; and, once having determined that the lesser post-shift solution is the only solution available, acceptance of the lesser reward. In support, Flaherty (1999) provided evidence that rats detect the lesser reward within the first bout of licks, after which intake precipitously declines. Microstructural analysis shows that shifted rats initiate more drinking bursts, but fewer licks per burst, than unshifted rats (Grigson et al., 1993), indicating that a shifted rat repeatedly initiates intake of the less preferred post-shift solution, but stops intake soon after

initiation. Flaherty (1999) also showed that, when downshifted in a radial arm maze, rats literally 'search' for the missing reward.

In addition to the cognitive component, evidence indicates that SNC also involves an emotional process akin to anxiety. That is, conflict is thought to ensue as the hungry rat comes to accept a lesser reward than that which was expected (Becker et al., 1984; Flaherty and Rowan, 1986, 1989). This 'conflicted' acceptance appears to begin on the 2nd postshift day. In accordance, SNC involves an increase in corticosterone levels on the second post-shift day (Flaherty et al., 1985; Mitchell and Flaherty, 1998) and recovery from the loss of reward is facilitated by pretreatment with the anxiolytic agent, such as chlordiazepoxide (CDP), in a dose and time dependent manner (Rosen and Tessel, 1970; Flaherty et al., 1980; Flaherty, 1990; Flaherty et al., 1990). Specifically, benzodiazepine pretreatment reduces contrast when administered on the 2nd, but not on the 1st post-shift day (Flaherty et al., 1980; Flaherty et al., 1990). Together, these data led Flaherty (1999) to hypothesize that the loss of reward elicits an emotional component which is associated with an increase in analgesic/anxiolytic steroids that activate an, as of yet, uncharacterized GABAergic system. Activation of this GABAergic system is thought to facilitate recovery from the loss of reward and to allow for the effectiveness of anxiolytic agents on the 2nd post-shift day. Recovery generally occurs by the 3rd post-shift trial. Successive negative contrast has been found to depend upon an intact pontine parabrachial nucleus (Grigson et al., 1994), amygdala (Becker et al., 1984), and gustatory thalamus (Reilly and Trifunovic, 1999).

Anticipatory Contrast

In anticipatory contrast, rats are given brief 3 minute access to a weak reward (such as 0.15% saccharin) and then a second 3 minute access period to a greater reward (such as 1.0 M

sucrose). Over repeated pairings, intake of the weak reward decreases compared with a control group that receives only the weak reward (Flaherty and Checke, 1982). This decrease in intake is thought to occur due to the rats' anticipation of future access to the greater reward (Flaherty and Rowan, 1985) and may also model certain aspects of feeding and foraging behavior in which the animal must choose between settling for a lesser, immediate reward or waiting for access to a better reward at some point in the future (Timberlake et al., 1987, 1988; Flaherty, 1996).

Anticipatory contrast effects are based on the relative difference between the rewards that are being compared and, as such, are influenced not only by the rewards themselves but by details of their presentation. For example, changes in the intensity or access time for either reward (Flaherty and Checke, 1982; Flaherty et al., 1991; Flaherty et al., 1994; Flaherty et al., 1996), changes in the interstimulus interval between rewards (Flaherty and Checke, 1982; Flaherty and Rowan, 1988; Lucas et al., 1990), and the addition of a response contingency to the first reward (Flaherty and Grigson, 1988) all alter the strength and expression of anticipatory contrast.

While anticipatory contrast is a robust phenomenon (Flaherty, 1996), the theoretical mechanisms which govern it have yet to be fully determined. Lesion studies show that this phenomenon depends upon an intact gustatory thalamus (Reilly and Pritchard, 1996; Schroy et al., 2005) and cortex (Geddes, 2010) and a number of theories attempt to explain the suppression of intake observed in the weaker reward when it is paired with a stronger reward. However, although these theoretical mechanisms may contribute to the decreased intake, they can not solely explain the suppression of intake. For example, the response competition theory states that the decreased intake of the weak reward is a result of the rats' preference for the location of the stronger reward. That is, the rats prefer to spend more time in the place associated with the stronger reward and, consequently, take less of the weaker reward. While this most likely contributes to the observed suppression, it is not adequate to explain the suppression as experiments in which the weak and strong rewards are presented sequentially in the same location

fail to eliminate contrast, although they do attenuate it (Flaherty and Checke, 1982; Flaherty et al., 1996). Another possible mechanism is that the presentation of the weaker reward (after it has been paired with the stronger reward) initiates an inhibitory, anxiogenic mechanism which decreases intake. While there is evidence for such a mechanism in other forms of contrast (i.e., successive negative contrast), this does not appear to be the case with anticipatory contrast. Anticipatory contrast experiments involving pretreatment with disinhibitory drugs (e.g. GABAergic or serotonergic anxiolytics such as chlordiazepoxide or cyproheptadine) failed to alter contrast (Flaherty and Rowan, 1988; Flaherty et al., 1994). Finally, the hedonic devaluation theory states that suppression of intake occurs because the weaker reward loses hedonic value due to its association with the stronger reward. While this theory may be the most parsimonious explanation (as anticipatory contrast is dependent on relative reward differences), several studies have made it difficult to conclusively accept hedonic devaluation as the primary mechanism in anticipatory contrast. Specifically, when contrast is allowed to develop and the weaker reward (which has been paired with a cue that predicts access to the greater reward) is then tested in a preference test against the same weak reward paired with a cue that predicts access to the same weak reward, devaluation is not observed (Flaherty et al., 1995). However, these studies have also demonstrated that anticipatory contrast is extremely sensitive to the cues and contexts in which it develops. For example, Flaherty, et al. (1995) have demonstrated that the presence of additional flavor or odor cues associated with the weak reward will disrupt the development of anticipatory contrast while additional contextual cues, temporal alternations of the weak or strong reward as the second reward, and additional drinking spout cues with the weak reward allow contrast to develop normally. It is possible that while the weak reward may become devalued in the context of the anticipatory contrast procedure, this devaluation may not express itself outside of the learned situation and therefore, would not translate to preference tests conducted after its development. Clearly, further testing is required to determine if hedonic devaluation occurs in

anticipatory contrast and if it is a contextual, as opposed to an absolute, devaluation of the weak reward.

Reward Comparison

In general, animals do not engage in more than one motivated behavior at a time. Potential rewards are compared so that the animal can best choose which behavior to engage in and this decision is influenced, in part, by the context, the need state of the animal, and by the perceived value of the incentive. The effect of exposure to more than one reward, or even different levels of the same reward, on subsequent responding for a reward has been clearly demonstrated in natural rewards (e.g., contrast effects) (Flaherty, 1999). This framework is, however, also useful when evaluating the comparison of natural rewards with drugs of abuse. The idea that drugs of abuse are not experienced in isolation, but are compared with other rewards in the environment is the basis of the reward comparison hypothesis. In reward comparison, intake of a natural reward is suppressed when it is paired with a drug of abuse. This suppression is thought to be due to the anticipation of the highly rewarding, as opposed to aversive, properties of the drug. In addition, this drug-induced suppression of a natural reward serves as the only known animal model of the devaluation of natural rewards observed in drug addiction.

Drug-induced suppression of intake of a conditioned stimulus

In the reward comparison paradigm, two groups of rats receive access to an otherwise palatable saccharin cue for 5 minutes daily over a number of days. Immediately following daily access to saccharin, one group of rats receives an intraperitoneal (ip) injection of saline while the other is injected with a drug of abuse such as cocaine. The group that receives saline continues to

drink saccharin over trials while the group that receives cocaine comes to avoid intake of the taste cue in anticipation of the administration of the drug (Grigson, 1997; Grigson and Twining, 2002; Risinger and Boyce, 2002). Although both rewards could be consumed (i.e., access to one reward is not contingent on responding to the other), intake of the reward cue is avoided following pairings with the drug of abuse because, at least initially (see Wheeler et al., 2008), the value of the gustatory stimulus pales in comparison to the potent rewarding properties of the drug (Grigson, 1997). Similarly, suppression of intake of the saccharin cue is observed when administration of the drug is active as well as passive. That is, when rats are implanted with an intravenous (IV) jugular catheter and have operant control over drug infusions, intake of saccharin is suppressed when it is paired with the opportunity to self-administer drug (Grigson and Twining, 2002). This active model of reward comparison allows not only for evaluation of changes in saccharin intake, but in drug intake as well. Consequently, it has been shown that suppression of saccharin intake is positively correlated with drug infusions. Rats that exhibit the greatest suppression of saccharin intake self-administer the most drug (Grigson and Twining, 2002).

Reward Comparison and Conditioned Taste Aversion.

The reward comparison hypothesis maintains that the rewarding properties of the drug are responsible for suppression of CS intake. However, the original interpretation of the mechanism behind drug-induced suppression was posited to presumed acute, aversive properties of drugs and was thought to be a form of conditioned taste aversion (CTA). Conditioned taste aversions occur when intake of a gustatory CS is reduced as it comes to predict the occurrence of an aversive unconditioned stimulus (US) such as x-radiation or LiCl (Garcia et al., 1955; Garcia et al., 1961; Smith et al., 1964; Nachman and Ashe, 1973). While illness-inducing stimuli

commonly served as the US, gustatory cues began to be paired with drugs of abuse in the 1970's (Garcia et al., 1961; Carroll and Smith, 1974; Wise et al., 1976; Reicher and Holman, 1977; White et al., 1977; Spector et al., 1986) and the resultant suppression of CS intake also was interpreted as a CTA. That is, it was believed that rats avoided intake of the taste cue because it predicted aversive properties of the drugs of abuse (Nachman et al., 1970; Riley and Tuck, 1985).

This interpretation of drug-induced suppression led to a number of paradoxical conclusions. For example, investigators have reported both simultaneous positive reinforcement and aversive conditioning when drugs of abuse served as the US. Specifically, Wise, Yokel, & DeWit (1976) found that rats decreased consumption of a gustatory cue that predicted the opportunity to self-administer amphetamine or apomorphine, but then readily lever-pressed for the drug. A similar pattern was reported by Reicher & Holman (1977) when rats developed a preference for a location paired with amphetamine while simultaneously avoiding the saccharin solution that predicted its availability. Finally, White, Sklar, and Amit (1977) showed that rats increased running speed in a runway for a food source that was paired with the administration of morphine, but then did not consume the food source upon reaching the goal box. In addition, discrepancies of this nature have not been adequately explained using the CTA interpretation despite continued investigation (Sherman et al., 1980; Corrigan et al., 1986; Gaiardi et al., 1991).

Utilization of the reward comparison framework, in which the rewarding, not the aversive, properties of the drug are hypothesized to mediate suppression, potentially resolves these paradoxical findings because the same mechanism that drives the increase in instrumental behavior is thought to drive the decrease in consumption if the drug-associated taste cue. There is a growing body of literature that aligns the suppressive effects of drugs of abuse with those of other rewards. For example, as is the case for anticipatory contrast effects (i.e., the suppressive effects of a sucrose US on CS intake), the suppressive effects of drugs of abuse depend upon the nature and intensity of the gustatory cue (Flaherty et al., 1994; Grigson, 1997, 2008a; Huang and

Hsiao, 2008), the deprivation state of the animal (Grigson et al., 1999), and a history of previous chronic morphine exposure (Grigson et al., 2001) while CTA does not (but see Huang and Hsiao, 2008). In addition to these behavioral data, lesion data also dissociate drug-induced suppression from CTA. For example, like sucrose-induced suppression (ACE), drug-induced suppression also is dependent on an intact gustatory thalamus and gustatory cortex, but CTAs are not (Mackey et al., 1986; Reilly and Pritchard, 1996; Grigson et al., 2000; Geddes et al., 2008). In fact, recent data indicate that communication within this thalamocortico loop may be essential for reward driven suppression but not suppression by aversive stimuli (Geddes, 2010).

Reward Comparison as a model for behavior in drug addiction.

A major advantage of the reward comparison paradigm is that it provides an animal model for a common behavior observed in human addiction, specifically the devaluation of natural rewards as a result of exposure to drugs of abuse. It has been well documented that human addicts are more likely to weigh less, to lose their jobs, and to have their children removed from their home due to neglect (Jones et al., 1995; Santolaria-Fernandez et al., 1995; Nair et al., 1997). In addition, it has also been demonstrated that addicts respond inappropriately to monetary rewards (Goldstein et al., 2006; Goldstein et al., 2008; Wilson et al., 2008). To date, the reward comparison model provides the only testable framework in which to investigate the factors and mechanisms that contribute to this devaluation of natural rewards by drugs of abuse.

In addition, the reward comparison model also provides a framework to test two other aspects of drug addiction: the possible protective effects of natural rewards on drug-taking behavior and the development of cue-induced craving and withdrawal. Cross-sensitization between drugs of abuse and natural rewards has been demonstrated (Avena and Hoebel, 2003a, b), but recent evidence indicates that natural rewards, such as sweets and enriched environments,

can disrupt drug self-administration through simultaneous presentation as well as through prior exposure to the alternative natural rewards (Bardo et al., 2001; Twining and Grigson, 2004; Stairs et al., 2006; Lenoir et al., 2007; El Rawas et al., 2008; Solinas et al., 2008). Indeed, sweets have been shown to attenuate a number of drug-related behaviors in rodents including drug acquisition (Carroll et al., 1989; Carroll and Lac, 1993; Ahmed, 2005; Lenoir et al., 2007), tolerance (D'Anci, 1999), withdrawal (Jain et al., 2004), and relapse (Liu and Grigson, 2005). This disruptive effect of natural rewards has already demonstrated clinical significance as addiction treatments that incorporate alternative incentives into their programs have proven successful in reducing responding for drugs (Donny et al., 2004) and in increasing abstinence periods in human addicts (Higgins et al., 1993; Donny et al., 2004).

Finally, the reward comparison paradigm also provides a testable framework for the cue-induced craving and withdrawal that develops in addiction. As drug addiction is a brain disease characterized by chronic relapse (DeJong, 1994; Leshner, 1996; Koob et al., 1998), the cues involved in reinstatement of drug-taking behavior are paramount to successful treatment and management of this disorder. In the reward comparison paradigm, the taste cue becomes the best predictor of the drug and evidence suggests that not only is the taste cue devalued by the drug, but the cue can, by its association, elicit cue-induced craving and withdrawal (Grigson, 2008b). In support, avoidance of the taste cue is associated with an elevation of circulating corticosteroids (Gomez et al., 2000) and a blunting of accumbens dopamine (Grigson and Hajnal, 2007a). Moreover, the greater the avoidance of the taste cue, the greater the elevation of circulating corticosterone and, as mentioned, the more drug the rats take when given the opportunity to self-administer (Grigson and Twining, 2002). In addition, recent evidence has demonstrated that rats exhibit aversive taste reactivity (i.e., gaping) during 30 minutes of intra-orally infused saccharin when it is paired with the opportunity to self-administer drug (Wheeler et al., 2008). These data most likely reflect a conditioned aversive state initiated, not by the cue itself (i.e., saccharin), but

to the wait for the drug self-administration session associated with the cue. Indeed, humans have been shown to make aversive faces when they have to wait for drug (Sayette et al., 2003). This conditioned negative affect, which may serve as a model for the cue-induced craving observed in addiction, may contribute to the suppression of intake of a cue associated with drug. As such, the reward comparison paradigm provides a promising model for future evaluation of the devaluation of natural rewards by drugs of abuse in the context of conditioned craving and withdrawal.

Reward Comparison and Sensitivity to Rewards

While numerous aspects of reward comparison have yet to be elucidated, this dissertation will focus on the effects of differential preference for rewards in selected strains of rats and mice. The literature demonstrates that a number of aspects of drug addiction can be altered by the relative preference for reward. For example, conditioned place preference (CPP), a model of preference for places associated with drugs of abuse, is greater and more persistent in the relatively reward preferring C57BL/6J mice compared with 129/J mice (Zhang et al., 2002; Schlussman et al., 2008). Similarly, nicotine supports CPP in reward sensitive Lewis rats but not in Fischer 344 rats (Horan et al., 1997). In addition, black agouti rats, which are more sensitive to methamphetamine due to poor metabolism as a result of a polymorphism of the CYP2D1 gene, demonstrate greater cue-induced reinstatement of extinguished drug-taking behavior than either Lewis or Fischer 344 rats (Xi and Kruzich, 2007). However, to date, the effects of differences in reward preference on the devaluation of natural rewards by exposure to drugs of abuse have yet to be examined. Based on the current body of literature, however, several predictions can be made. First, strains of rats and mice with increased preference for rewards should find drugs of abuse more rewarding and this increased preference should translate into greater suppression of intake of a gustatory CS paired with a drug of abuse. Second, this relative increase in suppression should

be observable in comparisons between disparate natural rewards as well (e.g., in consummatory contrast effects). To this end, Chapters 2 and 3 will use Lewis and Fischer rats to evaluate how differences in preference for rewards affect suppression of CS intake in reward comparison when a drug such as morphine or cocaine serve as the US, conditioned taste aversion when the illness-inducing agent LiCl serves as the US, anticipatory contrast when a highly preferred sucrose solution is used as the US, and in successive negative contrast when rats are unexpectedly downshifted from a high to a low reward. Similarly, Chapter 4 will evaluate differences in the suppression of CS intake in drug-induced reward comparison and LiCl-induced conditioned taste aversion in two mouse strains which differentially prefer rewards: C57BL/6 and DBA/2J mice. In addition, it was hypothesized that selective increases in reward preference within a mouse strain via transgenic manipulation would result in a similar pattern of data (e.g., greater suppression of intake of a gustatory CS when paired with a drug of abuse). Chapter 5, therefore, will examine drug-induced suppression of CS intake in transgenic mice with elevations of Δ FosB, a transcription factor known to increase sensitivity and responsiveness to rewards. Finally, in response to unexpected findings obtained in previous chapters, Chapter 6 will evaluate the effect of changes in the hedonic value of either the CS or the US on the relative suppression of intake of a gustatory cue in reward preferring C57BL/6 and less preferring DBA/2J mice.

Chapter 2

The suppressive effects of sucrose and cocaine, but not LiCl, are greater in Lewis than Fischer rats: Evidence for the reward comparison hypothesis.

Chapter 2 will evaluate the suppression of intake of a gustatory cue in two strains of rat that differ in sensitivity to rewards: Lewis and Fischer 344 rats. Behaviorally, Lewis rats have a high preference for drugs of abuse and will readily self-administer cocaine, ethanol, and opiates compared with the relatively low self-administration behavior of the Fischer 344 rats (Li and Lumeng, 1984; Suzuki et al., 1988; George and Goldberg, 1989; Guitart et al., 1993; Kosten et al., 1997). Lewis rats also work harder (i.e., demonstrate higher breaking points) when tested on progressive ratio schedules of self-administration (Martin et al., 1999) and demonstrate greater conditioned place preference to a number of drugs of abuse (Guitart et al., 1992; Kosten et al., 1994; Horan et al., 1997). In addition, Lewis rats demonstrate greater drug-induced reinstatement compared with Fischer 344 rats (Kruzich and Xi, 2006a, b). Lewis rats also are more sensitive to natural rewards as Lewis rats tend to consume more sucrose, demonstrate greater operant responding for food, and develop excessive wheel running behavior compared with Fischer 344 rats (Kosten et al., 1994; Werme et al., 1999; but see Kosten and Ambrosio, 2002; Martin et al., 2003). In sum, Lewis rats are more sensitive to rewards and are more willing to work for them.

Differences in the sensitivity to drugs of abuse and natural rewards between the Lewis and Fischer 344 rat strains can be traced to neurochemical differences in the mesolimbic dopamine system, a major reward pathway in the brain. In fact, drug-naïve Lewis rats demonstrate many of the molecular adaptations observed with chronic exposure to drugs of abuse. example, Lewis rats, compared with Fischer 344 rats, demonstrate lower levels of tyrosine hydroxylase (TH) expression, dopamine (DA), D2 and D3 dopamine receptors, DA transporters,

and the G-proteins $G\alpha_{1/2}$ and $G\alpha_3$ in the nucleus accumbens (NAc) (Beitner-Johnson et al., 1991; Guitart et al., 1993; Strecker et al., 1995; Flores et al., 1998; Haile et al., 2001), possess fewer spontaneously firing dopaminergic neurons in the ventral tegmental area (VTA) (Minabe et al., 1995), and have higher levels of adenylyl cyclase (AC), protein kinase A (PKA), and Δ FosB in the NAc (Guitart et al., 1993; Haile et al., 2001). In addition, Lewis rats also have fewer neurofilaments and greater levels of glial fibrillary acidic protein (GFAP) in the VTA compared with Fischer 344 rats (Beitner-Johnson et al., 1991; Guitart et al., 1992; Beitner-Johnson et al., 1993).

The differential response to rewards between Lewis and Fischer 344 rats supports a very specific prediction regarding the means by which drugs of abuse suppress intake of a gustatory CS. That is, if the suppressive effects of drugs of abuse are mediated by aversive properties (i.e., CTA), then the degree of suppression should not differ between the two strains of rats. If, on the other hand, the rewarding properties of the drugs of abuse mediate the suppression, then the magnitude of the suppression should be greater in the drug-preferring Lewis rats. The following experiments were designed to test this hypothesis and the results have been published (Grigson and Freet, 2000).

Experiment 2.1

Although it is generally accepted that Lewis rats are more sensitive to rewards, there has been some controversy as to whether this predisposition results in differential suppression of intake of a gustatory cue by cocaine between Lewis and Fischer 344 rats. Kosten, Miserendino, Chi, and Nestler (1994) found that while both Lewis and Fischer 344 rats acquired a cocaine-induced conditioned place preference, neither group learned a cocaine-induced CTA. Glowa, Shaw, and Riley (1994), on the other hand, did obtain strain differences. Lewis rats readily

acquired a cocaine-induced CTA, while Fischer 344 rats did so only when using a high, 50 mg/kg dose of cocaine. The disparity between the results of these studies may be due, in part, to the mode of US administration. That is, the subcutaneous (sc) injections, used by Glowa et al. (1994), have been found to sustain larger cocaine-induced CTAs than the intraperitoneal (ip) injections employed by Kosten et al. (1994), (Ferrari et al., 1991; Mayer and Parker, 1993). The first experiment, then, was designed to resolve this controversy by replicating the experiment using the more effective sc injection procedure. All of the parameters employed are standard to this laboratory (Grigson, 1997) and we selected a dose of cocaine (10 mg/kg) that is effective in our hands and is within the range dictated by the reports discussed above.

Methods

Subjects.

The subjects were 12 naïve male Fischer rats (Harlan Laboratories) and 13 naïve male Lewis rats (Harlan Laboratories) with the Fischer rats weighing between 204 and 272 g and the Lewis rats weighing between 280 and 349 g at the beginning of the experiment. All animals were housed individually in stainless steel hanging cages in a temperature-controlled (21°) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 a.m.). All experimental manipulations were conducted 3.5 h into the light phase of the cycle. The rats were maintained with free access to dry Harlan Teklad rodent diet and water, except where noted otherwise.

Apparatus.

All experimental manipulations were conducted in the home cages using inverted

Nalgene graduated cylinders and stainless steel spouts affixed to the front of the cage. Total intake was recorded to the nearest 0.5 ml.

Procedure.

The rats were placed on a water deprivation schedule for 10 days prior to the start of the experiment in which they received 5 min access to distilled water (dH₂O) in the morning and 1 h in the afternoon. During testing, the subjects received 5 min access to 0.15% saccharin, followed 5 min later, by a sc injection of either 10 mg/kg cocaine (1.5 mg/kg stock solution adjusted for body weight) or vehicle (0.15 M NaCl). There was one such taste-drug pairing a day, occurring every other day, for a total of 8 trials. All subjects received 1 h access to dH₂O each afternoon and 5 min access each morning between conditioning trials. Saccharin was obtained from the Sigma Chemical Company, St. Louis, MO and sodium chloride was obtained from Fisher Chemical, Pittsburgh, PA. Cocaine HCl was generously provided by the National Institute on Drug Abuse.

Results

CS Intake.

As predicted, only the Lewis rats reduced intake of the gustatory CS following saccharin-cocaine trials (see Figure 2.1). Post hoc assessment of the Strain x Drug x Trials interaction,

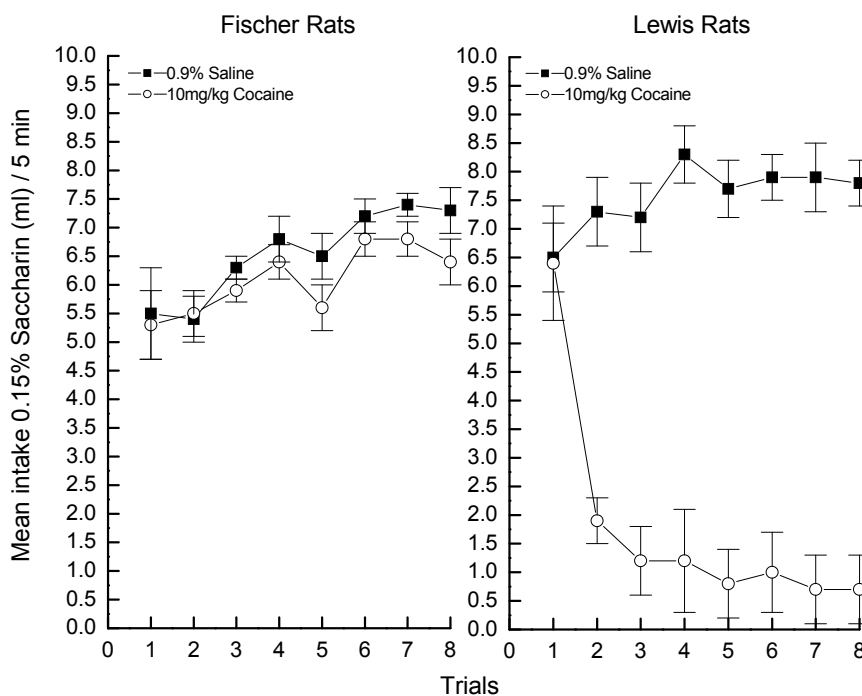


Figure 2.1: Mean (+SEM) intake (ml/5 min) of 0.15% saccharin in Fischer 344 and Lewis rats injected subcutaneously with either saline or 10 mg/kg cocaine hydrochloride across eight taste-drug pairings in Experiment 2.1.

$F(7,147) = 6.65$, $p < .0001$, revealed that the Lewis rats suppressed intake of the saccharin CS following a single taste-drug pairing, $p < .05$. In contrast, there was no significant suppression of saccharin intake by the Fischer rats following as many as 8 taste-drug pairings, $p > .05$. This finding is consistent with that of Glowa et al. and suggests that the failure to acquire suppressive effects in the Kosten et al. report was related to the use of ip, rather than sc, injections.

Our data did differ somewhat from those of Glowa et al., however. In the present study a 10 mg/kg dose of cocaine exerted robust suppressive effects, while a similarly injected 18 mg/kg dose induced only a moderate reduction in CS intake by the Lewis rats in the Glowa et al. report. Two differences exist between the procedures of Glowa et al. and those used in the current study.

First, we employed only a 5 min access period, whereas Glowa et al. used a 20 min access period to the gustatory CS. Second, in the Glowa et al. paper, the rats were restricted to only 20 min access to fluid daily. The rats in the present report, on the other hand, had 5 min access in the morning and 1 h access to dH₂O each afternoon to rehydrate. These differences in the water deprivation regimen are most likely to account for the larger suppressive effects obtained in the present report. Specifically, the rats in the Glowa et al. paper may have demonstrated smaller suppressive effects because the need for fluid facilitated intake of the saccharin CS even when paired with an 18 mg/kg dose of cocaine. Regardless of the magnitude of the suppressive effect, however, both reports that used the sc administration found that the Lewis rats demonstrated a far greater propensity for cocaine-induced suppression of saccharin intake than did the Fischer rats.

Experiment 2.2

Greater suppression of CS intake by the Lewis, relative to the Fischer rats, was interpreted by Glowa et al. as a CTA and, therefore, as evidence that the Lewis rats are not only more sensitive to the rewarding properties of the US, but also to the aversive properties as well. If this conclusion is correct, then the Lewis rats also should show larger LiCl-induced CTAs. The next experiment was designed to test the validity of this hypothesis.

Methods

Subjects.

The subjects were 12 male Fischer rats and 12 male Lewis rats with the Fischer rats weighing between 241 and 271 g and the Lewis rats weighing between 312 and 384 g at the

beginning of the experiment. They were obtained and housed as described in Experiment 2.1. Subjects had prior experience with either a 0.15 M NaCl or a .009 M LiCl injection (for details, see below).

Apparatus.

The apparatus was the same as that described in Experiment 2.1.

Procedure.

In our original CTA experiment with this set of rats, the Lewis and Fischer rats were injected ip with saline or LiCl. This procedure, however, yielded suppression in all groups, including the saline injected controls. A subsequent review of the literature revealed that Fischer rats, at least, find saline injections stressful, perhaps leading to the reduction in CS intake by the saline injected controls (Izumi, Washizuka, Hayashi-Kuwabara, Yoshinaga, Tanaka, Ikeda, Kiuchi, & Oguchi, 1996, 1997). Thus, in an effort to avoid the apparent stress associated with the ip injection, the USs (saline or LiCl) were ingested, rather than injected. As a consequence, these stimuli served as both the CS and the US in the present experiment. In a cross-over design, rats that were injected with LiCl in the original iteration ingested saline in this phase of the study; while rats that were initially injected with saline, ingested LiCl.

Rats were placed on a water deprivation schedule 10 days prior to the start of the experiment during which they were given 5 min access to dH₂O in the morning and 1 h in the afternoon. During testing, subjects received 5 min access to either .009 M LiCl (n=6 Lewis, n=6 Fischer) or 0.15 M Sodium Chloride (n=6 Lewis, n=6 Fischer) in the morning. Total intake was limited to 5 ml/trial and the trials occurred every other day, for a total of 3 conditioning trials. On

trials 4-6, the concentration of LiCl was increased to the standard dose (0.15 M). To maintain proper hydration, all subjects received 1 h access to dH₂O each afternoon and 5 min access to dH₂O each morning between conditioning trials.

Lithium chloride was obtained from the Sigma Chemical Company, St. Louis, MO and sodium chloride was obtained from Fisher Chemical, Pittsburgh, PA.

Results

Both the Lewis and the Fischer rats demonstrated a robust LiCl-induced CTA (see Figure 2.2). This observation was confirmed by post hoc analysis of a significant Drug x Trials

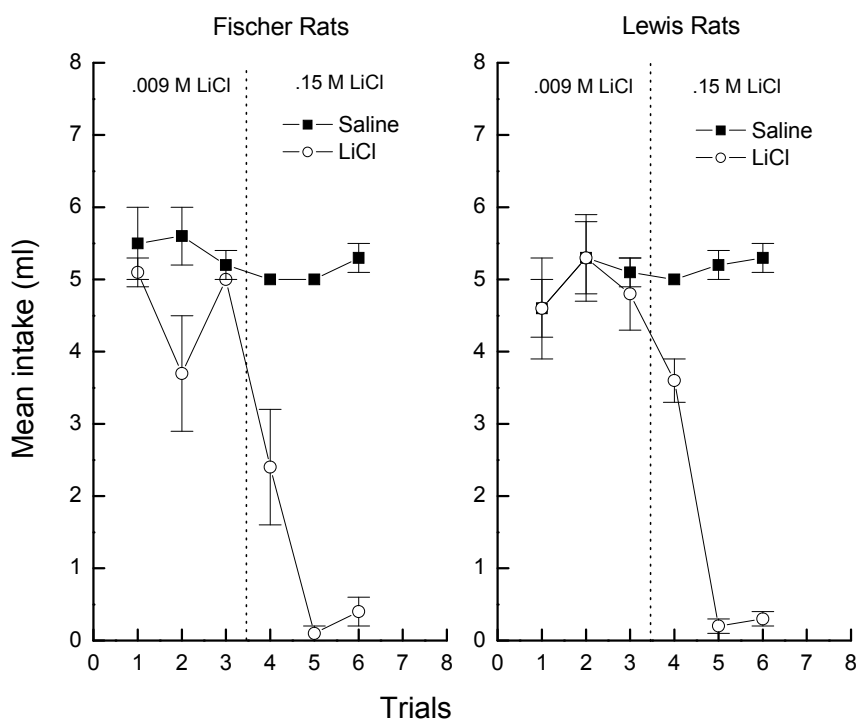


Figure 2.2: Mean (\pm SEM) intake (ml/5 min) of saline or LiCl by Fischer 344 and Lewis rats, across six trials in Experiment 2.2. The concentration of LiCl was 0.009 M during Trials 1-3 and 0.15 M during Trials 4-6.

interaction, $F(5,100) = 36.22$, $p < .0001$, which revealed that all rats consuming LiCl significantly reduced intake by the 4th trial, $p_s < .05$. Neither the main effect of strain, the Strain x Drug, nor the Strain x Drug x Trials interaction, however, achieved statistical significance, $p_s > .05$. This finding supports the conclusion that LiCl-induced CTAs are equivalent in the Fischer and Lewis rats. Therefore, the suppression of CS intake by the Lewis rats, and the lack of suppression by the Fischer rats, follow saccharin-cocaine pairings in Experiment 2.1, cannot be accounted for with a CTA explanation.

Experiment 2.3

The results of Experiment 2.1 showed that the Lewis rats suppressed intake of a saccharin CS when paired with a 10 mg/kg dose of cocaine, but that the Fischer rats did not. As discussed, this finding is consistent with that of Glowa et al. Their interpretation that Lewis rats are more sensitive to both the rewarding and the aversive properties of drugs of abuse, however, no longer seems tenable given the results of Experiment 2.2. Both the Lewis and the Fischer rats acquired a LiCl-induced CTA and the magnitude of the effects was equivalent between the two strains. The reward comparison hypothesis cannot address the CTA data of Experiment 2.2, but accurately predicted the greater cocaine-induced suppression obtained in the Lewis rats in Experiment 2.1. A further prediction, however, must follow. That is, if the suppressive effects of drugs of abuse are mediated by a reward comparison process, then the Lewis rats also should show greater sucrose-induced suppression of saccharin intake (i.e., ACEs) than the Fischer rats. Experiment 2.3 was designed to test this final hypothesis.

Methods

Subjects.

The subjects were 12 naïve male Fischer rats and 12 naïve male Lewis rats with the Fischer rats weighing between 194 and 211 g and the Lewis rats weighing between 230 and 291 g at the beginning of the experiment. All animals were obtained and housed as described in Experiment 2.1.

Apparatus.

The rats were trained in one of four identical modular operant chambers (MED Associates, Inc., St. Albans, VT), measuring 30.5 x 24.0 x 29.0 cm (length x width x height). All chambers had a clear Plexiglas top, front, and back wall. Side walls were made of aluminum. The grid floors consisted of nineteen 4.8-mm stainless steel rods spaced 1.6-cm apart (center to center). Each chamber was equipped with two retractable sipper tubes that could enter the chamber through 1.3-cm diameter holes spaced 16.4-cm apart (center to center). In the extended position, the tip of the sipper tube was aligned in the center of the hole, flush with the right end wall. A lickometer circuit was used to monitor licking. A shaded bulb, which reflected light off the ceiling, was located to the right of the cage and a white noise speaker was on the left-end wall, opposite to the sipper tubes. Each chamber was housed in a light and sound attenuated cubicle that was fitted with a ventilation fan and a white noise source that provided a background noise level of 75 dB, below required levels (>105 dB) for activation of the audiogenic stress pathway and elevation of CORT in rats (Palkovits et al., 2004; Palkovits et al., 2009). Control of events in the chamber and collection of the data were carried out on-line using a 33-MHz computer. Programs were written in the Medstate notation language (MED Associates, Inc.).

Procedure.

The rats were deprived to 82% of their free-feeding body weight and, once stabilized, were habituated to the modules for 5 min/day for 3 days. During testing, each subject was taken from his home cage, weighed, and placed into the module. Immediately following the start command, the house light came on and the first bottle was inserted containing a 0.15% saccharin solution. All rats (12 Fischer, 12 Lewis) were given 3 min access to this bottle of saccharin. Immediately thereafter, half of the rats received 3 min access to a second bottle containing more 0.15% saccharin (n=6 Lewis, n=6 Fischer) or a preferred 1.0 M sucrose solution (n=6 Lewis, n=6 Fischer). There was one such pairing a day for a total of 10 days in succession. All rats were fed 45 minutes after the end of their daily test session. The mean number of licks made on bottle 1 and bottle 2 served as the dependent measures. Saccharin was obtained from the Sigma Chemical Company, St. Louis, MO and sucrose was obtained from Fisher Chemical, Pittsburgh, PA. Sucrose was prepared 24 h prior to testing and all solutions were presented at room temperature.

Results***Bottle 1.***

The Lewis, but not the Fischer, rats demonstrated sucrose-induced suppression of saccharin intake, see Figure 2.3.1. This observation was confirmed by post hoc assessment of a significant Strain x Solution x Trials interaction, $F(10,190) = 6.55$, $p < .0001$, which revealed that Lewis rats in the saccharin-sucrose group consumed less first bottle saccharin than their saccharin-saccharin controls beginning with trial 3, $ps < .05$. As with the cocaine US in Experiment 2.1, the Fischer rats failed to show any evidence of suppression of CS intake, $ps > .05$.

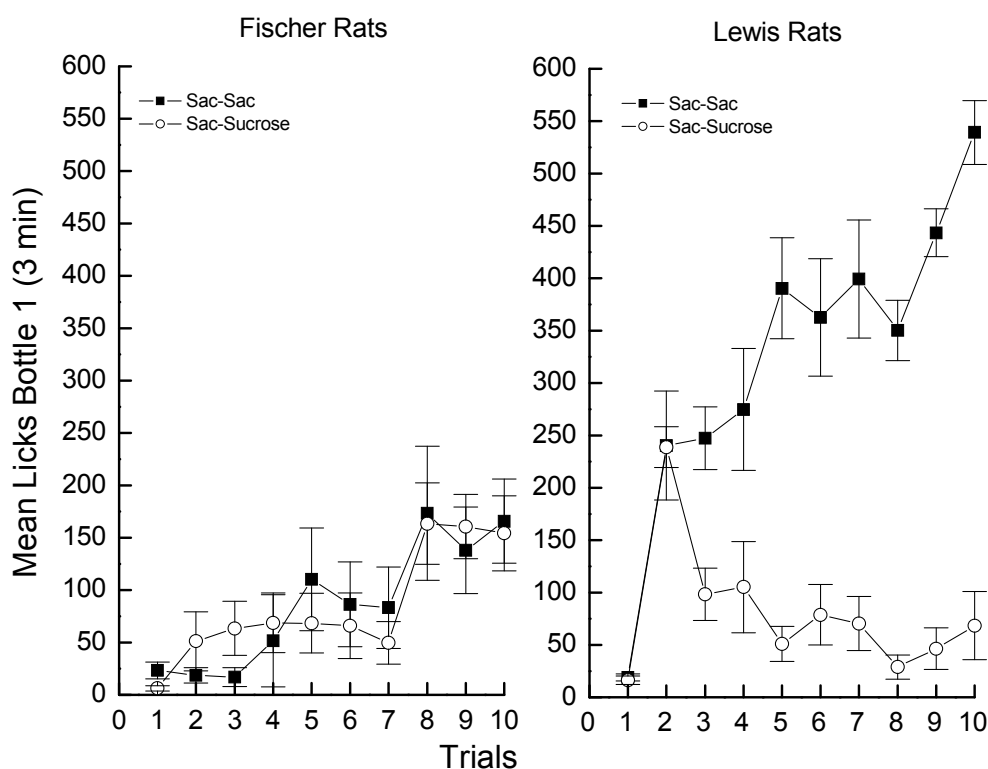


Figure 2.3.1: Mean (\pm SEM) number of licks/3 min elicited for Bottle 1 (0.15% saccharin [Sac]) by Fischer and Lewis rats in the Sac-Sac or the Sac-Sucrose condition of Experiment 2.3. The break along the x axis indicates that the data from Trial 8 were omitted due to experimenter error.

Bottle 2.

The Lewis and the Fischer rats both consumed more sucrose than saccharin in bottle 2 (see Figure 2.3.2). The Strain x Solution x Trials interaction was significant, $F(9, 180) = 5.42$, $p < .0001$, however, and a post hoc analysis revealed some differences between the strains. Specifically, the Lewis rats consumed significantly more sucrose than did the Fischer rats, beginning with the first trial, $p_s < .05$. Lewis rats also consumed more second bottle saccharin, but this effect did not achieve statistical significance until the 8th trial, $p_s < .05$. Furthermore, the

Lewis rats drank more sucrose than saccharin beginning with trial 1. The Fischer rats displayed a similar pattern, but this effect obtained significance only on trials 3, 4, and 6 - 10, $p < .05$.

Together, the data show that while the Lewis rats consumed more than the Fischer rats, both strains of rat evidenced a clear preference for sucrose over saccharin. As a consequence, the failure of the Fischer rats to develop an ACE cannot be attributed to a simple inability to detect or to respond to the hedonic properties of the gustatory stimuli.

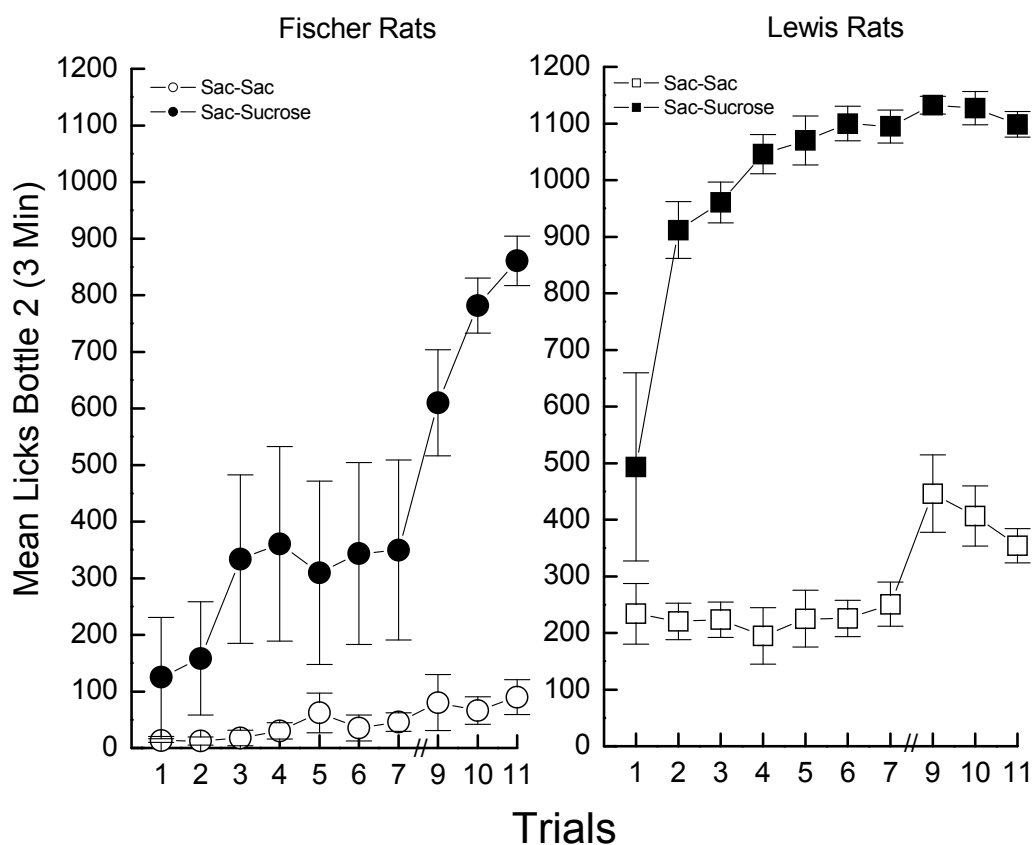


Figure 2.3.2: Mean (+SEM) number of licks/3 min elicited for Bottle 2 (0.15% saccharin [Sac]) or 1.0 M sucrose by Fischer and Lewis rats in the Sac-Sac or the Sac-Sucrose condition of Experiment 2.3. The break along the x axis indicates that the data from Trial 8 were omitted due to experimenter error.

Discussion

Drugs of abuse, drugs that rats and humans readily self-administer, suppress intake of a gustatory CS following once daily pairings (Cappell and LeBlanc, 1971; Cappell et al., 1973). Given the procedural similarities, these data have long been accepted as CTAs and, thereby, as evidence for the aversive properties of drug of abuse (Nachman et al., 1970; Riley and Tuck, 1985). More recent data, however, have called this line of thinking into question (Grigson, 1997). That is, not only an illness-inducing agent, but also a highly rewarding sucrose solution, can lead to a reduction in intake of a saccharin CS following once daily pairings (Flaherty and Checke, 1982). This observation, then, raises the possibility that the rewarding, rather than the aversive, properties of the drug of abuse may mediate the reduction in CS intake.

The evidence presented in Chapter 2 are consistent with other data standing in support of this hypothesis (Grigson, 1997). Specifically, in Experiment 2.1, a 10 mg/kg dose of cocaine selectively suppressed intake of the saccharin CS in the Lewis, but not in the Fischer rats. As stated, this dissociation has been taken as evidence that the Lewis rats are at once more sensitive to both the rewarding and the aversive properties of drugs of abuse (Glowa et al., 1994). The present data, however, do not support this analysis. That is, both the Lewis and the Fischer rats readily acquired a LiCl-induced CTA in Experiment 2.2 and the magnitude of the aversion was indistinguishable between the strains. The alternative hypothesis, reward comparison, however, was supported by the results of Experiment 2.3. That is, parallel to those effects obtained when using a cocaine US in Experiment 2.1, the Lewis, but not the Fischer rats, also evidenced robust sucrose-induced suppression of saccharin intake.

Together, these data support the conclusion that Lewis rats suppress intake of a saccharin CS when paired with sucrose or cocaine because the perceived value of the saccharin CS pales in

comparison to the anticipated highly preferred 1.0 M sucrose solution or sc administration of cocaine. This is not to suggest, however, that either the central process or the neural substrates mediating ACEs with sucrose are necessarily identical to those mediating rewarding comparison effects with drugs of abuse. Indeed, we have found some relatively small differences in Sprague-Dawley rats whereby drug-induced reward comparison effects are riddled with more individual variability than are sucrose-induced ACEs (unpublished findings).

Investigation of the neurochemistry in the Lewis and Fischer rats has yielded results that have proven useful in elucidating the behavioral differences seen when rewarding stimuli are involved. These neurochemical differences are present in the mesolimbic system, specifically the nucleus accumbens (NAc) and the ventral tegmental area (VTA), the same pathway that has been implicated in mediating the rewarding effects of drugs of abuse (Kuhar et al., 1991; Koob, 1992; Nestler, 1992). Beitner-Johnson, Guitart, & Nestler (1991) have found 5 phosphoproteins in the VTA that differ in concentration between the Lewis and Fischer rats, including 58 kDa phosphoprotein, also known as tyrosine hydroxylase. Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of dopamine and is found in higher concentrations in the Lewis rat. As a result, higher levels of dopamine are present in the VTA. Another difference between Lewis and Fischer rats appears in the NAc. It has been shown that Lewis rats possess higher levels of adenylate cyclase and cAMP dependent protein kinases than do Fischer rats (Guitart et al., 1993) and Lewis rats demonstrate greater dopamine release in the NAc core to morphine, amphetamine, and cocaine compared with Fischer rats and, depending upon on the dose, in the NAc shell as well (Cadoni and Di Chiara, 2007). Lower levels of dopamine transporter (DAT) and slower clearance of dopamine has also been observed in Lewis rats compared with Fischer rats (Gulley et al., 2007). According to molecular mechanisms of drug addiction (Nestler, 1992, 2001a, 2004a) and the molecular theory of addiction (Nestler, 1995, 2008), these substances are part of an intracellular cascade that is activated when a drug of abuse binds to a neurotransmitter-

receptor site. In fact, it has been shown that protein kinase A (PKA, a cAMP dependent protein kinase) is involved in the mediation of cocaine self-administration. Infusion of a PKA antagonist into the NAc produces a decrease in base-line measurements of cocaine self-administration in rats, while infusion of a PKA agonist produces an increase (Self et al., 1998). These differences in neurochemistry ultimately result in different basal levels of dopamine between Lewis and Fischer rats. Strecker, Eberle, & Ashby, Jr. (1995) have shown through in vivo microdialysis that Lewis rats have significantly lower basal levels of extracellular dopamine metabolites in the NAc than Fischer rats and that Lewis rats demonstrate greater sensitization (defined as an enhanced increase in dopamine) to repeated exposure to cocaine. As stated previously, dopamine is an active component in the reward pathway. It has been shown that dopamine levels increase when rats receive a rewarding stimulus, whether it is a natural reward such as food or water, or a drug of abuse such as cocaine (Hernandez and Hoebel, 1988; Yoshida et al., 1992). Therefore, it is possible that genetic conditions which allow for potentially higher dopamine increases (such as a low basal level) allow the Lewis rats to perceive a stimulus as more rewarding than it may be perceived by a Fischer rat.

Interestingly, it has been shown that extracellular dopamine decreases 40% in the NAc when a CS is presented after a learned taste aversion (Mark et al., 1991). This effect is not seen when LiCl alone is given, and so is a result of the learned aversion to saccharin. This seems to suggest that the rat's perception, or learned perception, of a solution influences subsequent neurochemistry in the mesolimbic system. If thinking that a stimulus is less rewarding results in changes that makes the stimulus less rewarding from a neurochemical standpoint, then it may begin to explain the neural substrate at work when something that is naturally rewarding in nature is overridden or devalued in the presence of drugs of abuse. It would be interesting to observe this phenomenon (i.e., dopamine levels in the NAc) in a strain of rats with an unusually high avidity for drugs of abuse and sucrose such as the Lewis rats and in another strain that approaches

these stimuli with little regard such as the Fischer rats. In Sprague-Dawley rats, suppression of saccharin intake following a single saccharin-morphine pairing is associated with a blunting of accumbens dopamine to baseline (Grigson and Hajnal, 2007a). Thus, we would predict that greater blunting would occur in a similar analysis in Lewis vs. Fischer 344 rats. Studies of this kind will increase our understanding of the cellular and molecular differences mediating these differences in behavior in these strains of rat. This, in turn, will increase our understanding of the processes by which powerful drugs of abuse come to override stimuli that are by nature rewarding.

While we benefit from a discussion of these data as they relate to the selected strains, we must finally note the potential impact of these data on our current understanding of the suppressive effects of drugs of abuse. That is, from a fundamental perspective, these data, along with others (Grigson, 1997; Grigson et al., 2009), suggest that rats compare the rewarding properties of drugs of abuse with those of natural rewards and that, depending upon the circumstances, the natural reward can be devalued in the process. Of course, in the literature, scientists have been looking at data of this nature for 35 years, only from a different venue. To the degree that this new perspective proves correct, much of the existing “CTA” data involving drugs of abuse will provide information regarding these cross-modal reward comparison processes. Further, a single example whereby powerful rewards, such as drugs of abuse, override natural rewards, such as saccharin, serves as indisputable evidence that different rewards (at least those conferred by food and drugs) are ultimately mediated by some common substrate. To date, there has been no paradigm with which to directly evaluate these processes or the underlying neurocircuitry. Reward comparison provides a paradigm.

Chapter 3

Lewis rats are more sensitive than Fischer rats to successive negative contrast, but less sensitive to the anxiolytic and appetite-stimulating effects of chlordiazepoxide.

The data presented in Chapter 2 demonstrate that reward preferring Lewis rats, compared with the relatively reward insensitive Fischer 344 rats, show greater suppression of intake of a gustatory CS that has been paired with a known rewarding US such as sucrose, but show similar suppression when the CS has been paired with a known aversive agent such as LiCl. In addition, the data in Chapter 2 also aligned the suppressive effects of cocaine with rewarding, as opposed to aversive, US properties. Taken together, this data was interpreted as evidence that rats suppress intake of a gustatory CS that has been paired with a drug of abuse because the rats compare the rewarding properties of the CS and US and suppress intake of the CS in anticipation of the drug in a process akin to sucrose anticipatory contrast (Grigson 1997). In addition, Lewis rats demonstrated greater suppression of intake of the CS because the perceived rewarding value of the US, whether it be cocaine or sucrose, was greater in the Lewis rats compared with the Fischer 344 rats.

The aim of Chapter 3 is to extend these findings to another form of contrast, successive negative contrast (i.e., the abrupt decrease in responding for a lesser reward in rats with a history of having received a stronger, more preferred reward). If Lewis rats are truly reward-preferring, we would predict that SNC effects also may be greater in Lewis rats than Fischer rats. However, regardless of their reward-preferring status, larger SNC effects also can be predicted for the Lewis rats on the basis of their performance in anticipatory contrast (Experiment 2.3). Anticipatory contrast, like SNC, involves the comparison of two rewards and the effect that this comparison has on subsequent intake. Evidence suggests that these phenomena depend upon

similar neural substrates, as both SNC and anticipatory contrast depend upon an intact gustatory thalamus (Reilly and Trifunovic, 2003; Reilly et al., 2004; Schroy et al., 2005). Thus, we hypothesize that Lewis rats also will demonstrate larger SNC as well. Finally, it is possible that Fischer rats may recover faster from the unexpected loss of reward due to larger corticosterone responses observed in this strain (Glowa et al., 1992; Dhabhar et al., 1993; Stohr et al., 2000). This larger corticosterone response may activate the GABAergic system to a greater degree, which could, according to Flaherty (Flaherty, 1999), result in faster recovery from the loss of reward in Fischer 344 rats compared to Lewis rats. The following experiments were conducted to explore the validity of these predictions and the results have been published (Freet et al., 2006).

Experiment 3.1

As previously stated, Experiment 3.1 was designed to test whether Lewis rats would exhibit greater SNC effects following an unexpected downshift in reward magnitude relative to the less reward sensitive Fischer rat. Lewis and Fischer rats were compared across four variables: the presence of successive negative contrast, the magnitude of the negative contrast effect, the rate of recovery from the loss of reward, and the effectiveness of the anxiolytic agent, chlordiazepoxide (CDP), on recovery.

Methods

Subjects.

The study was run in two separate replications, an initial pilot study and a larger replication. With the data collapsed, the subjects were 34 naïve male Fischer 344 rats (Harlan

Laboratories) and 36 naïve male Lewis rats (Harlan Laboratories), with the Fischer rats weighing between 189 and 238 grams and the Lewis rats weighing between 214 and 281 grams at the beginning of the experiment. They were housed individually in stainless steel hanging cages in a temperature-controlled (21°C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 am). All experimental manipulations were conducted three hours into the light phase of the cycle. The rats were maintained with free access to dry Harlan Teklad rodent diet and water except where otherwise noted. An Institutional Review Committee for the use of Animal Subjects approved the experimental protocol and the procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Apparatus.

The apparatus was identical to that described in Experiment 2.3.

Procedure.

Prior to the experiment, rats were food deprived to 82% of their free-feeding body weight, maintained by a once per day feeding. The Lewis and Fischer rats were divided into two groups (unshifted and shifted). The unshifted group (n=18 Lewis, n=17 Fischer) was given 5 minutes access to 0.1 M sucrose daily for 14 days. The shifted group (n=18 Lewis, n=17 Fischer) was given 5 minutes access to 1.0 M sucrose daily for 10 days and then was shifted to 0.1 M sucrose on days 11-14. On postshift day 2 (or day 12), half of the shifted and unshifted rats (shifted: n=9 Lewis, n=8 Fischer; unshifted: n=9 Lewis, n=8 Fischer) were injected intraperitoneally (ip) with 0.9% saline 30-35 minutes prior to being placed into the test chamber.

The other half of the rats (shifted: n=9 Lewis, n=9 Fischer; unshifted: n=9 Lewis, n=9 Fischer) were injected ip with a 10 mg/kg dose of the anxiolytic agent, CDP. The 10 mg/kg dose of CDP was selected because it has been shown to be a highly effective in reducing SNC effects when administered on the 2nd postshift day in Sprague-Dawley rats (Flaherty et al., 1990).

Sucrose (saccharose), obtained from Fisher Chemical, Pittsburgh, Pennsylvania, was prepared 24 hours in advance and presented at room temperature. Chlordiazepoxide was obtained from the Sigma Chemical Company, St. Louis, Missouri and was mixed in 0.9% saline 1-2 hours before administration.

Results

Intake.

To examine the preference for sucrose within and between the strains, the intake data for the first 10 trials were analyzed using a 2 x 2 x 10 mixed factorial ANOVA varying strain, sucrose concentration, and trials. Post hoc Newman-Keuls tests on the significant 3-way interaction, $F(9,486) = 1.95$, $p = .04$, revealed that Lewis rats made more licks for 1.0 M sucrose on all preshift days (trials 1-10) than did Fischer rats, $p_s < 0.001$; Lewis rats also drank more 0.1 M sucrose than Fischer rats, $p_s > 0.03$. Prior to the shift, Lewis rats drank more 1.0 M than 0.1 M sucrose on all trials, $p_s < .004$. Fischer rats also made more licks for 1.0 M than 0.1 M sucrose on all trials ($p < .005$) except Trial 9 ($p = .06$) and Trial 10 ($p = .07$) (see Figure 3.1).

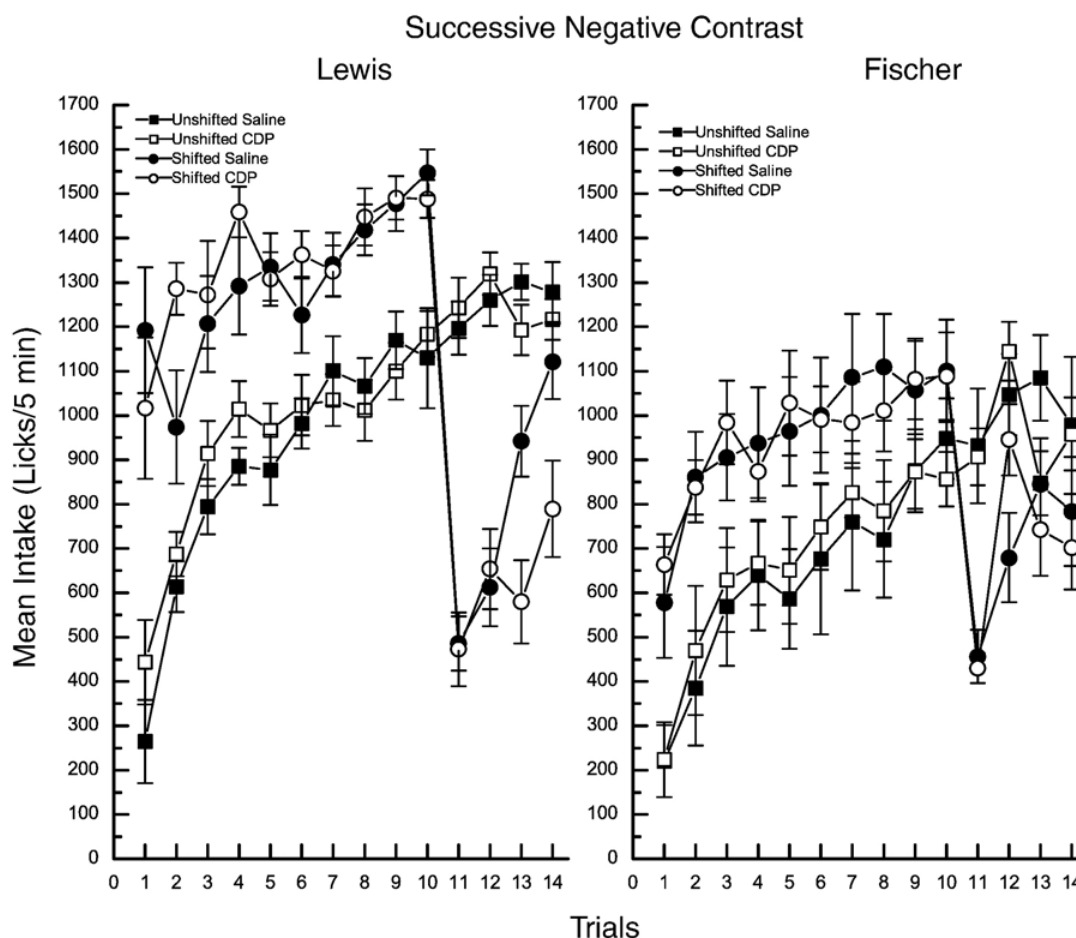


Figure 3.1: Mean intake for the preshift days (trials 1–10) and postshift days (trials 11–14) for Lewis (left panel) and Fischer (right panel) rats. In both strains, half of the rats were given 5 min access to 0.1Msucrose throughout the study (unshifted, squares). The other half of the rats were given 5 min access to 1.0Msucrose for 10 trials and were then downshifted to 0.1 M sucrose across trials 11–14 (shifted, circles). On trial 12, half of the rats from each shift condition were pretreated with saline (closed symbols) and the other half with chlordiazepoxide (CDP, open symbols).

To evaluate SNC, the intake data were analyzed using a $2 \times 2 \times 2 \times 2 \times 14$ mixed factorial ANOVA varying replication, strain, shift, drug, and trials. As the 5-way interaction just missed significance, $F(13,702) = 1.71$, $p > .05$, the replication factor was omitted and the data were reanalyzed using a $2 \times 2 \times 2 \times 14$ mixed factorial ANOVA. The results showed that the 4-way strain \times shift \times drug \times trials interaction was significant, $F(13,702) = 1.79$, $p < .04$. On the day of the shift (trial 11), both strains demonstrated successive negative contrast as defined by a

significant decrease in intake from trial 10 to trial 11 (shift magnitude, $p < .0001$) and a significant decrease in intake relative to their respective unshifted controls (contrast magnitude, $p < .0002$). In addition, a shift ratio (trial 11/ trial 10) was calculated for the shifted group in each strain to better compare the magnitude of the contrast effect across strains. The data were collapsed across drug treatment for each strain, as the drug was not presented until trial 12. The shift ratios were then compared using independent t-tests. The results of this analysis showed that the Lewis rats exhibited a smaller shift ratio (i.e., a larger shift) than the Fischer rats (Lewis = 0.32 (+/- .03); Fischer = 0.42 (+/- .03) and this difference was statistically significant, $t(33) = 2.42, p < .01$). Taken together, these data show that the unexpected loss of reward led to contrast in both strains of rats and the magnitude of this effect tended to be larger in the reward sensitive Lewis rats.

As indicated by examination of the data for the vehicle treated controls, recovery from the loss of reward also was slower in the Lewis, relative to the Fischer, rats. Shifted Fischer rats recovered from contrast by the 3rd postshift day (i.e., intake by the shifted Fischer rats did not differ from that of their unshifted controls by trial 13, $p = .73$). Shifted Lewis rats, on the other hand, continued to consume less of the 0.1 M sucrose solution than their unshifted controls on trial 13, $p < .05$, and did not recover from reward downshift until trial 14, $p = .90$. In the Fischer rats, the administration of CDP on trial 12 led to a complete recovery of contrast relative to unshifted CDP-treated controls, $p = .93$. A single exposure to the same dose of the drug, however, exerted no contrast-reducing effect in the shifted Lewis rats. Intake of the 0.1 M sucrose solution by the CDP treated shifted Lewis rats remained below that of their unshifted CDP, $p < .0001$, or saline, $p < .0001$, treated controls and statistically similar to the shifted saline group, $p = .99$. Finally, for Lewis rats, the administration of CDP served to prevent recovery from the unexpected loss of reward over the 4-day postshift period. Thus, the shifted CDP-treated Lewis rats made

fewer licks for the lesser postshift solution than the unshifted controls on trial 13 and on trial 14 as well, $p_s < .0001$.

In addition to anxiolytic effects, CDP, and benzodiazepines in general, also induce known appetite stimulating effects (Berridge and Treit, 1986; Flaherty, 1999). Despite these rather potent effects, CDP supported only a small trend toward an appetite stimulating effect in the unshifted Fischer rats and no appetite stimulating effect at all in the unshifted Lewis rats. These data may indicate that both strains of rats are insensitive to the appetite stimulating effects of CDP. Alternatively, Fischer rats may be more sensitive than Lewis rats, but the magnitude of the effect may have been occluded in the Fischer rats by a ceiling effect. Thus, Fischer rats may have been making their maximum number of licks in the 5-min test session, and licking could not be further augmented by the administration of CDP. Experiment 3.2 will eliminate this potential confound to better evaluate the appetite stimulating effect of CDP in both strains of rat.

Experiment 3.2

The results of Experiment 3.1 showed that both Lewis and Fischer rats demonstrate successive negative contrast effects. The magnitude of this effect tended to be larger in Lewis rats and Lewis rats also were slower to recover from the loss of expected reward. In addition, while the administration of CDP facilitated the rate of recovery in Fischer rats on postshift day 2, it failed to do so in Lewis rats. In fact, the rate of recovery in Lewis rats was delayed following the administration of the drug. In addition to these findings, the results of Experiment 3.1 also showed that CDP-induced a small, non-significant appetite-stimulating effect in the Fischer rats and no appetite stimulating effect what-so-ever in the Lewis rats. Experiment 3.2 revisited this issue in naïve Lewis and Fischer rats using procedures described by Twining et al. (Twining et al., 2005). Specifically, the rats were tested in a non-deprived state so that ceiling effects could

not impede the data for either the Lewis or the Fischer rats. The 10 mg/kg dose of CDP was used, as this dose has been found to support robust appetite stimulating effects in Sprague-Dawley rats using identical procedures (Twining et al., 2005).

Methods

Subjects.

The subjects were 12 naïve male Fischer rats (Harlan Laboratories) and 12 naïve male Lewis rats (Harlan Laboratories) with the Fischer rats weighing between 207 and 230 grams and the Lewis rats weighing between 216 and 244 grams at the beginning of the experiment. All rats were housed individually in stainless steel hanging cages in a temperature-controlled (21°C) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 a.m.). All experimental manipulations were begun 5.5 to 6.5 hours into the light phase of the cycle (12:30pm to 1:30pm). The rats were maintained with free access to dry Harlan Teklad rodent diet and water, except where otherwise noted. An Institutional Review Committee for the use of Animal Subjects approved the experimental protocol and the procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Apparatus.

The apparatus was the same as that described in Experiment 3.1.

Procedure.

Across trials 1-12, each subject was taken from his home cage, weighed, and placed in the chamber. Immediately after initiating the trial, the house light came on and a bottle containing a 0.1 M sucrose solution was advanced. Each subject was given 5 minutes access to this bottle at which time the bottle was retracted, the house light shut off, and the subject was removed from the box and placed back in the home cage. On trials 6, 7, 9, 10, and 12, each subject received an ip injection of 0.9% saline 30-35 minutes prior to run time. On trials 8 and 11, each subject received an ip injection of the 10 mg/kg dose of CDP 30-35 minutes prior to the start of the experimental session.

Sucrose and CDP were obtained and prepared as described in Experiment 3.1.

Results

The benzodiazepine, CDP, induced significant appetite-stimulating effects in Fischer, but not in Lewis, rats (see Figure 3.2). This observation was confirmed by a 2 x 12 mixed factorial

Selected Strains and CDP

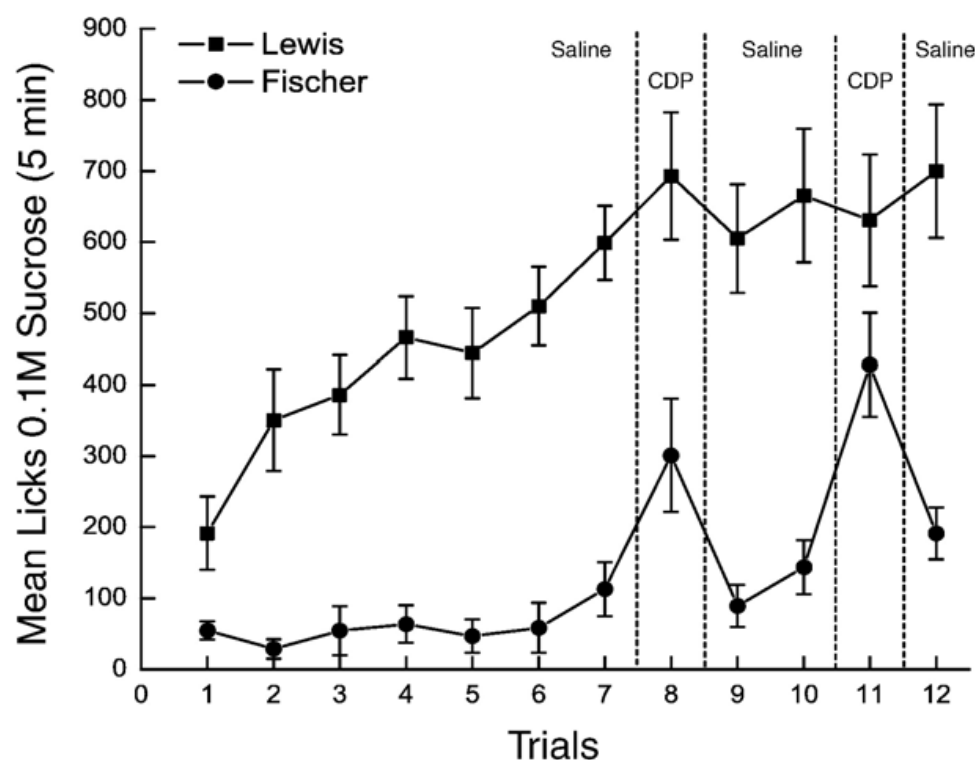


Figure 3.2: Mean intake for Lewis (closed square) and Fischer (open circle) rats is presented for trials 1–12. All rats received an ip injection of saline on trials 7, 9, 10, and 12 and 10 mg/kg CDP on trials 8 and 11.

ANOVA which demonstrated a significant Strain x Trials interaction, $F(11,242) = 4.03$, $p < .0001$, as well as significant main effects of Strain, $F(1,22) = 41.98$, $p < .0001$, and Trials, $F(11,242) = 16.88$, $p < .0001$. Newman-Keuls post hoc comparisons of the significant Strain x Trials interaction demonstrated that, in Lewis rats, there was no significant difference between intake on trial 8 (CDP) and trial 7 (saline; $p = .54$) or trial 8 and trial 9 (saline; $p = .48$) or between trial 11 (CDP) and trial 10 (saline; $p = .57$) or trial 11 and trial 12 (saline; $p = .68$). Intake by the Fischer rats, however, was significantly different across these comparisons. The number of licks on trial 8 (CDP) was greater than that generated on trial 7 (saline; $p = .02$) and that generated on trial 9 (saline; $p = .01$) and the number of licks emitted on trial 11 (CDP) was greater than the number emitted on trial 10 (saline; $p = .0001$) and on trial 12 (saline; $p = .001$).

These data confirm that CDP induces potent appetite stimulating effects in the Fischer rats, but is fully ineffective in the Lewis rats.

Discussion

The data from Experiments 3.1 and 3.2 demonstrate that Lewis and Fischer rats both show successive negative contrast effects, yet they respond differently on two other major indices: Rate of recovery and response to CDP (i.e., to both the anxiolytic and appetite-stimulating effects of the drug). The data from Experiment 3.1 clearly show that both the Lewis and the Fischer rats exhibit robust SNC effects relative to their own unshifted controls. The magnitude of the drop in intake is larger in the Lewis rats (as indicated by the shift ratio), but the interpretation of this finding is not straightforward. That is, while the Fischer rats displayed a smaller reduction in intake when shifted to the lesser reward, it is obvious that these rats also made fewer licks for the 1.0 M preshift solution than did the Lewis rats. Ceiling effects, then, may have contributed to the smaller contrast effect (i.e., to the higher shift ratio) in the Fischer rats. Even so, it also is clear that the shifted Fischer rats could have made fewer licks than 400 licks during the first 5 min post-shift period, but they did not. Thus, when taken together, the data demonstrate that, relative to Fischer rats, SNC effects tend to be larger in Lewis rats following the unexpected loss of reward. Future studies may revisit this issue using longer access to the preshift solution. Acquisition in various paradigms has been shown to be slower in Fischer than Lewis rats (Kosten et al., 1997; Kosten and Ambrosio, 2002). Manipulations known to support smaller contrast effects also may be implemented because strain differences in the anticipatory contrast paradigm were revealed between the Lewis and Fischer rats only when using procedures that supported small anticipatory contrast effects and, thereby, allowed for an assessment of the effect (Experiment 2.3).

The other major finding was the differential effects of the common anxiolytic agent CDP. In Experiment 3.1, CDP failed to attenuate contrast in the Lewis rats. This dose, which facilitated recovery in the Fischer rats, also is known to facilitate recovery in outbred rats in a dose and time dependent manner when administered on the second postshift day (Rosen and Tessel, 1970; Flaherty et al., 1980; Flaherty, 1990; Flaherty et al., 1990). In general, this finding is consistent with the literature. Lewis rats are reportedly less sensitive than Fischer rats to benzodiazepines in several other paradigms including the development of physical dependence to diazepam, elevated plus-maze performance, and the anxiolytic-like effect of the NK-1 receptor antagonist NKP608 (Suzuki et al., 1992; Takahashi et al., 2001; Vendruscolo et al., 2003). Pretreatment with CDP, however, not only failed to facilitate recovery, but actually retarded recovery from the loss of reward. This is of interest as it suggests that CDP may activate a mechanism that directly opposes recovery from the loss of reward.

In Experiment 3.2, differences in CDP-induced hyperphagia also were observed between Lewis and Fischer rats. Again, a review of the literature suggests that the failure to demonstrate CDP-induced appetite stimulating effects by the Lewis rats is not due to a general failure to exhibit drug-induced appetite stimulating effects, per se, but to a more specific failure to respond to the benzodiazepine. Thus, Lewis rats have been shown to exhibit larger, rather than smaller, appetite stimulating effects following the administration of morphine (Gosnell and Krahn, 1993) and they exhibit clear appetite-stimulating effects following the administration of Delta(9)-THC (Koch, 2001). Lewis rats, then, are less sensitive than Fischer rats to a range of effects associated with the administration of a benzodiazepine.

In sum, it has been demonstrated previously that Lewis and Fischer rats behave differently in the reward comparison and anticipatory contrast paradigms (Chapter 2). Here we have extended this finding to include another contrast paradigm, successive negative contrast. Lewis rats tended to exhibit a larger SNC than Fischer rats. Relative to saline-treated Fischer rats,

saline-treated Lewis rats showed a slower rate of recovery from contrast. This increase in SNCs in reward-preferring Lewis rats is parallel to the increase in ACE found in the Lewis rats in Chapter 2. While these phenomena differ in many ways (Flaherty, 1996), both require comparison of the perceived value of an available reward with the memory of another. In ACE the contrast effect depends upon an anterograde comparison process, while in SNC the contrast effect depends upon a retrograde comparison process. As occurs with Sprague-Dawley rats (Flaherty, 1999), pretreatment with CDP served to eliminate contrast when administered on the 2nd postshift day in Fischer 344 rats. Pretreatment with CDP in Lewis rats, on the other hand, failed to attenuate contrast and ultimately served to delay recovery. Lewis rats also were found to be fully insensitive to the appetite stimulating effects of the benzodiazepine, while non-deprived Fischer rats demonstrated a profound hyperphagic response to CDP. Published data show that this effect relates to a specific failure to appropriately respond to the benzodiazepine, rather than to a general failure to exhibit appetite-stimulating effects, per se. Additional studies are required to determine whether these strain differences in behavior are due to known strain differences in the responsiveness of the HPA axis and/or in the GABA/benzodiazepine chloride ionophore.

Chapter 4

Cocaine-induced suppression of saccharin intake is attenuated in C57BL/6 mice compared with DBA/2J mice.

The data presented in Chapters 2 and 3 demonstrate that reward preferring Lewis rats, compared with Fischer 344 rats, show greater suppression of a gustatory CS in a number of paradigms including anticipatory contrast, successive negative contrast, and reward comparison. Current literature demonstrates that mice suppress intake of a gustatory cue paired with a drug of abuse in a dose-dependent manner similar to that observed in rats (Risinger and Boyce, 2002) and it can therefore be predicted, based on the data from the previous chapters, that reward preferring mouse strains will also demonstrate greater suppression of a gustatory CS paired with a drug of abuse. As comparisons between strains of mice with differential preference for rewards have not been evaluated, the aim of Chapter 4 is to more closely examine drug-induced suppression of CS intake in two such strains of mice: C57BL/6 and DBA/2J mice. An assessment of these inbred mouse strains will allow us to evaluate possible differences in the effect of differential reward preference on drug-induced suppression across species as well as provide a foundation for the use of transgenic mouse strains in future investigations of this phenomenon.

C57BL/6 mice demonstrate increased preference for rewards compared with DBA/2J mice. For example, C57BL/6 mice demonstrate increased oral consumption of a number of rewards compared with DBA/2J mice including alcohol (Belknap et al., 1993; Meliska et al., 1995; Misra and Pandey, 2003), morphine (Berrettini et al., 1994), and amphetamine (Meliska et al., 1995). Similarly, C57BL/6 mice consume more natural rewards such as saccharin and sucrose compared with a range of mouse strains (Bachmanov et al., 2001; Lewis et al., 2005). In addition, C57BL/6 mice also demonstrate greater conditioned place preference effects to morphine and

cocaine (Orsini et al., 2005) and greater cocaine self-administration compared with DBA/2J mice (Carney et al., 1991; Kuzmin and Johansson, 2000).

Neurochemically, C57BL/6 mice, compared with DBA/2J mice, share many of the characteristics associated with reward-preferring rat strains and rats with chronic exposure to drugs of abuse. That is, C57BL/6 mice have decreased expression of cAMP responsive element binding protein (CREB) in the shell of the NAc (Misra and Pandey, 2003) and decreased expression of the dopamine transporter (DAT) and tyrosine hydroxylase (TH) in the VTA (D'Este et al., 2007). C57BL/6 mice have also been shown to have higher levels of DAT in the NAc and Δ FosB in the dorsal striatum compared with A/J mice, another relatively reward insensitive mouse strain (Brodtkin et al., 1998). Finally, C57BL/6 mice have higher levels of GABA_A α 1 subunit mRNA and lower GABA_A α 2 subunit mRNA levels in the striatum compared with DBA/2J mice (Korostynski et al., 2006).

Experiment 4.1

Although drug-induced suppression of CS intake has been extensively studied in rats, investigation of this phenomenon in mice has remained largely unexplored. Risinger & Boyce (2002) evaluated differences in the suppression of saccharin or NaCl paired with morphine, cocaine, ethanol, or LiCl in DBA/2J mice and demonstrated greater suppression of saccharin, compared with NaCl, when the US was cocaine or morphine. In contrast, DBA/2J mice demonstrated greater suppression of NaCl, compared with saccharin, when the US was ethanol or LiCl. These differences indicate that, at least in DBA/2J mice, suppression of a saccharin CS may be more sensitive to a rewarding US while a NaCl CS may be more sensitive to an aversive US. However, to date, drug-induced suppression of CS intake has not been evaluated in C57BL/6 mice and the questions of strain differences, specifically in relation to reward preferences, in

drug-induced suppression remain. Therefore, Experiment 4.1 was conducted to compare the suppressive effects of cocaine on saccharin intake in the reward preferring C57BL/6 mice and the relatively reward-insensitive DBA/2J mice.

Methods

Subjects.

The subjects were 30 male C57BL/6 mice and 30 DBA/2J mice obtained from Jackson Laboratories. Mice weighed between 17.9 and 27.5 grams at the beginning of the experiment. They were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 2 hours into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water except where otherwise noted.

Apparatus.

All experimental manipulations were conducted in the home cages. Modified serological pipettes (Fisher Scientific; 07-200-574) were used to provide dH₂O and saccharin access. Pipettes were converted to plastic cylinders by removing the tapered ends. A stainless steel spout (Girton Manufacturing Co., Inc.; ST300C) was then placed in the bottom of the cylinder while a silicon stopper (Fisher Scientific; 09-704-1D) sealed the top of the cylinder. Intake of dH₂O and saccharin was recorded in 1/10 milliliters.

Procedure.

Saccharin-cocaine pairings. All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on a water deprivation schedule which consisted of access to dH₂O for 1 hour in the morning and 2 hours in the afternoon. Baseline intake and body weight were recorded for 1 week. Mice remained on a water deprivation schedule as described above. On each taste-drug pairing (which occurred every 48 hours), all mice received 1 hour access to 0.15% saccharin followed by an i.p. injection of either saline (n=10/cell), 10 mg/kg cocaine (n=10/cell), 20 mg/kg cocaine (n=10/cell), or 30 mg/kg cocaine (n=10/cell). Taste-drug pairings occurred in the morning (2 hours into the light phase) for 5 trials. A sixth trial was conducted with CS only access (no injection). The saccharin solution was presented at room temperature and cocaine HCl was provided by the National Institute on Drug Abuse. *Two-bottle intake test.* Five days following the final reward comparison trial, mice were tested for saccharin preference using a two-bottle intake test. All mice simultaneously received a bottle of 0.15% saccharin and a bottle of dH₂O for 1 hour. The position of bottles was counter-balanced across groups.

Results***CS Intake.***

Intake was analyzed using 2 x 4 x 6 mixed factorial analyses of variances (ANOVAs) varying strain (C57BL/6 or DBA/2J), drug (saline, 10, 20, or 30 mg/kg cocaine), and trials (1-6). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with alpha = .05. Observation of Figure 4.1.1 shows that while DBA/2J mice demonstrate dose-dependent suppression of saccharin intake, suppression is significantly attenuated in the C57BL/6 mice.

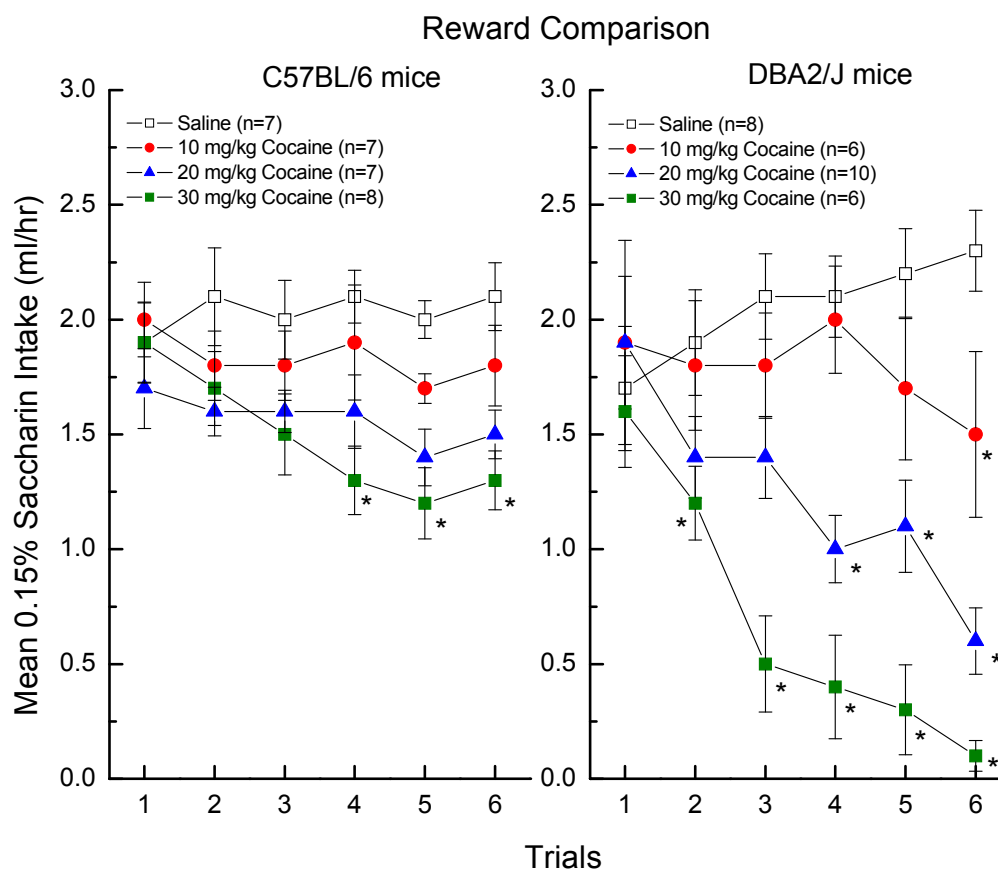


Figure 4.1.1: Mean (\pm SEM) intake (ml/1 hr) of 0.15% saccharin following 5 pairings with the intraperitoneal injection of saline, 10 mg/kg, 20 mg/kg, or 30 mg/kg cocaine in C57BL/6 and DBA/2J mice. A final CS only test was conducted on the 6th trial.

Support for this observation was provided by post hoc analysis of a significant treatment x drug x trials interaction, $F(15,255)=2.21$, $p<.01$. Specifically, while cocaine was effective in reducing CS intake in DBA/2J mice at 10 mg/kg (Trial 6, $p<.02$), 20 mg/kg (Trials 4-6, $ps<.0001$), and 30 mg/kg (Trials 2-6, $ps<.0001$), C57BL/6 mice only suppressed intake of saccharin when paired with the 30 mg/kg dose (Trials 4-6, $p<.05$). In addition, there was a significant main effect of strain, $F(1,51)=6.25$, $p<.02$, and drug, $F(3,51)=13.03$, $p<.0001$. While saline and 10 mg/kg cocaine were not significantly different between the strains ($ps>.05$), suppression was greater in

DBA/2J mice compared with C57BL/6 mice with the 20 mg/kg (Trials 4 and 6, $p < .02$) and 30 mg/kg cocaine (Trials 3-6, $p < .001$).

Body weight.

Body weight was analyzed using a 2 x 4 x 19 mixed factorial analyses of variances (ANOVAs) varying strain (C57BL/6 or DBA/2J), drug (saline, 10, 20, or 30 mg/kg cocaine), and trials (1-19). Drug exposure did not significantly altered body weight (data not shown). This conclusion was supported by a non-significant 3-way interaction, $F < 1$, and main effect of drug, $F < 1$. The main effect of trials was significant, $F(18,918)=52.42$, $p < .0001$, indicating that body weight increased over successive trials. The main effect of strain also was significant, $F(1,51)=7.38$, $p < .01$, confirming that C57BL/6 mice were significantly heavier than DBA/2J mice throughout the study.

AM water intake.

Morning intake of dH₂O (ml/hr) on the days between conditioning trials (Trials W1-W5) is presented in Figure 4.1.2 (top left and right panels). A 2 x 4 x 5 mixed factorial ANOVA revealed that drug exposure did not significantly alter morning dH₂O intake as indicated by a non-significant strain x drug x trials interaction, $F(12,204)=1.18$, $p = .30$. In addition, the main effect of strain was not statistically significant, $F(1,51)=2.17$, $p = .15$. The main effect of drug, $F(5,51)=2.94$, $p < .05$, and trials, $F(4,204)=9.05$, $p < .001$, however, were statistically significant. Post hoc test on the main effect of drug revealed that, overall, morning water intake was dose-dependently reduced between conditioning trials, $p < .05$. As evidenced by the figure, this effect was carried by the DBA/2J mice suggesting a degree of non-specific suppression in this strain.

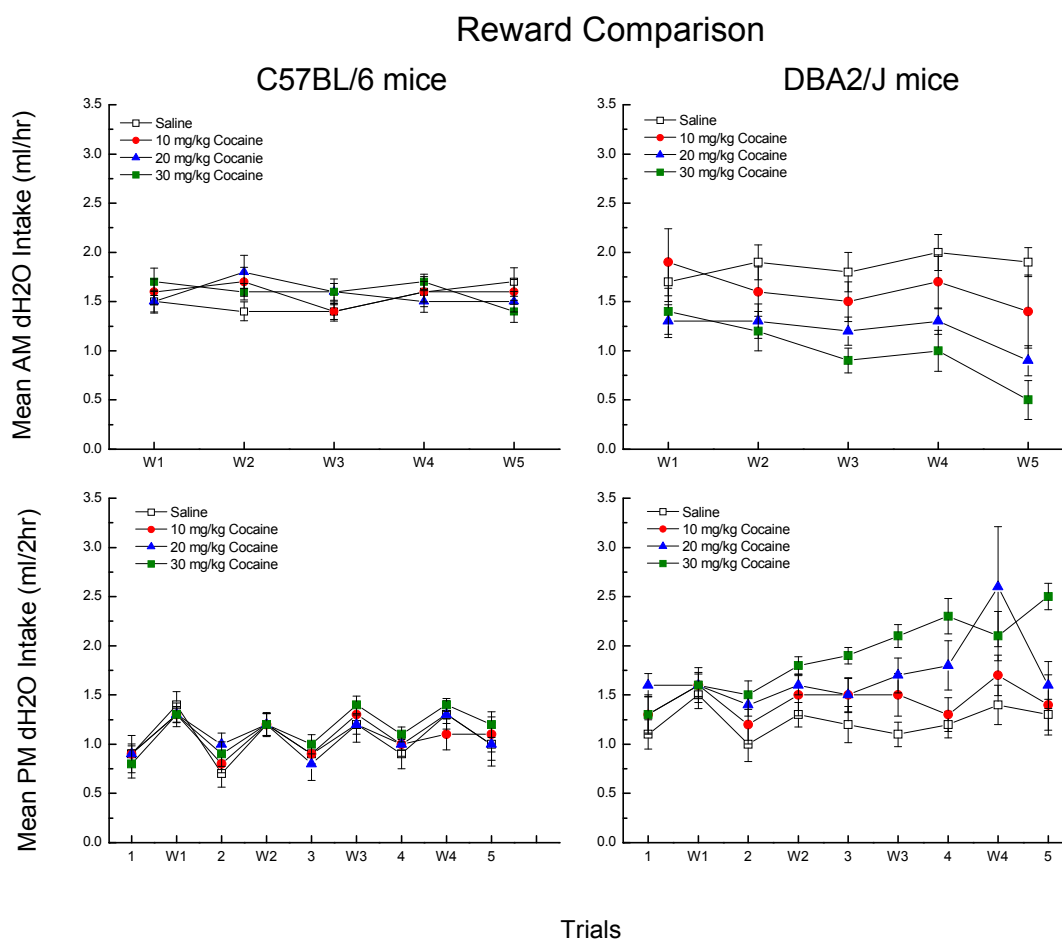


Figure 4.1.2: Mean (\pm SEM) intake of dH₂O in the morning (ml/1 hr; top panels) and afternoon (ml/2 hr; bottom panels) in C57BL/6 and DBA/2J mice as a function of drug group (saline, 10 mg/kg, 20 mg/kg, or 30 mg/kg cocaine) and trials. * denotes significant difference from saline controls.

PM water intake.

Intake of dH₂O for the 2 hour access period in the afternoon for all trials is presented in Figure 4.1.2 (bottom left and right panels). Although a 2 x 4 x 9 mixed factorial ANOVA did not reveal a significant 3-way interaction, $F(24,408)=.95$, $p=.53$, the main effect of strain, $F(1,51)=36.82$, $p<.001$, drug, $F(3,51)=4.25$, $p<.01$, and trial, $F(8,408)=10.08$, $p<.001$, did attain

statistical significance. The significant main effect of strain shows that the DBA/2J mice drank more water during the afternoon session than did the C57BL/6 mice overall. Further, significant main effect of drug suggests that afternoon water intake is increased to compensate for lower intake in the drug-treated groups. Again, this effect appears to be carried largely by the behavior of the DBA/2J mice.

Two-bottle intake test.

The results of the two-bottle intake test for saccharin against dH₂O in C57BL/6 and DBA/2J mice are presented in Figure 4.1.3 Preference for saccharin was analyzed using t-tests with alpha = .05 for each cell. C57BL/6 mice significantly preferred saccharin over dH₂O ($p < .05$) with the exception of the 30 mg/kg cocaine group ($p > .05$). In contrast, DBA/2J mice did not demonstrate a significant preference for saccharin in the saline and 10 mg/kg cocaine groups ($p > .05$). In addition, DBA/2J mice preferred dH₂O over saccharin in the 20 mg/kg and 30 mg/kg cocaine groups ($p < .05$). The advantage of the two-bottle test over the one-bottle test is that the two-bottle test provides a ready water alternative, thereby reducing the impact of need state on the data. Regardless, and contrary to our original hypothesis, the results here show, unequivocally, that the “more reward-preferring” C57BL/6 mice exhibited less avoidance of a cocaine-associated saccharin cue than did the “less reward-sensitive” DBA/2J mice.

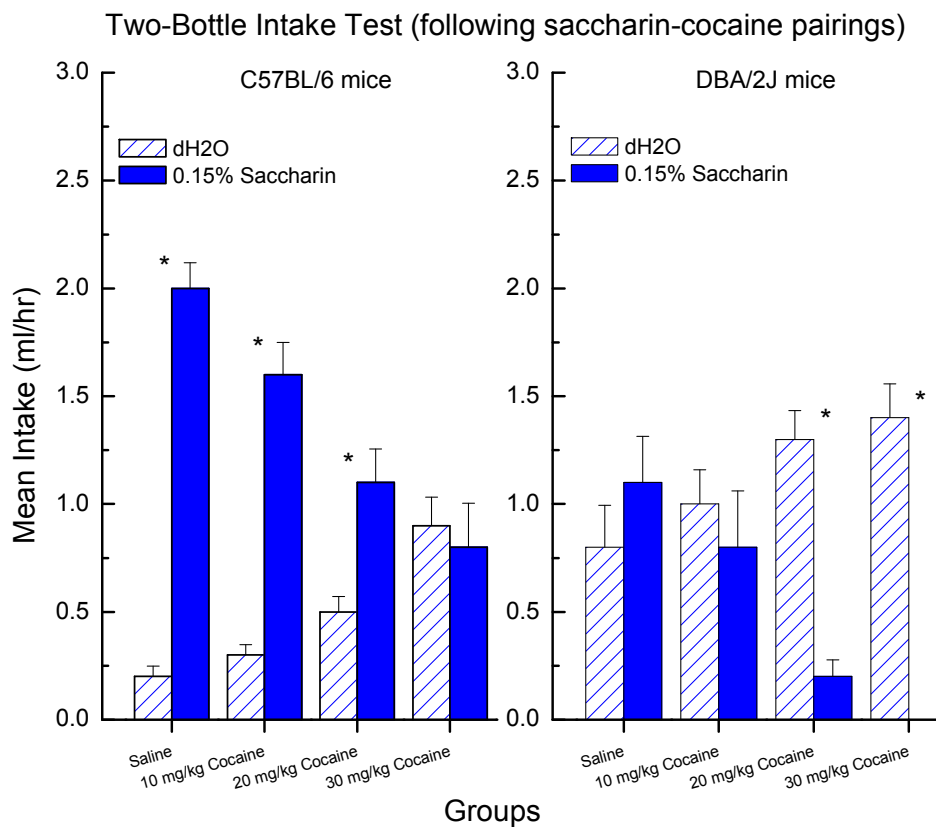


Figure 4.1.3: Mean (\pm SEM) intake (ml/1 hr) in a two-bottle test of 0.15% saccharin against dH₂O following saccharin-cocaine pairings in C57BL/6 and DBA/2J mice. * indicates significant difference between dH₂O and saccharin ($p < .05$).

Experiment 4.2

The results in Experiment 4.1 demonstrate that, compared with DBA/2J mice, C57BL/6 mice demonstrate attenuated suppression of intake of a saccharin CS paired with cocaine. However, it is possible that this attenuated effect may be independent of reward preference and may reflect differences in impulsivity between the strains. That is, if C57BL/6 mice are more impulsive than DBA/2J mice, the observed attenuated suppression may be a result of the inability

of C57BL/6 mice to suppress intake regardless of reward preference. Experiment 4.2 was designed to evaluate impulsivity in C57BL/6 and DBA/2J mice.

Methods

Subjects.

The subjects were 15 male C57BL/6 mice and 15 male DBA/2J mice obtained from Jackson Laboratories. Mice weighed between 21.0 and 28.2 grams at the beginning of the experiment. They were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 1 hour into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604.

Apparatus.

The mice were trained in one of four identical modular operant chambers (MED Associates, Inc., St. Albans, VT), measuring 30.5 x 24.0 x 29.0 cm (length x width x height). All chambers had clear Plexiglas top, front, and back walls, while the side walls were made of aluminum. The grid floors consisted of nineteen 4.8-mm stainless steel rods spaced 1.6-cm apart (center to center). Each chamber was equipped with three retractable sipper tubes that could enter the chamber through 1.3-cm diameter holes spaced 16.4-cm apart (center to center). A lickometer circuit was used to monitor licking. A shaded bulb, which reflected light off the ceiling, was located to the right of the cage. Each chamber was housed in a light and sound attenuated cubicle that was fitted with a ventilation fan and a white noise source that provided a

background noise level of 75 dB, below levels (110 dB) shown to be insufficient to elevate CORT levels in C57BL/6 mice (Spehner et al., 1996; de Wazieres et al., 2000). Control of events in the chamber and collection of the data were carried out by computer. Programs were written in the Medstate notation language (MED Associates, Inc.).

Procedure.

All subjects were weighed once a day throughout the study a half hour before experimental manipulations. For baseline measurements, all mice were placed on a water deprivation schedule which consisted of access to dH₂O for 1 hour in the morning and 2 hours in the afternoon. Baseline intake and body weight were recorded for 1 week. *Training.* Each subject was taken from his home cage and placed in the modular chamber. Immediately after starting the program, a bottle containing 0.15% saccharin advanced and a corresponding cue light was turned on. Following completion of a 3-lick requirement (FR3), the saccharin bottle retracted and both the saccharin bottle and another bottle containing dH₂O advanced. The position of the saccharin and dH₂O bottles was counter-balanced across and within groups. During simultaneous presentation of the bottles, each subject could choose to lick either the 0.15% saccharin bottle or the dH₂O bottle. Choice of a particular bottle was defined by completion of an FR20 requirement on that bottle. Once a choice was established, the other bottle would retract and the subject was allowed an additional 5 seconds access to the chosen bottle. The chosen bottle then retracted and, following a 60 second intertrial interval, the procedure repeated for a total of 6 trials/session each day. After each daily session, the subject was removed from the box and placed back in the home cage. All mice received 2 hour access to dH₂O in the home cage each day 1 hour after training. All mice received 5 days of training (Days 1-5). *Impulsivity Testing.* Impulsivity testing was identical to training sessions with the exception of increasing delays in the advancement of the

saccharin bottle during the choice phase of each trial. Immediately upon completion of the FR3 requirement on the saccharin bottle, both bottles retracted and the dH₂O bottle immediately advanced into chamber. Advancement of the 0.15% saccharin bottle was delayed depending on the interval indicated by the test day and each delay was tested for 3 days. Consequently, advancement of the 0.15% saccharin bottle was delayed for 2 seconds on days 6-8, 4 seconds on days 9-11, 6 seconds on days 12-14, 8 seconds on days 15-17, and 12 seconds on days 18-20. As such, mice had a choice between an immediately available, less preferred water reward and a more preferred, but increasingly delayed, saccharin reward.

Results

Intake.

The results of impulsivity testing in C57BL/6 and DBA/2J mice are presented in Figure 4.2. Intake of 0.15% saccharin and dH₂O was analyzed using a 2 x 2 x 20 repeated measures ANOVA varying strain (C57BL/6 or DBA/2J), solution (saccharin or dH₂O), and trials (1-20). Newman-Keuls post hoc analysis of a significant strain x solution x trials interaction, $F(19,1064)=5.30$, $p<0.0001$, indicated that, in the C57BL/6 mice, intake of 0.15% saccharin was significantly greater than dH₂O intake for days 1-13, $ps<0.0001$, when the delay was 6 sec or less. There was no significant difference between saccharin and dH₂O intake for days 14-16, $ps>0.09$, when the delay was between 6 and 8 sec, and dH₂O intake was significantly greater than saccharin intake for days 17-20, $ps<0.002$, when the delay was increased from 8 to 12 sec. In the DBA/2J mice, saccharin intake was significantly greater than dH₂O intake for days 1-5 and 7, $ps<0.04$, when the delay was 2 sec or less. Saccharin and dH₂O intake were not significantly different for days 6, 8-12, $ps>0.34$, when the delay was 6 sec or less, and dH₂O intake was

significantly greater than saccharin intake for days 13-20, $p < 0.001$, when the delay was between 6 and 12 sec. In sum, the C57BL/6 mice were less impulsive than were the DBA/2J mice and were, as such, more willing to wait for access to the sweet. These data provide additional independent evidence that C57BL/6 mice perceive saccharin as more valuable than do DBA/2J mice.

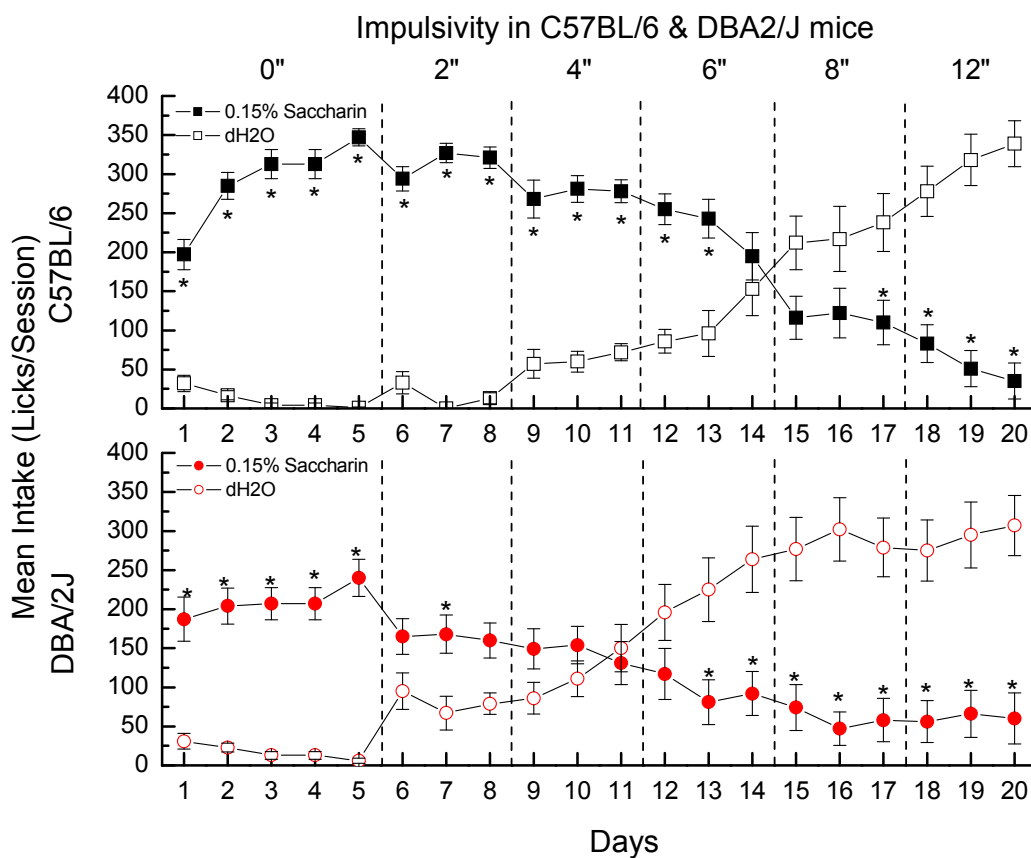


Figure 4.2: Mean (\pm SEM) intake (licks/session) of immediately available dH₂O and increasingly delayed 0.15% saccharin in C57BL/6 and DBA/2J mice. * denotes significant difference between saccharin and dH₂O intake during the same session/day.

Body Weight.

Body weight was analyzed using a 2 x 20 repeated measures analyses of variances (ANOVA) varying strain (C57BL/6 or DBA/2J) and trials (1-20). Body weight over trials (data not shown) did not significantly differ between strains as indicated by a non-significant 2-way interaction, $F(19, 513)=0.71$, $p=0.81$. However, the main effects of trial, $F(19, 513)=42.74$, $p<.0001$, and strain, $F(1, 27)=11.24$, $p=.002$, were significant confirming that body weight increased over successive trials and that C57BL/6 mice were significantly heavier than DBA/2J mice throughout the study.

Discussion

Previous data have demonstrated that rats and mice suppress intake of a CS paired with a drug of abuse. Although the majority of these data have been conducted with rats, mice have been shown to suppress intake in a similar dose-dependent manner. DBA/2J mice will suppress intake of a rewarding saccharin or neutral NaCl CS when paired with morphine, cocaine, or ethanol (Risinger and Boyce, 2002). Similarly, C57BL/6 and DBA/2J mice from Experiment 4.1 suppressed intake of a saccharin CS that had been paired with cocaine. The rat data in Experiment 2.1 demonstrate that suppression of intake of a saccharin CS paired with cocaine is greatly facilitated in a reward-preferring rat strain (i.e., Lewis rats) when compared with a relatively reward-insensitive strain (i.e., Fischer 344 rats). However, data in Experiment 4.1 demonstrated an inverse relationship in mice. That is, reward-preferring C57BL/6 mice demonstrated attenuated suppression compared with DBA/2J mice. It is clear that, although mice and rats similarly suppress intake of a CS paired with a drug of abuse, a relative increase in sensitivity to rewards is differentially expressed across species.

One possibility that can be reasonably excluded for the attenuated suppression observed in reward preferring mice is an increase in impulsivity. For example, if C57BL/6 mice were more impulsive than DBA/2J mice, the observed attenuated suppression following taste-drug pairings could be accounted for by the inability of the C57BL/6 mice to suppress intake of the taste cue regardless of the preference for the drug reward. The data in Experiment 4.2, as well as the impulsivity literature, argue against this account. In our experiment, C57BL/6 mice were found to be less impulsive compared with DBA/2J mice and C57BL/6 mice were able to forego intake of an immediately available, less preferred water reward in favor of a delayed, more preferred saccharin reward significantly longer than DBA/2J mice. This finding supports the conclusion that the ability of C57BL/6 mice, compared with DBA/2J, to suppress intake of a saccharin CS in Experiment 4.1 was not impeded by impulsivity. Indeed, to the contrary, thirsty C57BL/6 mice were willing to give up an immediately available water to wait relatively long periods of time for access to the preferred saccharin reward.

While differences in impulsivity likely do not account for the attenuated suppression observed in Experiment 4.1, there are a myriad of differences, whether genetic, physiological, or behavioral, between these inbred mouse strains with the potential to confound either the expression or our interpretation of the interaction between drug-induced suppression and reward preference in these strains. A selective increase in reward preference within a single mouse strain would eliminate these concerns as experimental and control groups would be identical with the exception of the manipulated variable, reward preference. To this end, the experiments in Chapter 5 were designed to evaluate drug-induced suppression in NSE-tTA x TetOp- Δ FosB mice, an inducible transgenic mouse strain that can selectively increase preference for rewards.

Chapter 5

Over expression of Δ FosB is associated with attenuated cocaine-induced suppression of saccharin intake in mice.

The data in Experiment 4.1 demonstrate that C57BL/6 mice, compared with DBA/2J mice, exhibit attenuated suppression of CS intake following pairing with a drug of abuse. This finding appears to run contrary to the data observed in Experiments 2.1 and 3.1 in which reward-preferring Lewis rats demonstrate greater suppression of intake compared with the relatively reward-insensitive Fischer 344 rats and it is clear that species differences exist regarding the effect of relative reward preference on drug-induced suppression of a gustatory CS. The aim of Chapter 5 is to focus on the evaluation of drug-induced suppression in a single mouse strain: transgenic NSE-tTA x TetOp- Δ FosB mice. NSE-tTA x TetOp- Δ FosB mice can be induced to selectively over express the nuclear transcription factor Δ FosB in the striatum (Chen et al., 1998) and such elevations of Δ FosB have been shown to increase the perceived rewarding value of drugs of abuse and natural rewards (Nestler et al., 1999; Nestler et al., 2001; Olausson et al., 2006). It has also been demonstrated that Δ FosB is elevated in the NAc of Lewis rats compared with Fischer 344 rats (Haile et al., 2001) and in the dorsal striatum of C57BL/6 mice compared with A/J mice (Brodkin et al., 1998), suggesting a possible molecular mediator for the increased reward preference in these strains. Use of NSE-tTA x TetOp- Δ FosB mice will allow for a more selective evaluation of the effect of changes in reward preference on drug-induced suppression of CS intake and control for any possible confounds that may have arisen in previous experiments due to strain differences other than reward preference.

Δ FosB is a member of the Fos family of transcription factors and has received a great deal of attention as a possible molecular switch for the long-term neuronal plasticity observed in

drug addiction (Nestler et al., 1999; Nestler et al., 2001; McClung et al., 2004). Δ FosB can homodimerize (Jorissen et al., 2007) or heterodimerize with JunD (and to a lesser extent JunB) (Hiroi et al., 1998; Perez-Otano et al., 1998) to form activator protein-1 (AP-1) complexes (Curran and Franza, 1988; Chen et al., 1995; Nestler et al., 2001). AP-1, then, binds at the AP-1 consensus site (TGAC/GTCA) to promote or inhibit transcription of various genes including, but not limited to, dynorphin, the AMPA glutamate receptor subunit GluR2, cyclin-dependent kinase 5 (cdk-5), and nuclear factor kappa B (NF- κ B) (Dobrazanski et al., 1991; Nakabeppu and Nathans, 1991; Yen et al., 1991; Chen et al., 1997). In the nucleus accumbens, elevation of Δ FosB inhibits transcription of dynorphin (McClung et al., 2004), but see (Andersson et al., 2003), and promotes transcription of GluR2 (Kelz and Nestler, 2000), cdk-5 (McClung and Nestler, 2003), and NF- κ B (Ang et al., 2001). Manipulation of many of these genes (and/or their products) has been found to influence sensitivity to drugs of abuse. For example, over expression of GluR2 using viral-mediated gene transfer in rats or blockade of dynorphin by the κ -receptor antagonist nor-BNI in mice increases the rewarding effects of cocaine and morphine, respectively (Kelz et al., 1999; Zachariou et al., 2006).

A number of factors can elevate Δ FosB in the brain and the elevation can be region specific. Chronic stress, antipsychotic drugs, and drugs of abuse all elevate Δ FosB in the dorsal (caudate/putamen) and ventral striatum (Atkins et al., 1999; Perrotti et al., 2004; Perrotti et al., 2008). In the ventral striatum (i.e., nucleus accumbens), however, each of these factors differentially elevates Δ FosB in specific cell types. For example, chronic stress elevates Δ FosB in the dynorphin+/substance P+ and enkephalin+ subsets of medium spiny dopamine neurons in the ventral striatum (Perrotti et al., 2004). Antipsychotic drugs elevate Δ FosB in the enkephalin+ dopamine neurons in the ventral striatum (Hiroi and Graybiel, 1996; Atkins et al., 1999) and drugs of abuse elevate Δ FosB in the dynorphin+/substance P+ dopamine neurons in the ventral striatum (Nye et al., 1995; Moratalla et al., 1996; Perrotti et al., 2008). Although increase of

Δ FosB varies with drug, dose, and regimen, cocaine self-administration has been shown to increase the number of Δ FosB+ cells up to 150,000 in the NAc shell and core (Perrotti et al., 2008). It is this latter pattern of Δ FosB expression in the dorsal striatum and in the dynorphin+/substance P+ dopamine neurons in the nucleus accumbens that we will refer to as “striatal” expression in this dissertation (unless otherwise noted), as it is this pattern of expression that is most relevant to natural rewards, drugs of abuse, and addiction (Werme et al., 2002; Colby et al., 2003; McClung et al., 2004; Olausson et al., 2006) and it is this pattern of expression found in the transgenic mice used in the studies in the current chapter (Kelz et al., 1999). In addition, elevation of Δ FosB in transgenic mice is significantly above that observed with physiological induction by drugs of abuse as the NSE-tTA x TetOp system has been shown to increase activity of the target gene to 700,000/mg of protein (Chen et al., 1998).

Interestingly, the elevation of Δ FosB by drugs of abuse requires chronic, rather than acute, exposure (Nye et al., 1995; Nye and Nestler, 1996; McClung et al., 2004). Thus, while acute exposure to drugs rapidly increases many Fos family proteins in the striatum, such as cFos and FosB (Sheng and Greenberg, 1990; Hope et al., 1992; Persico et al., 1993; Daunais and McGinty, 1994), there is only a very small increase in Δ FosB (Nestler et al., 1999; Nestler, 2001b). However, once generated, Δ FosB is relatively stable (Ulery et al., 2006) and has an in vivo half-life of greater than 1 week compared with 10-12 hours for other Fos proteins (Chen et al., 1997). This stability allows for the slow accumulation of Δ FosB with chronic exposure to drug. Other Fos proteins, in comparison, demonstrate a desensitized response over time (Hope et al., 1992; Hope et al., 1994; Nye et al., 1995; Moratalla et al., 1996). Chronic drug exposure, then, allows Δ FosB to reach levels where it can affect gene expression and become behaviorally relevant.

Importantly, there is a growing body of literature demonstrating that elevation of Δ FosB increases the perceived reward value of drugs of abuse. For example, preference for drug-

associated locations, modeled by conditioned place preference, is increased in mice with elevated Δ FosB in the striatum (Kelz et al., 1999). Acquisition and maintenance of drug taking behavior, as well as motivation to get drug, are similarly increased in mice with elevated Δ FosB (Colby et al., 2003). While progress has been made in understanding the effects of Δ FosB in numerous aspects of drug addiction, one area that has not been investigated is the effect of Δ FosB on drug-induced devaluation of natural rewards. According to the reward comparison hypothesis, any condition/circumstance that augments the perceived value of the drug reward should augment avoidance of the lesser saccharin cue. Indeed, the results of Experiment 2.1 demonstrated that drug sensitive Lewis rats exhibited greater avoidance of the saccharin cue following saccharin-cocaine pairings than the less sensitive Fischer rats. In addition, Sprague-Dawley rats also exhibit greater avoidance of a taste cue paired with cocaine or sucrose following a history of chronic morphine treatment (Grigson et al., 2001). However, the data from Experiment 4.1 demonstrated that reward preferring C57BL/6 mice exhibit less avoidance of a saccharin cue paired with cocaine compared with DBA/2J mice. Therefore, Experiment 5.1 will more selectively examine the effect of an increase in perceived reward value via over expression of Δ FosB in drug-induced suppression of CS intake by evaluating cocaine-induced suppression of intake of a saccharin cue in mice that over express Δ FosB in the striatum. The results of the experiments in Chapter 5 have been published (Freet et al., 2009).

Experiment 5.1

Previous studies have demonstrated that mice suppress intake of a taste cue when paired with a drug of abuse in a manner similar to that seen in rats (Grigson & Schroy, unpublished findings, Risinger and Boyce, 2002). Much like the rat data, these studies utilized restricted access to water and used a preferred 0.15% saccharin solution as the CS (Bachmanov et al., 2001;

Tordoff and Bachmanov, 2003). In these experiments, intake of a saccharin cue was suppressed when access to saccharin was followed by the injection of 10 mg/kg (in DBA/2 mice) or 20 mg/kg (in DBA/2 and C57BL/6 mice) cocaine (Grigson & Schroy, unpublished findings, Risinger and Boyce, 2002). Therefore, Experiment 5.1 evaluated the suppression of intake of a 0.15% saccharin cue when paired with saline, 10 mg/kg, or 20 mg/kg cocaine in water deprived NSE-tTA x TetOp- Δ FosB Line A mice. These adult transgenic mice (SJL x C57BL/6 background) demonstrate selective over expression of Δ FosB in the striatum upon removal of doxycycline from the water (Chen et al., 1998). Based on the data obtained in rats, it was hypothesized that elevation of Δ FosB in these mice would augment the rewarding effects of the drug and, thereby, facilitate drug-induced suppression of intake of the saccharin cue relative to Δ FosB normal controls.

Methods

Subjects.

The subjects were 60 naïve male NSE-tTA x TetOp- Δ FosB Line A bitransgenic mice. The mice were generated by the animal facility at the University of Texas Southwestern Medical Center in Dallas, Texas and maintained on 100 μ g doxycycline/ml in the drinking water. This approach maintains full repression of transgenic Δ FosB expression and thereby allows for normal development (as described in Chen et al., 1998). The mice were then shipped to the animal facility at the Pennsylvania State University College of Medicine in Hershey, Pennsylvania and quarantined for 2 months (all mice were maintained on doxycycline during transport and during quarantine). Upon release from quarantine, half of the mice (n=30) had doxycycline removed and Δ FosB over expression was allowed to proceed for 8 weeks prior to testing, the time required for

maximal Δ FosB action (McClung and Nestler, 2003). The rest of the mice (n=30) remained on doxycycline for the duration of the studies. Mice weighed between 31.2 and 45.0 grams at the beginning of the experiment and were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 2 hours (9 a.m.) and 7 hours (2 p.m.) into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water, except where otherwise noted.

Apparatus.

All experimental manipulations were conducted in the home cages and water and saccharin was provided using modified Mohr graduated pipettes as described above.

Procedure.

All subjects were weighed once a day throughout the study. Following release from quarantine, and as described, the Δ FosB over expression groups (n=30) were taken off of 100 μ g/ml doxycycline. These mice received unadulterated dH₂O for the remainder of the study while the other half of the mice (n=30), the Δ FosB normal groups, continued on doxycycline. After eight weeks of Δ FosB over expression, baseline water intake was evaluated. For baseline measurements, all mice were placed on a water-deprivation schedule which provided access to dH₂O (with or without doxycycline depending on the treatment group) for 1 hour beginning at 9:00 in the morning and for 2 hours beginning at 2:00 in the afternoon. Baseline intake and body weight were recorded for 1 week. During testing, all mice received 1 hour access to 0.15% saccharin in the morning followed immediately by an i.p. injection of either saline (n=10/cell), 10

mg/kg cocaine (n=10/cell), or 20 mg/kg cocaine (n=10/cell). Taste-drug pairings occurred every 48 hours for 5 trials. To maintain hydration, all subjects received 2 hours access to dH₂O or 100 µg/ml doxycycline each afternoon and 1 hour access to dH₂O or 100 µg/ml doxycycline each morning between conditioning trials, as specified by the group assignment. Saccharin was obtained from the Sigma Chemical Company, St. Louis, MO, and cocaine HCl was generously provided by the National Institute on Drug Abuse. The saccharin solution was presented at room temperature.

Results

CS Intake.

Intake and body weight were analyzed using 2 x 3 x 5 mixed factorial analyses of variances (ANOVAs) varying treatment (normal vs. over expression of Δ FosB), drug (saline, 10, or 20 mg/kg cocaine), and trials (1-5). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with alpha = .05. Observation of Figure 5.1.1 shows that over expression of Δ FosB in the striatum is associated with a reduction, rather than an augmentation, of cocaine-induced suppression of intake of the saccharin cue. Support for this observation was provided by post hoc analysis of a significant treatment x drug x trials interaction,

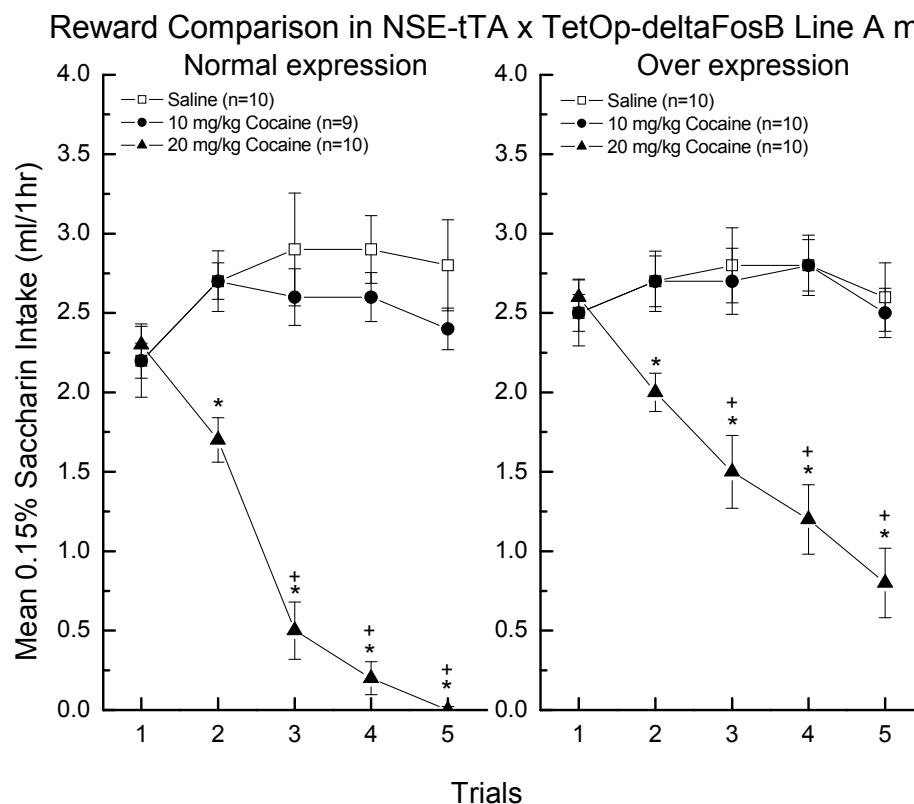


Figure 5.1.1: Mean (\pm SEM) intake (ml/1 hr) of 0.15% saccharin following 5 pairings with the intraperitoneal injection of saline, 10 mg/kg cocaine, or 20 mg/kg cocaine in NSE-tTA x TetOp- Δ FosB Line A mice with normal (left panel) or elevated (right panel) levels of Δ FosB in the striatum. * denotes significant difference from saline controls. + denotes significant difference across treatment groups within the same drug group.

$F(8,212)=2.08$, $p<.04$. Specifically, the results of post hoc Newman-Keuls tests showed that, while the 10 mg/kg dose of cocaine was ineffective in reducing CS intake in both treatment groups ($p>.05$), the 20 mg/kg dose was less effective in mice with elevated expression of Δ FosB (see Figure 5.1.1, right panel). Thus, while it is the case that treatment with the 20 mg/kg dose of cocaine significantly reduced intake of the saccharin cue relative to each group's saline treated controls on trials 2 – 5, $ps < .05$, mice with elevated expression of Δ FosB consumed significantly more of the saccharin cue that was paired with the 20 mg/kg cocaine than did the normal expressing controls. These group differences were significant on trials 3 – 5, $ps<.05$.

Body weight.

Neither over expression of Δ FosB in the striatum nor drug exposure significantly altered body weight (data not shown). This conclusion was supported by a non-significant main effect of treatment, $F < 1$, or drug, $F(2,53)=1.07$, $p=.35$. The main effect of trials was significant, $(5,265)=10.54$, $p<.0001$, indicating that body weight changed over successive trials. Finally, although the $2 \times 3 \times 6$ repeated measures ANOVA revealed a significant treatment \times drug \times trials interaction, $F(10,265)=4.35$, $p<.01$, the results of the post hoc tests were unremarkable.

AM water intake.

Morning intake of dH_2O (ml/h) on the days between conditioning trials (baseline, Trials W1-W4) is presented in Figure 5.1.2 (top left and right panels).

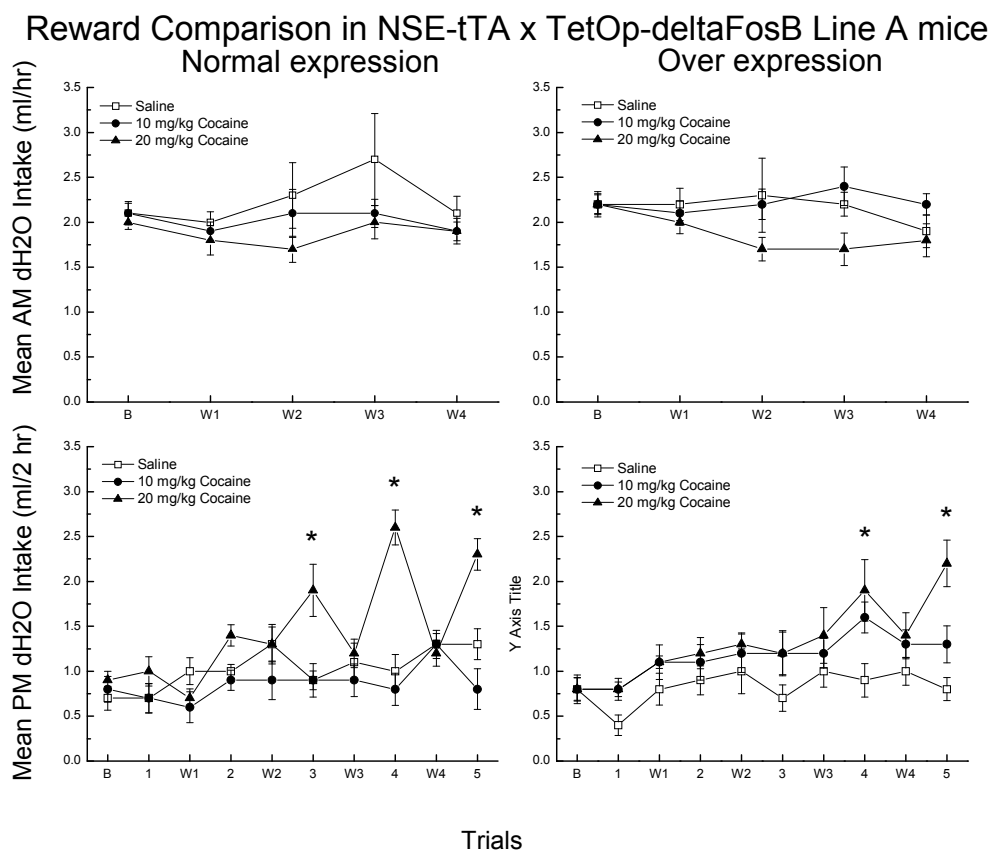


Figure 5.1.2: Mean (\pm SEM) intake of dH₂O in the morning (ml/1 hr; top panels) and afternoon (ml/2 hr; bottom panels) in NSE-tTA x TetOp- Δ FosB Line A mice with normal (left panels) or elevated (right panels) levels of Δ FosB in the striatum as a function of drug group (saline, 10 mg/kg, or 20 mg/kg cocaine) and trials. * denotes significant difference from saline controls.

A 2 x 3 x 5 mixed factorial ANOVA revealed that neither over expression of Δ FosB in the striatum nor drug exposure significantly altered morning dH₂O intake as indicated by a non-significant treatment x drug x trials interaction, $F < 1$ (see Figure 5.1.2, top panels). In addition, neither the main effect of treatment, $F < 1$, drug, $F(2,53)=2.55$, $p=.09$, nor the treatment x drug interaction, $F(8,212)=1.57$, $p=.14$, was statistically significant.

PM water intake.

Intake of dH₂O for the 2 hour access period in the afternoon for all trials is presented in Figure 5.1.2 (bottom panels).

The main effect of treatment was not significant, $F < 1$, suggesting that over expression of Δ FosB did not affect PM dH₂O intake overall. The main effect of drug, on the other hand, did attain statistical significance, $F(2,53)=7.95$, $p < .001$, as did the treatment x drug x trials interaction, $F(18,477)=2.12$, $p < .005$. Post hoc tests of this 3-way ANOVA revealed that PM dH₂O intake in the 10 mg/kg cocaine groups did not significantly differ from that of the saline controls ($p > .05$). However, PM dH₂O intake was significantly increased in the 20 mg/kg groups compared to their saline controls and this effect was significant on conditioning trials where mice had avoided intake of the saccharin cue in the morning (i.e., Trials 3, 4, and 5 in mice with normal Δ FosB and Trials 4 and 5 in mice with elevated Δ FosB, $p < .05$).

Experiment 5.2

Mice with elevated expression of Δ FosB exhibited lesser, rather than greater, avoidance of a saccharin cue following repeated saccharin-cocaine pairings. There are a number of possible explanations for these data. The most obvious, given the literature, is that this paradigm is sensitive to aversive, rather than rewarding, drug properties (Nachman et al., 1970; Riley and Tuck, 1985). Elevated Δ FosB, then, may not only increase responsiveness to rewarding drug properties, but may decrease responsiveness to aversive drug properties as well. Were this the case, then mice with elevated Δ FosB also may be expected to demonstrate smaller LiCl-induced CTAs than mice with normal expression of Δ FosB. To test this hypothesis, the same mice were run in a standard conditioned taste aversion paradigm in which they received 1 hour access to a

novel 0.1 M NaCl solution and, immediately thereafter, were injected intraperitoneally (ip) with saline, 0.018 M LiCl, or 0.036 M LiCl.

Methods

Subjects.

The subjects were 58 (n=29 over expressed Δ FosB, n=29 normal Δ FosB) male NSE-tTA x TetOp- Δ FosB Line A mice used in Experiment 5.1. Mice were counterbalanced to evenly distribute prior saccharin-saline or saccharin-cocaine experience among the groups. At the time of testing, mice in the experimental group had over expression of Δ FosB in the striatum for approximately 17 weeks and all mice weighed between 31.7 and 50.2 grams at the beginning of the experiment. They were housed individually and maintained as described above.

Apparatus.

The apparatus was the same as that described in Experiment 5.1.

Procedure.

All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on the water-deprivation schedule described above (1 h a.m., 2 h p.m.), with or without doxycycline as per group assignment. Baseline intake and body weight were recorded for 1 week. During testing, all mice received 1 hour access to 0.1 M NaCl in the morning followed immediately by an i.p. injection of either saline (n=9/cell), 0.018 M LiCl (n=10/cell), or

0.036 M LiCl (n=10/cell). In rats, the suppressive effect of a 0.009 M dose of LiCl has been matched to that of a 10 mg/kg dose of cocaine (Grigson, 1997). However, given the prior experience of the mice in Experiment 1, and evidence showing that such prior experience can retard the development and/or expression of a subsequent CS-US association (Twining et al., 2005), we used slightly higher doses of LiCl (0.018 M and 0.036 M). Taste-drug pairings occurred every 48 hours for 5 trials. All subjects received 2 hours access to dH₂O or 100 µg/ml doxycycline each afternoon and 1 hour access to dH₂O or 100 µg/ml doxycycline each morning between conditioning trials. NaCl was obtained from Fisher Chemical, Pittsburgh, PA; LiCl was obtained from the Sigma Chemical Company, St. Louis, MO. The NaCl solution was presented at room temperature.

Results

CS Intake.

Intake was analyzed using a 2 x 3 x 5 mixed factorial ANOVA varying treatment (normal vs. over expression of Δ FosB), drug (saline, 0.018 M, or 0.036 M LiCl), and trials (1-5). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with alpha = .05. The effect of the over expression of Δ FosB on LiCl CTA learning is shown in Figure 5.2., where all mice, regardless of Δ FosB expression, avoided intake of the NaCl CS that

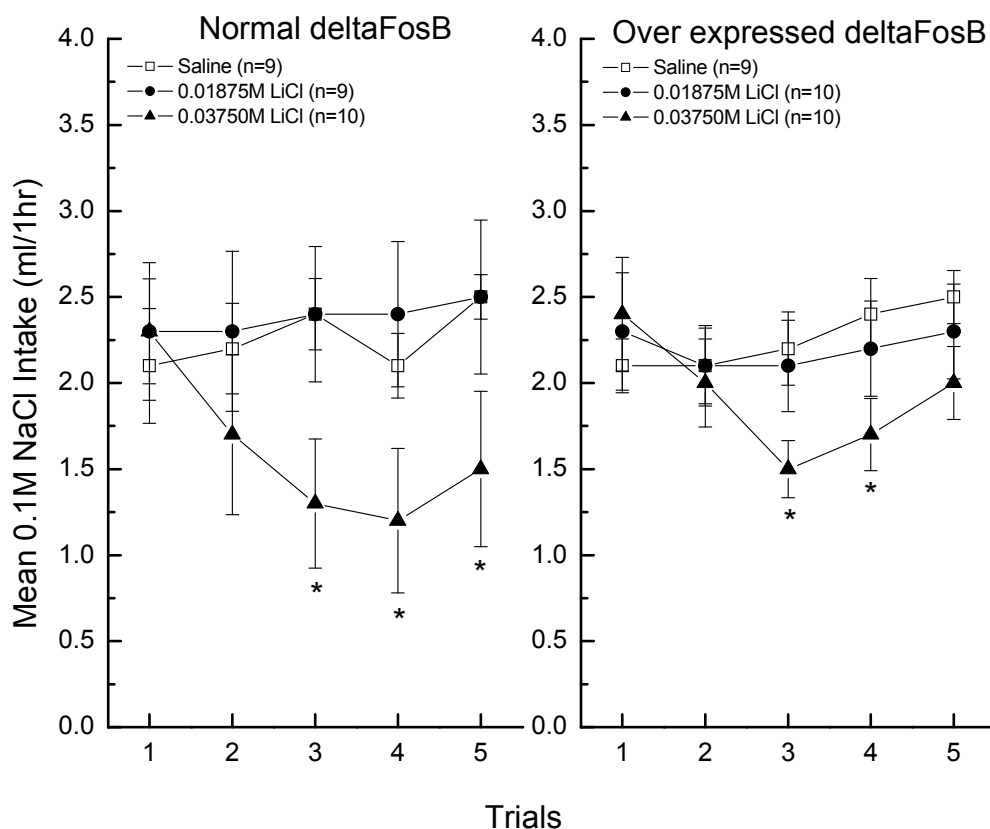


Figure 5.2: Mean (\pm SEM) intake (ml/1 hr) of 0.1 M NaCl following 5 pairings with the intraperitoneal injection of saline, 0.018 M LiCl, or 0.036 M LiCl in NSE-tTA x TetOp- Δ FosB Line A mice with normal (left panel) or elevated (right panel) levels of Δ FosB in the striatum.

had been paired with the illness-inducing agent LiCl relative to the saline treated subjects. Unlike the cocaine data described above, the 3-way ANOVA did not approach statistical significance, $F < 1$. In addition, there was no significant effect of treatment (i.e., doxy or water), $F < 1$, treatment x trial interaction, $F < 1$, or treatment x drug interaction, $F < 1$. Even so, observation of the data shown in Figure 5.2 suggests that the suppressive effect of LiCl, like that of cocaine, may have been smaller in the over expressing Δ FosB mice. Thus, we reanalyzed the treatment groups separately using 3 x 5 mixed factorial ANOVAs varying drug and trials. The results of these ANOVAs confirmed a significant drug x trials interaction for both the normal, $F(8,100)=3.48$,

$p < .001$, and the over expressed Δ FosB mice, $F(8,108) = 2.19$, $p < .033$. Post hoc tests showed a significant reduction in CS intake by the higher dose of LiCl on trials 3 – 5 for the normal mice, and on trials 3 and 4, for the over expressing mice, $ps < .05$.

Despite a relatively high sample size, the LiCl data are more variable than were the cocaine data in Experiment 5.1. The variability shown in Figure 5.2 likely relates to the history of saline or cocaine treatment in Experiment 5.1. In an effort to test this hypothesis, we reanalyzed the LiCl CTA data using a $2 \times 2 \times 3 \times 5$ mixed factorial ANOVA varying history (saline vs. cocaine), treatment (normal vs. over expression of Δ FosB), drug (saline, 0.018 M, or 0.036 M LiCl), and trials (1-5). For the sake of simplicity, the cocaine variable in the history factor consisted of data from mice with a history of experience with either the 10 or 20 mg/kg dose of cocaine. Similar to the results of the initial analysis, the 4-way interaction also failed to attain statistical significance, $F(8,180) = 1.34$, $p = .22$. A history of saccharin-saline or saccharin-cocaine pairings, then, likely contributes to variability in the data, but the impact is not uniform, and inclusion of the history factor is not helpful in revealing statistically significant differences in the magnitude of the LiCl-induced CTA between the normal Δ FosB mice and the mice with an over expression of Δ FosB. In sum, LiCl suppresses intake of the NaCl CS and, while there is a tendency for a slightly diminished effect in the over expressing Δ FosB mice, the difference between treatment groups was not statistically significant.

Taken together, the results from Experiments 5.1 and 5.2 show that mice with elevated Δ FosB consume significantly more of a saccharin CS following saccharin-cocaine pairings and tend to consume more of a NaCl CS following pairings with LiCl. The tendency to consume more of the drug-associated CSs (particularly in Experiment 5.1) may be due to an increase in sensitivity to the rewarding properties of the saccharin and/or the salt CS, as elevated levels of Δ FosB are known to be associated with an increase in responsiveness to other natural rewards such as food pellets (Olausson et al., 2006) and wheel running (Werme et al., 2002). The final

experiment will test whether these mice with elevated striatal levels of Δ FosB will respond more to the rewarding properties of a range of concentrations of sucrose and salt in two-bottle intake tests with water.

Body Weight.

Body weight was analyzed using a 2 x 3 x 7 repeated measures analyses of variances (ANOVA) varying treatment (elevated Δ FosB or normal Δ FosB), drug (saline, 0.018M LiCl, 0.037M LiCl) and trials. Although there was a significant 3-way interaction, $F(12, 306)=3.38$, $p<.0001$, this interaction was carried by a significant main effect of trials, $F(6,306)=127.12$, $p<.0001$. There was no significant main effect of either treatment, $F(1, 51)=1.28$, $p=.26$, or drug, $F(2, 51)=1.68$, $p=0.20$, confirming that body weight increased over successive trials but that mice with elevated Δ FosB were not significantly heavier than control mice throughout the study.

Experiment 5.3

Experiment 5.3 was designed to examine the hypothesis that the reduced suppression of CS intake by the over expressing Δ FosB mice in Experiment 5.1 was due to augmentation of the perceived reward value of not only the drug of abuse, but of the natural saccharin reward cue as well. To evaluate this hypothesis, we used the same mice and one- and two-bottle intake tests to examine the effect of over expression of Δ FosB on intake of a rewarding (sucrose) stimulus. In addition, given the tendency for these mice to over consume the NaCl CS following NaCl-LiCl pairings in Experiment 5.2, we also used one- and two-bottle intake tests to examine the effect of elevated Δ FosB on intake of a range of concentrations of the more “neutral” NaCl solutions. Three concentrations of NaCl (0.03 M, 0.1 M, 0.3 M) and sucrose (0.01 M, 0.1 M, 1.0 M) were

examined. It was hypothesized that if elevation of Δ FosB augments the rewarding value of natural rewards, intake of sucrose should be greater in the experimental mice compared with controls.

Methods

Subjects. The subjects were 28 (n=14 over expressed Δ FosB, n=14 normal Δ FosB) male NSE-tTA x TetOp- Δ FosB Line A mice used in Experiment 5.1. At the time of testing, mice in the experimental group had over expression of Δ FosB in the striatum for approximately 25 weeks. In addition, mice had prior experience with saccharin-sucrose pairings in an unsuccessful anticipatory contrast experiment (the parameters that support anticipatory contrast in mice are still under investigation). Mice weighed between 31.5 and 54.5 grams at the beginning of the experiment. They were housed and maintained as previously described.

Apparatus.

The apparatus was the same as that described in Experiment 5.1.

Procedure.

All subjects were weighed once daily. Over a 4-day habituation period, each mouse received one hour access to dH₂O in the morning and 2 hours access to dH₂O (with or without doxycycline) in the afternoon. Throughout the experiment, mice with elevated Δ FosB (n=14) received dH₂O to rehydrate each afternoon, while mice with normal Δ FosB (n=14) received 100 μ g/ml doxycycline in dH₂O. Three concentrations of NaCl (0.03 M, 0.1 M, and 0.3 M) and

sucrose (0.01 M, 0.1 M, and 1.0 M) were used as the tastants. Each concentration was presented to the mice during the AM 1 hour period for three consecutive days. The first two days were one-bottle presentations of the tastant while the third day consisted of a two-bottle presentation of the tastant and dH₂O. The position of the bottles was counterbalanced, left and right, within groups and across two-bottle test sessions. The solutions were presented in an ascending order and intake of NaCl was tested before sucrose. Two dH₂O only trials were conducted between NaCl and sucrose testing. Intake was measured on each day to the nearest 1/10 ml.

Data Analysis.

The data were analyzed using t-tests with $\alpha = .05$.

Results

The data from the two-bottle tests were most informative and, thus, will be presented here (see Figure 5.3). Baseline one-bottle water intake also is shown in the left most bar of each panel as a point of reference.

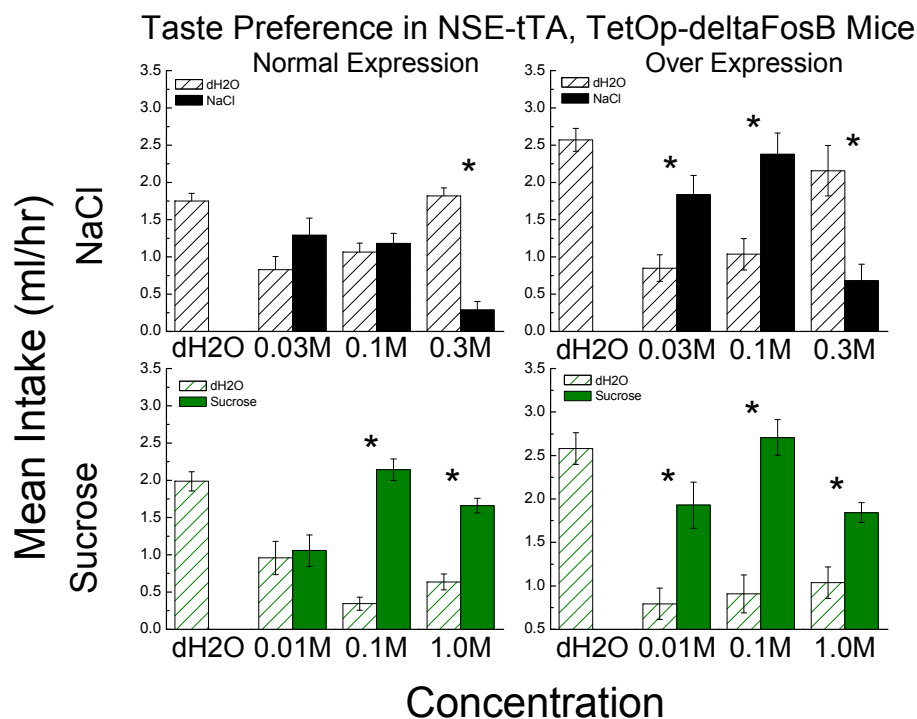


Figure 5.3: Mean (\pm SEM) intake (ml/1 hr) of a range of concentrations of NaCl (top panels) and sucrose (bottom panels) vs. dH₂O in NSE-tTA x TetOp- Δ FosB Line A mice with normal (left panels) or elevated (right panels) levels of Δ FosB in the striatum. * denotes significant difference from dH₂O intake.

NaCl preference.

In mice with normal Δ FosB (top left panel), intake of the two lowest concentrations of NaCl (0.03 M and 0.1 M) did not differ from intake of dH₂O in the two-bottle tests ($p > .05$). The highest concentration of NaCl (0.3 M), however, was significantly less preferred than dH₂O ($p < .0001$), consistent with the aversive nature of this concentration (Bachmanov et al., 2002). In the mice with elevated Δ FosB (top right panel), a similar pattern was evident with the 0.3 M concentration of NaCl ($p < .01$), indicating that elevation of Δ FosB did not significantly alter the response to this aversive stimulus. Compared to rats with normal Δ FosB, however, a different

pattern occurred with the lower concentrations of NaCl. Specifically, rats with elevated expression of Δ FosB demonstrated a preference for the lower 0.03 M and 0.1 M concentrations of NaCl relative to dH₂O in the two-bottle tests (p s < .03). Elevation of Δ FosB, then, may shift the preference for lower concentrations of NaCl from neutral to preferred.

Sucrose preference.

Analyses using t-tests for dependent samples indicated that, in mice with normal Δ FosB, intake of the lowest concentration of sucrose (0.01 M) was not significantly different than dH₂O ($p = .82$), see Figure 5.3, bottom panels. In contrast, the 0.1 M and 1.0 M sucrose concentrations were significantly preferred to dH₂O (p s < .0001). In the mice with elevated Δ FosB, sucrose was significantly preferred to dH₂O across all concentrations tested (p s < .02). This finding is parallel to that obtained for NaCl solutions and provides support for the conclusion that elevation of Δ FosB increases the preference for natural rewards.

Body Weight.

Body weight was analyzed using a 2 x 18 repeated measures analyses of variances (ANOVA) varying treatment (elevated Δ FosB or normal Δ FosB) and trials (1-18). Body weight over trials (data not shown) did not significantly differ between treatment as indicated by a non-significant 2-way interaction, $F(17, 357)=1.53, p=0.08$. The main effect of trials was significant, $F(17, 357)= 2.57, p<.001$, indicating that body weight increased over successive trials. The main effect of treatment, however, was not significant, $F(1, 21)=0.30, p=0.59$, confirming that mice with elevated Δ FosB were not significantly heavier than control mice throughout the study.

Discussion

The data in Chapter 5 demonstrate that elevation of Δ FosB in the striatum is associated with attenuated cocaine-induced suppression of saccharin intake. This finding runs counter to the data obtained in Experiment 2.1 with rats but aligns with the mouse data reported in Experiment 4.1. As stated earlier, subjects in the previous experiments not only possessed elevated Δ FosB and differential preference for rewards, but also the myriad of neuronal adaptations that result from exposure to drugs of abuse, the addiction-prone phenotype (Nestler, 1995; Nestler and Aghajanian, 1997; Nestler, 2001a), and from basic differences between strains. These factors undoubtedly contribute to behavior and present possible confounds when attempting to interpret the role of Δ FosB, per se, and reward preference in general, in drug-induced suppression of CS intake. The experiments in Chapter 5 controlled for these confounds (i.e., all subjects were the same with the exception of elevations in Δ FosB) allowing for a more direct interpretation of the role of Δ FosB and increased reward preference in the phenomenon, at least in mice. As stated above, the current data demonstrate that cocaine-induced suppression of saccharin intake occurred in the presence of elevated striatal Δ FosB, but the effect was attenuated relative to controls. Elevation of Δ FosB in the striatum, then, served to reduce, rather than enhance, cocaine-induced suppression of saccharin intake.

There are several interpretations for the attenuated effect that can be excluded fairly quickly. First, it is possible that elevations in Δ FosB decreased the rewarding value of cocaine. This seems an unlikely explanation given the extensive literature linking elevated Δ FosB to an increase in the perceived reward value of cocaine and other drugs of abuse (Kelz et al., 1999; Nestler et al., 1999; Nestler et al., 2001; Colby et al., 2003; McClung and Nestler, 2003; McClung et al., 2004). Second, the attenuation may reflect species differences in drug-induced suppression and the behavioral effects of Δ FosB. Again, the literature does not support this

possibility as rats and mice demonstrate similar trends in drug-induced suppression of CS intake (Grigson, 1997; Grigson and Twining, 2002; Risinger and Boyce, 2002) and behavioral sensitization by Δ FosB (Kelz et al., 1999; Werme et al., 2002; Olausson et al., 2006; Zachariou et al., 2006). Finally, it is possible that elevation of Δ FosB may create a general associative deficit that would attenuate cocaine-induced suppression of saccharin intake. This possibility, too, appears unlikely as disruptions of this nature are not seen in the learning or performance of operant behavior (Colby et al., 2003) and acquisition of the LiCl-induced CTA did not differ, significantly, as a function of Δ FosB expression in Experiment 5.2. The Δ FosB over expressing mice also behave normally in the Morris water maze and in conditioned place preference (Kelz et al., 1999).

Another possibility is raised by a traditional conditioned taste aversion interpretation of the data in Experiment 5.1. That is, if cocaine-induced suppression of intake of the saccharin cue were driven by aversive drug properties, then one would conclude that elevated Δ FosB reduced, at least in part, the impact of these aversive drug properties. In fact, there is evidence that drugs of abuse have aversive properties. Cocaine has been shown to potentiate panic-like flight responses (Blanchard et al., 1999) and defensive behaviors (Blanchard and Blanchard, 1999) in mice. Even so, most evidence suggests that drugs of abuse suppress CS intake via rewarding drug properties (Grigson and Twining, 2002; Grigson et al., 2008). In addition, in Experiment 5.3 and 5.2, the elevation of Δ FosB had no effect on either the unconditioned or the conditioned response to aversive stimuli, respectively. That is, relative to the normal mice, mice with elevated Δ FosB exhibited a similar aversion to the potent 0.3 M NaCl solution in Experiment 5.3 and a statistically similar aversion to the LiCl-associated CS in Experiment 5.2.

This evidence aside, in a recent study, we obtained evidence that cocaine-induced suppression of intake of a saccharin cue is associated with the onset of a conditioned aversive state (Wheeler et al., 2008). We hypothesize that the aversive state is mediated, in large part, by

the development of cue-induced withdrawal (Grigson et al., 2008; Wheeler et al., 2008). The possibility, then, might be considered that the increase in Δ FosB in the striatum leads to less avoidance of the drug-associated cue because the drug supports the development of less cue-induced withdrawal. Although possible, this conclusion also seems difficult to accept because, in rats, more aversion to the CS (as measured by an increase in aversive taste reactivity behavior) is associated with an increase in responsiveness to the drug (Wheeler et al., 2008). Thus, using this logic, we would be forced to conclude that mice with elevated Δ FosB are more responsive to the rewarding properties of the drug, as has been shown, but also exhibit less cue-induced craving or withdrawal. This seems unlikely.

A more heuristic explanation for the attenuated effect in the current data is that, while elevation of Δ FosB increased the rewarding effects of cocaine in these mice, it also increased the perceived rewarding value of saccharin. If Δ FosB increased the absolute reward value of saccharin and cocaine similarly, the perceived increase in the reward value of saccharin would be greater (compared to cocaine) as stated by Weber's Law (i.e., sensitivity to a perceived change inversely depends on the absolute strength of the stimuli, Weber, 1846). Such an increase in relative CS palatability would decrease the relative difference between the rewards and attenuate the reward comparison effect (Flaherty and Rowan, 1986; Flaherty et al., 1994). This interpretation is further supported by the literature showing that elevation of Δ FosB increases responding for natural rewards. For example, wheel running (Werme et al., 2002) and motivation for food pellets (Olausson et al., 2006) are both increased with elevation of Δ FosB. In addition, the data obtained in Experiment 5.3 also demonstrate that elevation of Δ FosB increases preference for sucrose (0.03 M, 0.1 M, and 0.3 M) and for lower concentrations of NaCl (0.01 M and 0.1 M) in two-bottle tests with water.

The goal of the current chapter was to obtain a more selective evaluation of the effect of increased reward preference in the reward comparison paradigm. The current data suggest that, in

mice, relative increases in reward preference may oppose drug-induced suppression of CS intake by reducing the perceived difference in reward value between natural rewards and drugs of abuse. In so doing, mice with this phenotype actually may be better protected from drug when it is presented with viable natural rewards. In support, access to saccharin blunts the nucleus accumbens dopamine response to the initial injection of morphine in Sprague-Dawley rats (Grigson and Hajnal, 2007a) and brief daily access to a palatable sucrose solution decreases rats' willingness to work for cocaine early in acquisition (Twining, 2007). In addition, female rats, which are more responsive to the rewarding effects of drug compared with males (Lynch and Carroll, 1999; Carroll et al., 2002; Hu et al., 2004; Jackson et al., 2006), also demonstrate attenuated suppression of a saccharin cue paired with drug (Cason and Grigson, *manuscript in prep*). Thus, while the elevation of Δ FosB may predispose rats and mice to drug-taking behavior in the absence of alternative rewards, it may protect the subject from drug-taking behavior in the presence of a viable alternative natural reward. If this is indeed the case, then the comparison of the two rewards should be critical to the attenuated suppression observed in the reward preferring mice and a specific prediction emerges. That is, if mice with elevated Δ FosB exhibit less cocaine-induced suppression of CS intake because of a strong preference for the sweet-tasting cue, then a shift in the hedonic valence of the CS should eliminate this observed strain effect. The experiments in Chapter 6 were designed to address this possibility.

Chapter 6

Attenuated CS suppression in C57BL/6 mice compared with DBA/2J mice is eliminated by changes in CS or US hedonic valence.

If an increased responsiveness to rewards increases the rewarding value of both the CS and US in the reward comparison paradigm, and this generalized increase is responsible for the attenuated suppression of CS intake observed in Experiments 4.1 and 5.1, then a change in the hedonic value of either the CS or the US should eliminate the attenuated suppression observed in the reward preferring mice. Consequently, it is predicted that drug-induced suppression of an aversive CS should be similar between reward preferring C57BL/6 mice and relatively less sensitive DBA/2J mice. Similarly, there should be no significant difference between these strains in the conditioned taste aversion paradigm as well when the putative aversive agent, LiCl, solution serves as the US. Therefore, Experiments 6.2 and 6.3, respectively, were designed to test these assumptions while Experiment 6.1 was conducted to evaluate voluntary intake of QHCl and to determine its suitability as an aversive conditioned stimulus.

Experiment 6.1

A number of studies have evaluated voluntary intake of rewarding and neutral solutions in various strains of mice (for example, Belknap et al., 1993; Berrettini et al., 1994; Bachmanov et al., 1998; Bachmanov et al., 2002; Lewis et al., 2005; Sclafani, 2006) but relatively few experiments have focused on intake of aversive solutions, especially between C57BL/6 and DBA/2J mice. Analysis of taste reactivity scores has demonstrated that C57BL/6 mice produce significantly more aversive reactions (and significantly less positive reactions) to 0.8 mM and 3

mM QHCl compared with DBA/2J mice although positive and aversive reactions were not significantly different between the strains for 0.03 mM and 0.3 mM QHCl (Cagniard and Murphy, 2009). C57BL/6 mice have been shown, however, to drink significantly less low (0.1mM) and high (1.1 mM) concentrations of QHCl compared with DBA/2J mice (Kotlus and Blizard, 1998; Frank and Blizard, 1999). Therefore, Experiment 6.1 was designed to evaluate voluntary intake of an aversive stimulus (QHCl) using a series of one-bottle and two-bottle intake tests to determine an appropriate concentration of QHCl that both strains will drink but still find aversive compared with dH₂O.

Methods

Subjects.

The subjects were 10 naïve male C57BL/6 mice and 10 naïve male DBA/2J mice obtained from Jackson Laboratories. Mice weighed between 17.9 and 23.6 grams at the beginning of the experiment. They were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 6 hours into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water except where otherwise noted.

Apparatus.

All experimental manipulations were conducted in the home cages. Modified serological pipettes were used to provide dH₂O and saccharin access. Pipettes were identical to those previously described. Intake of dH₂O and saccharin was recorded in 1/10 milliliters.

Procedure.

All subjects were weighed once daily. Over a 5-day habituation period, each mouse received 1 hour access to dH₂O six hours into the light cycle (test period, approximately 1:00 pm) and then 2 hours access to dH₂O eight hours into the light cycle (rehydration period, approximately 3:00 pm). Saccharin (0.15%) and QHCl (0.1 mM, and 1.0 mM) were used as the tastants for one-bottle and two-bottle preference tests. *Saccharin (0.15%) tests.* Saccharin was presented during the test period for three consecutive trials (trials 1-3). The first two trials were one-bottle presentations while the third trial consisted of a two-bottle presentation of saccharin and dH₂O. *QHCl (0.1 mM) tests.* QHCl was tested for four trials (trials 6-9). The first two trials were one-bottle presentations of 0.1 mM QHCl and the third trial consisted of a two-bottle presentation of 0.1 mM QHCl and dH₂O. Following a dH₂O only trial, an additional two-bottle intake test was conducted with 0.1 mM QHCl and dH₂O (trial 9). *QHCl (1.0 mM) tests.* QHCl was tested for two consecutive trials (trials 10 and 11). Trial 11 consisted of a one-bottle presentation of 1.0 mM QHCl while trial 12 consisted of a two-bottle presentation of 1.0 mM QHCl and dH₂O. The position of the bottles on the home cage was counterbalanced, left and right, within groups and across two-bottle test sessions. Two dH₂O only trials were conducted following changes in tastant or tastant concentration. Intake was measured on each day to the nearest 1/10 ml. The quinine HCl solution and dH₂O were presented at room temperature.

Results

One-bottle intake tests.

The results of the one-bottle intake test for saccharin and QHCl in C57BL/6 and DBA/2J mice are presented in Figure 6.1.2. Preference for saccharin or QHCl compared with dH₂O was analyzed using t-test for independent samples with alpha = .05 for each cell. Neither the C57BL/6 mice nor the DBA/2J mice drank significantly more 0.15% saccharin compared with dH₂O in one-bottle intake tests ($p > .05$). C57BL/6 mice, on the other hand, were found to drink significantly less 0.1 mM QHCl ($t = -2.14$, $p = .05$) and 1.0 mM QHCl ($t = -2.37$, $p = .03$) than dH₂O. In contrast, and consistent with the two-bottle test data, DBA/2J mice drank significantly more 0.1 mM QHCl than dH₂O ($t = 2.85$, $p = .01$), but did not differ in intake between dH₂O and 1.0 mM QHCl ($p > .05$). Taken with the results from the two-bottle tests, C57BL/6 mice significantly prefer saccharin over dH₂O and dH₂O over QHCl when given a choice while DBA/2J mice prefer, or are indifferent, to these tastants compared with dH₂O. Finally, although intake of both concentrations of QHCl is lower in C57BL/6 vs. DBA/2J mice, both strains drink adequate amounts of each tastant (between 1-1.5 ml). Testing, then, can proceed with QHCl serving as an aversive CS for the reward preferring C57BL/6 mice.

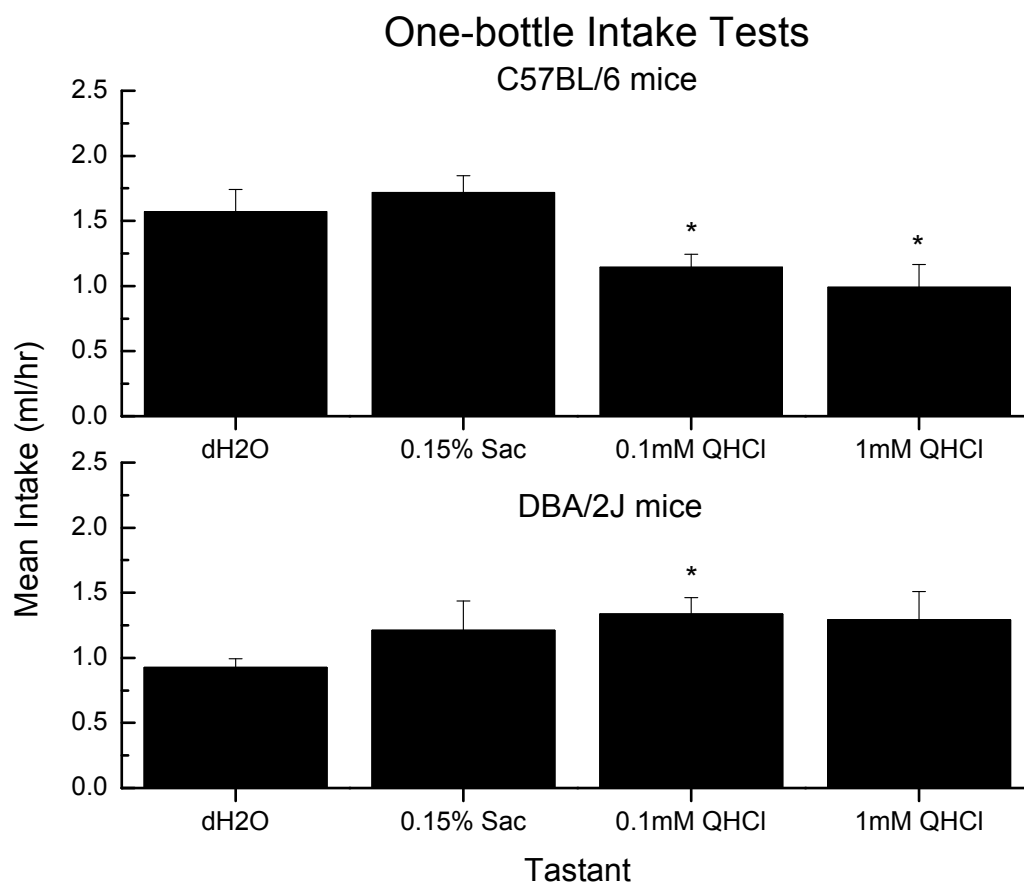


Figure 6.1.1: Mean (\pm SEM) intake (ml/1 hr) in one-bottle tests of dH₂O, 0.15% saccharin, 0.1 mM QHCl, and 1 mM QHCl in C57BL/6 and DBA/2J mice. * indicates significant difference between dH₂O and tastant ($p < .05$) within strain.

Two-bottle intake tests.

The results of the two-bottle intake test for saccharin and QHCl against dH₂O in C57BL/6 and DBA/2J mice are presented in Figure 6.1.1. Preference for saccharin and QHCl was analyzed using t-test for dependent samples with $\alpha = .05$ for each cell. C57BL/6 mice significantly preferred saccharin over dH₂O, $t=8.04$, $p < .0001$, while the DBA/2J mice did not demonstrate a significant preference for saccharin, $p > .05$. In addition, in the repeated two-bottle

tests, C57BL/6 mice significantly preferred dH₂O over 0.1 mM QHCl ($t=-2.39$, $p=.0403$ first test; $t=-6.73$, $p=.0403$ second test) and over 1.0 mM QHCl ($t=-16.50$, $p<.0001$). In contrast, DBA/2J mice demonstrated no significant preference for QHCl with the exception of the first two-bottle preference test for 0.1 mM QHCl, $t=4.46$, $p=.002$.

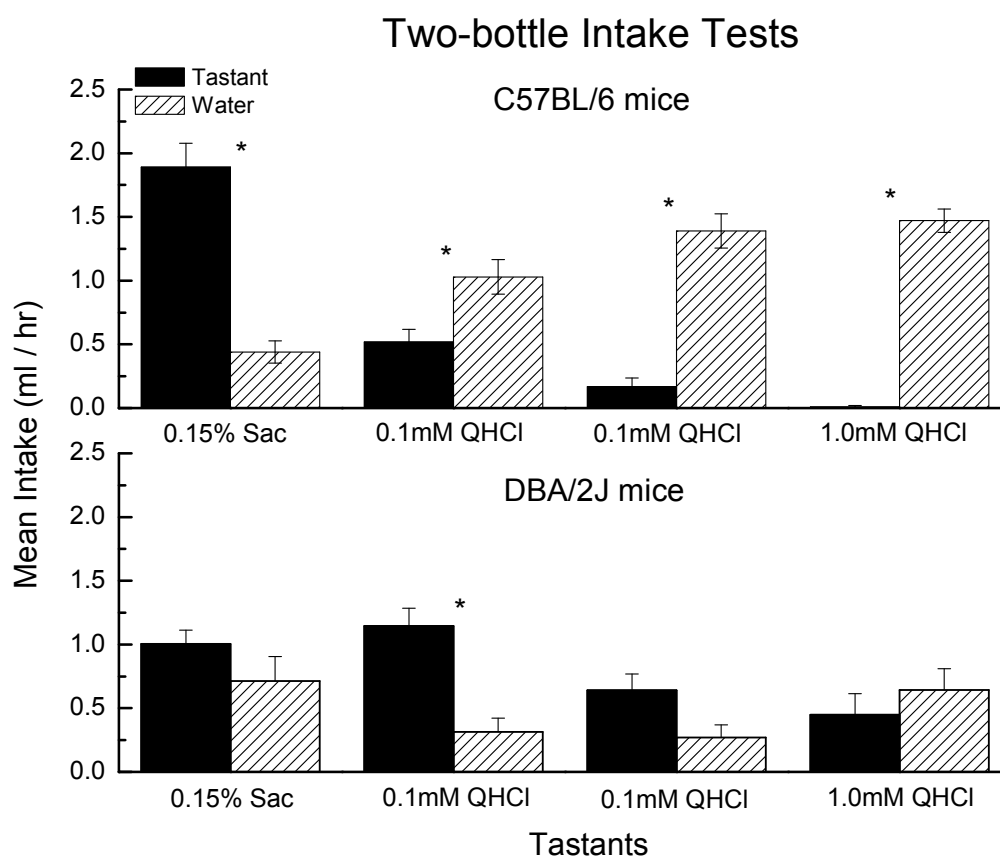


Figure 6.1.2: Mean (\pm SEM) intake (ml/hr) in two-bottle tests of 0.15% saccharin, 0.1 mM QHCl, and 1.0 mM QHCl against dH₂O in C57BL/6 and DBA/2J mice. * indicates significant difference between dH₂O and tastant ($p<.05$).

Body Weight.

Body weight was analyzed using a 2 x 14 repeated measures analyses of variances (ANOVA) varying strain (C57BL/6 or DBA/2J) and trials (1-14). Body weight over trials (data not shown) significantly differed between strains as indicated by a significant 2-way interaction, $F(13, 221)=2.07$, $p=0.02$. The main effects of trial, $F(13, 221)= 32.04$, $p<.0001$, and strain, $F(1, 17)=27.66$, $p<.0001$, were also significant indicating that body weight increased over successive trials and that C57BL/6 mice were significantly heavier than DBA/2J mice throughout the study.

Experiment 6.2

The data from Experiment 6.1 demonstrate that both C57BL/6 mice and DBA/2J mice drink 0.1 mM QHCl, despite the fact that C57BL/6 mice significantly avoid intake of QHCl in two-bottle tests against dH₂O. This finding allows us to test the hypothesis that the attenuated suppression of saccharin intake in mice with elevated Δ FosB and in C57BL/6 mice following saccharin-cocaine pairings in Experiments 4.1 and 5.1 was due to the high perceived rewarding value of the CS as well as the US. That is, although mice should suppress intake of an aversive CS as observed in rats (Grigson et al., 2009), differential sensitivity to rewards between the strains should result in differential preference for the US reward only and not affect the value of the aversive CS. Although greater preference for cocaine in C57BL/6 mice would predict greater suppression of intake of an aversive CS paired with cocaine, use of a non-reward CS (NaCl) in Experiment 5.1 slightly attenuated suppression in the experimental group with increased reward sensitivity. Therefore, it is predicted that compared with DBA/2J mice, C57BL/6 mice will demonstrate an elimination of the attenuated effect observed in the previous experiments (i.e.,

demonstrate equal suppression) when 0.1 mM QHCl is paired with cocaine. Experiment 6.2 was conducted to evaluate this hypothesis.

Methods

Subjects.

The subjects were 30 naïve male C57BL/6 mice and 30 naïve male DBA/2J mice obtained from Jackson Laboratories. Mice weighed between 20.3 and 30.3 grams at the beginning of the experiment. They were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 1 hour into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water except where otherwise noted.

Apparatus.

The apparatus was the same described in Experiment 6.1.

Procedure.

QHCl-cocaine pairings. All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on a water deprivation schedule which consisted of access to dH₂O for 1 hour in the morning and 2 hours in the afternoon. Baseline intake and body weight were recorded for 8 days. Mice remained on water deprivation schedule as described

above. *Phase 1.* On each taste-drug pairing (which occurred every 48 hours), all mice received 1 hour access to 0.1 mM QHCl followed by an i.p. injection of either saline (n=8/cell), 10 mg/kg cocaine (n=7/cell), 20 mg/kg cocaine (n=7/cell), or 30 mg/kg cocaine (n=8/cell). Taste-drug pairings occurred in the morning (2 hours into the light phase) for 5 trials (trials 1-5). A sixth trial (trial 6) was conducted with CS only access (no injection). *Phase 2.* Mice were run for an additional 8 trials in an identical procedure to that used in Phase 1 with the exception that all drug groups now received 40 mg/kg cocaine (trials 7-12; n=22/cell) and then 60 mg/kg cocaine (trials 13 and 14; n=22/cell). Trials 8 and 14 were CS only access (no injection). Previous saline groups continued to receive saline for all trials (n=8/cell; trials 7-14). Cocaine HCl was provided by the National Institute on Drug Abuse. *Two-bottle intake tests.* Two-bottle intake tests were conducted with QHCl vs. water following phases 1 and 2 to evaluate the QHCl preference in the presence of a water alternative. The position of the bottles was counter-balanced across groups.

Results

CS Intake, Phase 1.

Intake was analyzed using a 2 x 4 x 6 mixed factorial analyses of variances (ANOVAs) varying strain (C57BL/6 or DBA/2J), drug (saline, 10, 20, or 30 mg/kg cocaine), and trials (1-6). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with alpha = .05. Observation of Figure 6.2.1 shows that neither C57BL/6 nor DBA/2J mice demonstrated significant suppression of QHCl intake, regardless of drug group, compared with saline controls. Despite a significant 3-way interaction, $F(15, 260)=2.18$, $p=.007$, post hoc analysis provided no significant comparisons, $p>.05$. In addition, there was no significant main effect of drug, $F(3, 52)=2.28$, $p=.09$, and no significant strain x drug interaction, $F(3,52)=.30$, $p=.82$, indicating that

there was no difference in suppression of QHCl intake by drug or between the strains. A significant main effect of strain, $F(1, 52)=45.76, p<.0001$, and non-significant strain x trial interaction, $F(5, 260)=.82, p=.53$, indicates that although initial intake of QHCl was different between the strains, this difference was maintained across trials.

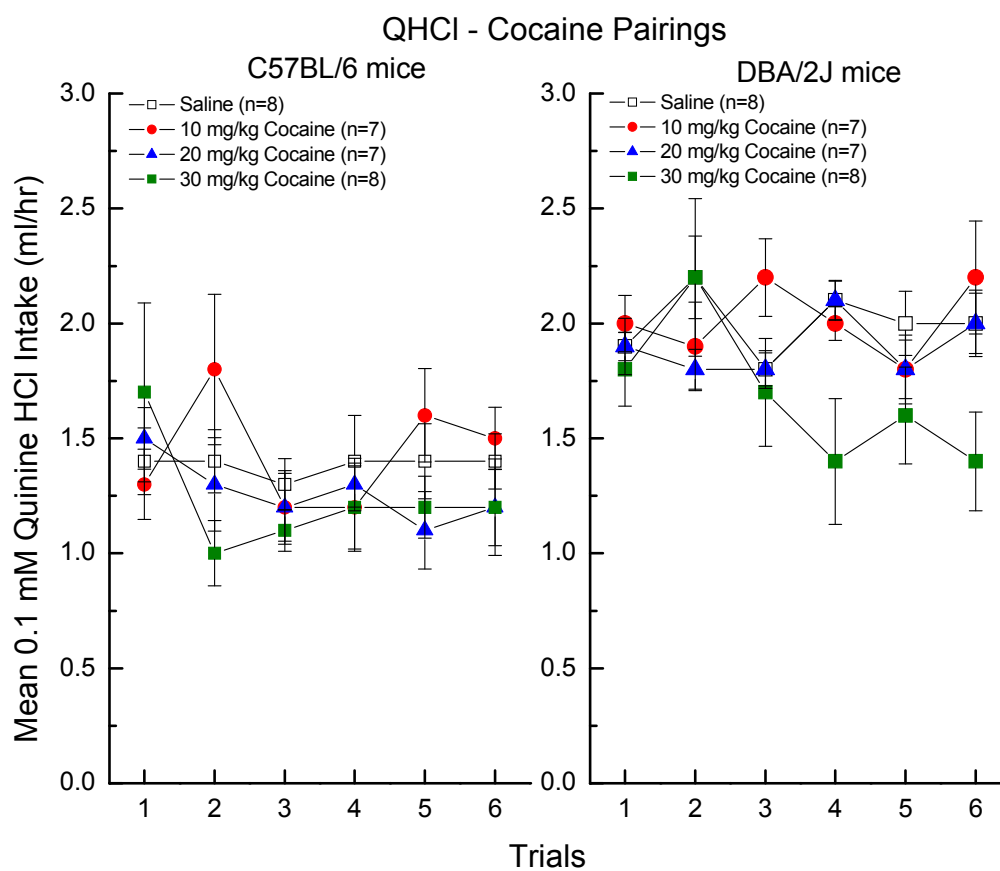


Figure 6.2.1: Mean (\pm SEM) intake (ml/1 hr) of 0.1 mM QHCl following 5 pairings with the intraperitoneal injection of saline, 10 mg/kg cocaine, 20 mg/kg cocaine, or 30 mg/kg cocaine in C57BL/6 and DBA/2J mice.

Two-bottle Intake test, Phase 1.

The results of the two-bottle intake test for QHCl against dH₂O in C57BL/6 and DBA/2J mice following QHCl-cocaine pairings are presented in Figure 6.2.2. A 2 x 4 x 2 mixed factorial analyses of variances (ANOVAs) varying strain (C57BL/6 or DBA/2J), drug (saline, 10, 20, or 30 mg/kg cocaine), and intake (QHCl and dH₂O) was conducted. Results demonstrate no difference between in intake between the strains or drug groups as indicated by non-significant main effects of strain, $F(2,40)=1.30$, $p=.28$, and drug, $F(6,80)=1.66$, $p=.14$ as well as a non-significant strain x drug interaction, $F(6,80)=.36$, $p=.90$.

Preference for QHCl was also analyzed using t-tests for dependent samples with alpha = .05 for each cell. C57BL/6 mice significantly preferred dH₂O over QHCl in the saline control group ($p<.05$), but did not demonstrate a significant preference or aversion in the drug groups ($ps>.05$). In contrast, DBA/2J mice did not demonstrate a significant preference between dH₂O and QHCl ($ps>.05$). The DBA/2J mice also failed to demonstrate a significant avoidance of the QHCl CS when paired with either the 20 or the 30 mg/kg dose of cocaine.

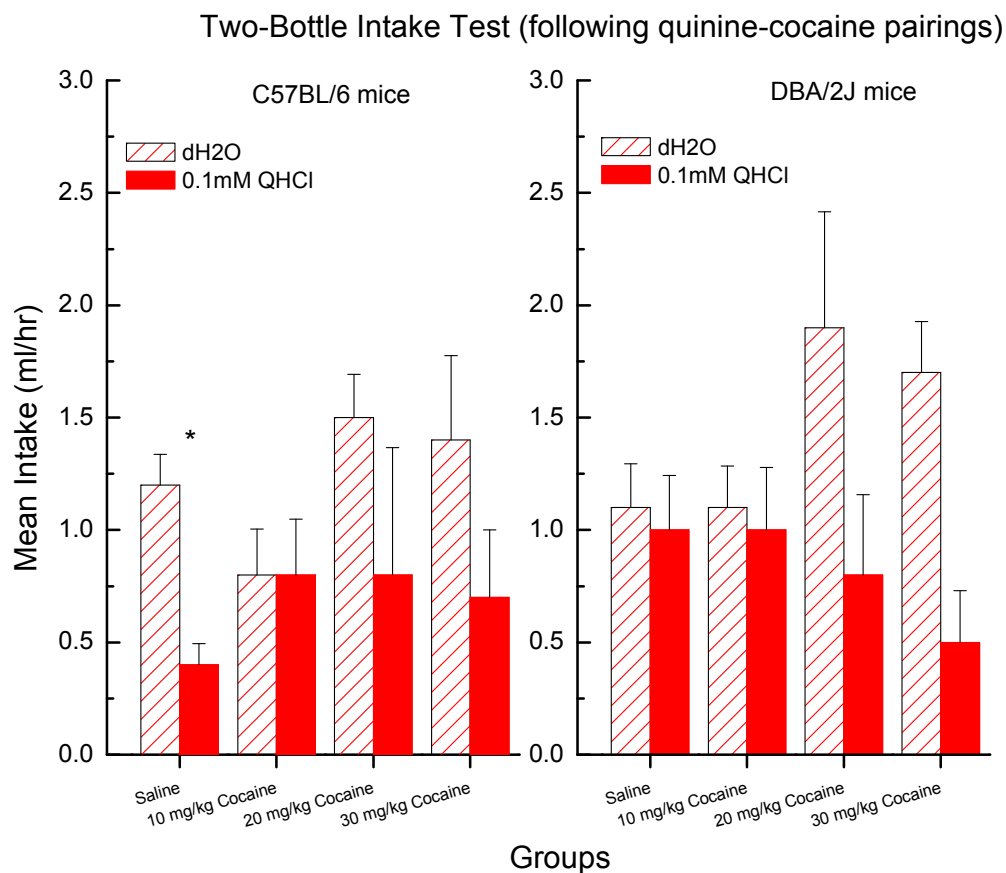


Figure 6.2.2: Mean (\pm SEM) intake (ml/1 hr) in a two-bottle test of 0.1 mM QHCl against dH₂O following QHCl-cocaine pairings in C57BL/6 and DBA/2J mice. * indicates significant difference between dH₂O and QHCl ($p < .05$).

CS Intake, Phase 2.

The results of QHCl-cocaine pairings during phase 2 in C57BL/6 and DBA/2J mice are presented in Figure 6.2.3. Intake was analyzed using a 2 x 2 x 8 mixed factorial analyses of variances (ANOVAs) varying strain (C57BL/6 or DBA/2J), drug (saline or cocaine), and trials (7-14). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with $\alpha =$

.05. Significant main effects of strain, $F(1, 56)=11.27, p<.001$, and drug, $F(1, 56)=12.68, p<.001$, indicate that DBA/2J mice, in general, consumed more QHCl compared with C57BL/6 mice and that cocaine was effective in suppressing intake, compared with saline, in all mice. Once again, however, even when greatly increasing the dose of the drug, suppression of QHCl intake following QHCl-cocaine pairings was not significantly different between C57BL/6 and DBA/2J mice across trials as indicated by a non-significant strain x drug x trials interaction, $F(7, 392)=1.13, p=.34$.

CS intake from phase 2 has also been reanalyzed on the basis of prior drug history from phase 1. A separate $2 \times 4 \times 8$ mixed factorial ANOVA varying strain (C57BL/6 or DBA/2J), drug (saline or history of 10, 20, or 30 mg/kg cocaine), and trials (7-14) was used to analyze intake. Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with $\alpha = .05$. As above, suppression of intake between the strains was not significantly different as indicated by a non-significant strain x drug x trials interaction, $F(21, 364)=.90, p=.60$.

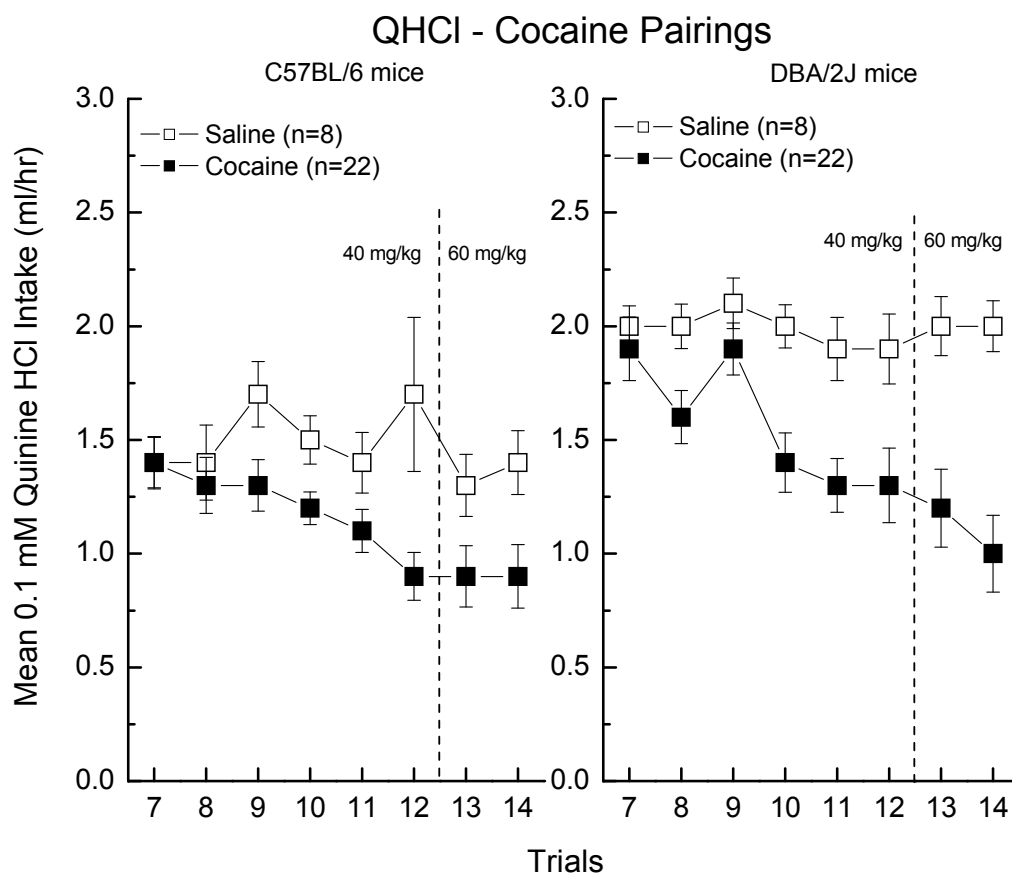


Figure 6.2.3: *Top panels.* Mean (\pm SEM) intake (ml/1 hr) of 0.1 mM QHCl following pairings with intraperitoneal injection of saline, 40 mg/kg cocaine (trials 7, 9-12) and 60 mg/kg cocaine (trial 13) in C57BL/6 and DBA/2J mice.

Two-bottle Intake test, Phase 2.

The results of the second two-bottle intake test for QHCl against dH₂O in C57BL/6 and DBA/2J mice following QHCl-cocaine pairings are presented in Figure 6.2.4. Preference for QHCl was analyzed using t-tests for dependent samples with $\alpha = .05$ for each cell. C57BL/6 mice significantly preferred dH₂O over QHCl in the saline control group as well as the cocaine group ($p < .05$). In contrast, DBA/2J mice did not demonstrate a significant preference between

dH₂O and QHCl in the saline control group ($p > .05$) but demonstrated a significant preference for dH₂O over QHCl in the cocaine group ($p < .05$).

Two-bottle intake data following phase 2 has also been reanalyzed on the basis of prior drug history from phase 1. Separate t-tests for dependent samples with $\alpha = .05$ for each cell indicated that, in C57BL/6 mice, all groups demonstrated preference for dH₂O compared with QHCl ($p < .001$), with the exception of mice with a history of 10 mg/kg cocaine in phase 1 ($p = .07$). In comparison, DBA/2J saline controls demonstrated similar preference for dH₂O and QHCl ($p = .22$) while DBA/2J mice with history of 10, 20, or 30 mg/kg cocaine in phase 1 demonstrated preference for dH₂O against QHCl ($p < .04$).

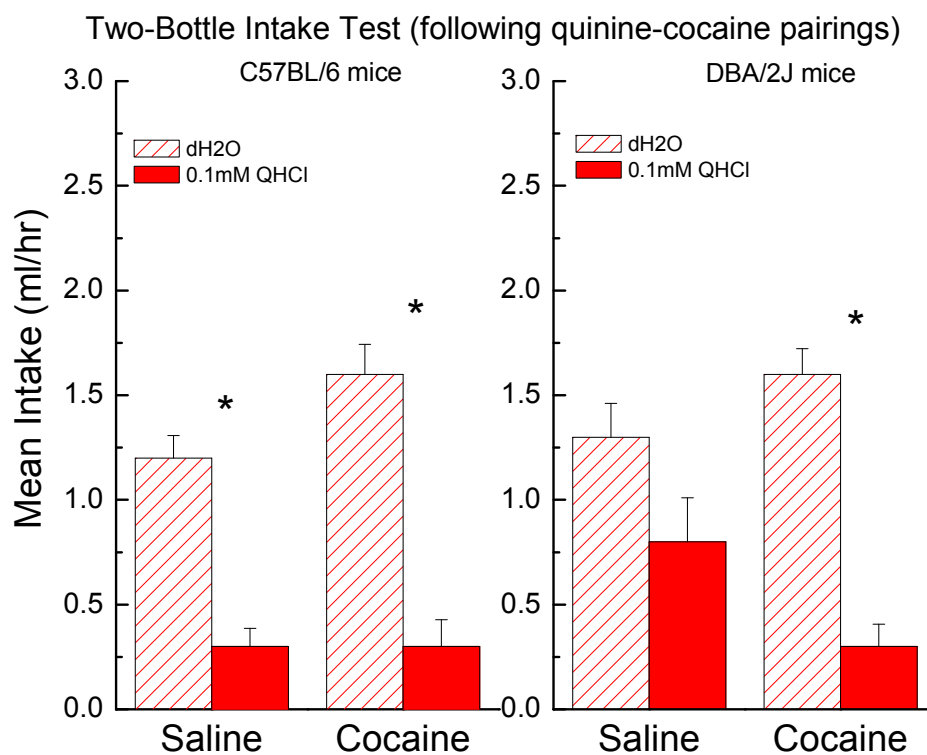


Figure 6.2.4: Mean (\pm SEM) intake (ml/1 hr) in a two-bottle test of 0.1 mM QHCl against dH₂O following QHCl-cocaine pairings in C57BL/6 and DBA/2J mice.

Body Weight.

Body weight was analyzed using a 2 x 4 x 31 repeated measures analyses of variances (ANOVA) varying strain (C57BL/6 or DBA/2J), drug (saline, 10 mg/kg cocaine, 20 mg/kg cocaine, or 30 mg/kg cocaine), and trials (1-31). Body weight over trials (data not shown) did not significantly differ between treatment as indicated by a non-significant 3-way interaction, $F(90, 1560)=0.97$, $p=0.57$. The main effect of drug was not significant, $F(3, 52)= 0.06$, $p=.98$. The main effect of trials was significant, $F(30, 1560)= 16.60$, $p<.0001$, indicating that body weight increased over successive trials. In addition, the main effect of strain was also significant, $F(1, 52)=11.41$, $p<.0001$, indicating that C57BL/6 mice were significantly heavier than DBA/2J mice throughout the study.

Experiment 6.3

The data from Experiment 6.2 demonstrates that the use of the aversive QHCl as the CS eliminates differences in cocaine-induced suppression between C57BL/6 mice and DBA/2J mice. It is further predicted that these strains should not be significantly different in CS suppression when an aversive agent, such as LiCl, serves as the US. The present experiment was designed to test this hypothesis. Although the literature shows that C57BL/6 and DBA/2J mice suppress intake of saccharin when paired with LiCl in a similar manner (Ingram, 1982), we failed to replicate this finding with saccharin (data not shown). Specifically, at the doses of LiCl needed to obtain suppression, C57BL/6 saline controls also demonstrated suppression of CS intake, likely due to high injection volumes. Even so, there are data demonstrating that, at least in DBA/2J mice, NaCl is a more effective CS than saccharin when LiCl serves as the US (Risinger and Boyce, 2002). Therefore, Experiment 6.3 used NaCl as the CS and ip administered saline or LiCl

as the US across trials 1 – 6 and then allowed for oral consumption of the NaCl or LiCl (i.e., the salts served as both the CS and the US) across trials 7 – 9. It is predicted that oral consumption of LiCl will lead to equivalent CTAs in C57BL/6 and DBA/2J mice similar to that observed in Lewis and Fischer rats in Experiment 2.2.

Methods

Subjects.

The subjects were 30 naïve male C57BL/6 mice and 30 naïve male DBA/2J mice. Mice weighed between 14.9 and 23.0 grams at the beginning of the experiment. They were housed individually in standard, clear plastic pan cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light-dark cycle (lights on at 7:00 am). All experimental manipulations were conducted 1 hour into the light phase of the cycle. The mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water except where otherwise noted.

Apparatus.

The apparatus was the same as described in Experiment 6.1.

Procedure.

NaCl - ip LiCl pairings. All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on a water deprivation schedule which consisted of access to dH₂O for 1 hour in the morning and 2 hours in the afternoon. Baseline intake and body

weight were recorded for 5 days. Mice remained on water deprivation schedule as described above. For trials 1-6, all mice received 1 hour access to 0.1 M NaCl followed by an i.p. injection of either saline (n=15 C57BL/6; n=15 DBA/2J) or LiCl (n=15 C57BL/6; n=15 DBA/2J). LiCl doses increased over trials and were 0.5 mEq/kg 0.0375 M LiCl (trials 1-3) and 2.0 mEq/kg 0.15 M LiCl (trials 4-6). Taste-drug pairings occurred in the morning every 48 hours for 6 trials. *Oral consumption*. For trials 7-9, mice were given a chance to drink either 0.15 M NaCl (n=15 C57BL/6; n=15 DBA/2J) or 0.15 M LiCl (n=15 C57BL/6; n=15 DBA/2J) for 1 hour in the morning. As such, the NaCl or LiCl served as both the CS and the US. Mice that previously received saline injections were given NaCl while mice with previous experience with LiCl injections received LiCl to drink. All mice were capped at 1 ml of intake for their respective tastant. Trials occurred every 48 hours.

LiCl was obtained from the Sigma Chemical Company, St. Louis, MO, and the NaCl was obtained from Fisher Chemical, Pittsburgh, PA. The NaCl and LiCl solutions were presented at room temperature.

Results

CS Intake.

The results of NaCl-LiCl pairings in C57BL/6 and DBA/2J mice are presented in Figure 6.3. Intake was analyzed using a 2 x 2 x 9 mixed factorial ANOVA varying strain (C57BL/6 or DBA/2J), drug (saline or LiCl), and trials (1-9). Post hoc tests were conducted, where appropriate, using Neuman-Keuls tests with alpha = .05. Significant main effect of strain, $F(1,56)=22.60$, $p<.0001$, and drug, $F(1,56)=4.48$, $p=.04$, indicated that C57BL/6 mice, compared with DBA/2J mice, consumed more NaCl in general and that LiCl was effective in suppressing

intake in both strains. However, C57BL/6 and DBA/2J mice did not differentially suppress intake of the NaCl CS as a function of drug group across trials as indicated by a non-significant strain x drug x trial interaction, $F(8, 448)=0.92$, $p=.50$, as well as a non-significant strain x drug interaction, $F(1,56)=0.34$, $p=.56$.

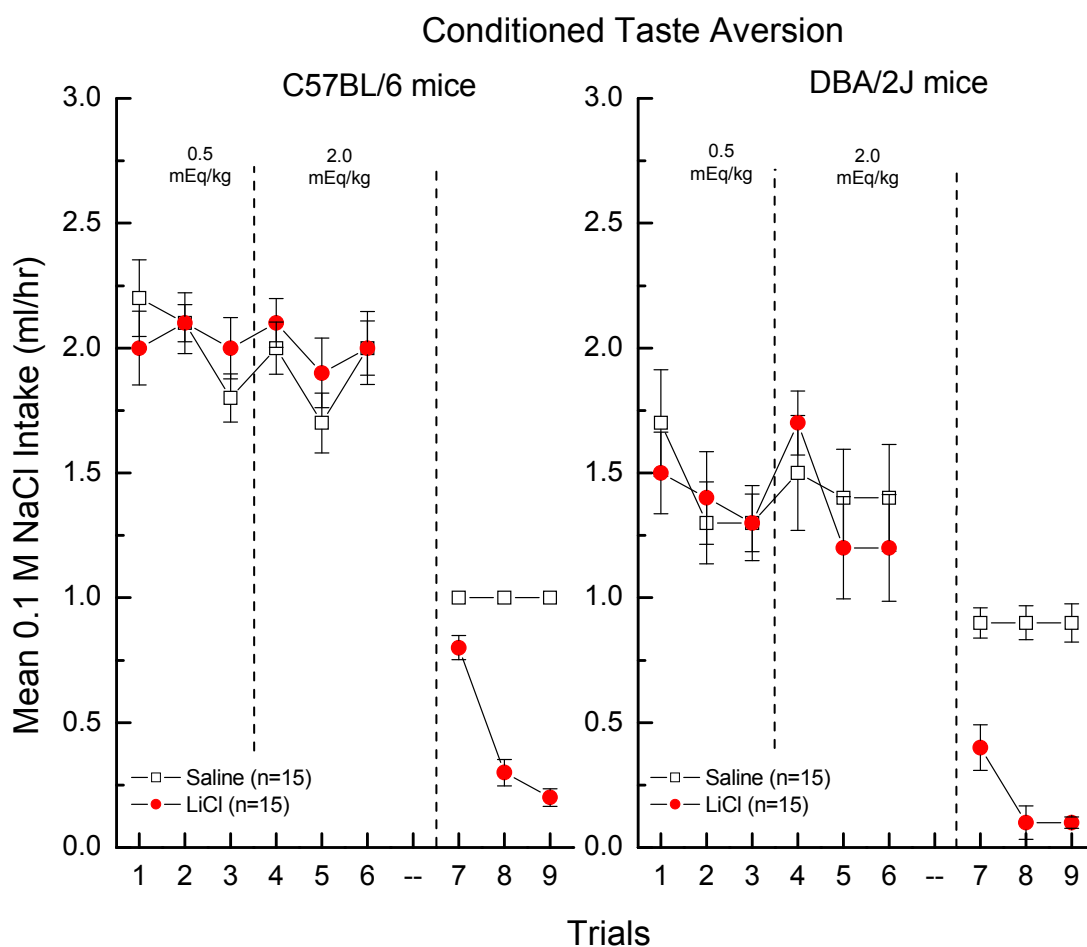


Figure 6.3: Mean (\pm SEM) intake (ml/1 hr) of 0.1 M NaCl following 5 pairings with the intraperitoneal injection of saline, 0.5 mEq/kg LiCl, or 2.0 mEq/kg LiCl followed by one CS only test in C57BL/6 and DBA/2J mice (Trials 1-6). Mean (\pm SEM) intake (ml/1 hr; intake capped at 1 ml) of 0.15 M NaCl or 0.15 M LiCl in C57BL/6 and DBA/2J mice (Trials 7-9).

Body Weight.

Body weight was analyzed using a 2 x 2 x 17 repeated measures analyses of variances (ANOVA) varying strain (C57BL/6 or DBA/2J), drug (saline or LiCl), and trials (1-17). Body weight over trials (data not shown) did not significantly differ between treatment as indicated by a non-significant 3-way interaction, $F(16, 896)=0.56$, $p=0.91$. The main effect of drug was not significant, $F(1, 56)= 0.03$, $p=.86$. The main effect of trials was significant, $F(16, 896)= 125.48$, $p<.0001$, indicating that body weight increased over successive trials. In addition, the main effect of strain was also significant, $F(1, 56)=10.81$, $p=.002$, indicating that C57BL/6 mice were significantly heavier than DBA/2J mice throughout the study.

Discussion

The aim of the experiments in Chapter 6 was to evaluate whether the attenuated suppression observed in Experiments 4.1 and 5.1 was dependent upon the comparison of a rewarding CS and US. That is, it was hypothesized that an increase in the responsiveness to rewards may increase the rewarding value of both the CS and US in the reward comparison paradigm, but that this generalized increase would result in a decrease in the relative reward difference between the stimuli and manifest as the attenuated suppression observed in mice in the previous experiments. It was predicted, then, that a change in the hedonic value of either the CS or the US should eliminate the attenuated suppression observed in the reward preferring mice. Experiment 6.1 determined that QHCl is voluntarily consumed by C57BL/6 and DBA/2J mice in one-bottle intake tests and that QHCl is aversive when compared with dH₂O in two-bottle intake tests. As such, QHCl was determined to be a suitable aversive conditioned stimulus and was

paired with cocaine in Experiment 6.2. The results demonstrated that drug-induced suppression of the intake of the aversive CS was similar between reward preferring C57BL/6 mice and relatively less sensitive DBA/2J mice. In addition, there was no significant difference in suppression between these strains in the conditioned taste aversion conducted in Experiment 6.3 using LiCl as the aversive US.

Although we conclude from the analysis of the data in Experiment 6.2 that suppression of intake of the aversive QHCl cue, when paired with cocaine, is not significantly different between C57BL/6 and DBA/2J mice, there are a number of things that must be considered. First, within the low to moderate doses (10-30 mg/kg) of cocaine used in phase 1 of Experiment 6.2, there was no suppression of intake of the QHCl cue in either the C57BL/6 or DBA/2J mice. It is unusual that mice in both strains would protect intake of a cue which is not preferred, especially when it is paired with a powerful drug of abuse. In fact, the two-bottle intake data in Figure 6.2.2 demonstrated that although the DBA/2J saline control mice were indifferent to QHCl compared with dH₂O, C57BL/6 control mice significantly preferred dH₂O over QHCl. In addition, it has been shown that rats suppress intake of an aversive malic acid cue when paired with the doses of cocaine used in Experiment 6.2 (unpublished data). This lack of suppression makes it difficult to interpret the data in Experiment 6.2 in terms of the stated hypothesis. That is, it is problematic to evaluate the effect of changes in the hedonic value of the CS on drug-induced suppression without the development of suppression. As a result, cocaine dosage was increased for phase 2 of Experiment 6.2. In an attempt to observe suppression, the QHCl cue was paired first with 40 mg/kg, and then 60 mg/kg, cocaine. Consequently, suppression was observed in both the C57BL/6 and DBA/2J mice (Figure 6.2.3). However, the use of higher doses of cocaine, itself, presents a second problem with regards to interpretation. Specifically, cocaine (and drugs of abuse in general) is a complex US with both rewarding and aversive properties (see Blanchard and Blanchard, 1999; Blanchard et al., 1999 for some acute, aversive properties of cocaine in

mice). Although it is reasonable to assert that any aversive properties of the drug are minimized at low and moderate doses (i.e., the rewarding properties of the drug are primarily responsible for suppression as explained in Chapter 1, Introduction), the same can not be said for higher doses of the drug. The probability that suppression, at least in part, may begin to reflect aversive properties of the drug (i.e. conditioned anxiety, increased toxicity, etc.) increases with higher doses of cocaine. Again, this may present difficulties in interpretation as it becomes less clear as to whether we are evaluating suppression resulting from rewarding or aversive mechanisms or a combination of both.

However, it should be noted that, despite these concerns in phases 1 and 2 of Experiment 6.2, the results were always similar between the strains. The aim of the experiment was to determine whether the differential suppression between the strains observed in Experiments 4.1 and 5.1 would remain if either the CS or US (or both) were not rewards. It did not. The expression of drug-induced suppression (or lack thereof) of intake of the CS cue was similar between the strains when the CS was an aversive QHCl solution. This similarity in suppression between the strains was also observed in Experiment 6.3 when the aversive agent LiCl was used as the US, despite initial difficulties in obtaining suppression. In a study by Ingram (1982), C57BL/6 and DBA/2J mice did not suppress intake of a saccharin CS when it was paired with LiCl until a large 6.0 mEq/kg dose was used (standard LiCl dose is 2.0 mEq/kg); at that point, however, suppression was similar between the strains. Our initial attempt to replicate this finding was not successful. Although we were able to obtain suppression at the 6.0 mEq/kg dose of LiCl, intake of the saline controls also decreased. Therefore, Experiment 6.3 evaluated suppression of intake of a NaCl CS paired with LiCl, as NaCl has been shown to be a particularly effective CS when LiCl serves as the US, at least in DBA/2J mice (Risinger and Boyce, 2002). Similar to the Ingram study, C57BL/6 and DBA/2J mice did not suppress intake of the CS at low to standard doses (0.5-2.0 mEq/kg) of LiCl but, when allowed to orally consume either NaCl or LiCl, both

strains equally suppressed intake of LiCl following a single pairing. Taken together with the data from Experiment 6.2, the evidence would appear to support the position that the increased sensitivity to reward in the C57BL/6 mice does not significantly alter suppression of intake of a CS compared with DBA/2J mice unless both the CS and the US are hedonically rewarding.

Chapter 7

General Discussion

The goal of the current dissertation was to evaluate the effect of changes in preference and/or sensitivity to rewards in drug-induced suppression of a natural reward cue and to interpret and apply these findings to the complex behavioral disease of drug addiction. As stated earlier (see Chapter 1, Introduction), the reward comparison paradigm serves as a model for a devastating consequence of drug addiction, specifically the devaluation of natural rewards. In addition, continued refinement of the reward comparison paradigm has led to insights into cue-induced withdrawal, and even the protective effects of natural rewards, observed in drug addiction. The data indicate that increases in sensitivity to reward can facilitate or attenuate drug-induced suppression of intake of a non-drug CS depending upon whether the increased preference is limited to the drug of abuse or whether it also affects sensitivity to other natural rewards.

Sensitivity to Rewards and the Devaluation of Natural Rewards by Drugs of Abuse

The experiments conducted in the current dissertation provide data demonstrating that increased sensitivity to rewards can result in either facilitation or attenuation of drug-induced suppression of a natural reward. In the case of the augmented suppression obtained in the Lewis rats, this effect appears to be due to a selective increase in the perceived reward value of the drug of abuse. In fact, microstructural analysis demonstrates no difference in intake of sucrose, citric acid, or quinine between Lewis and Fischer rats using a brief access lick test (Wheeler, 2003). The attenuated suppression seen in the mice, on the other hand, appears to be due to increased sensitivity to the natural reward cue as well as the drug of abuse. In addition, the development of

both facilitation and attenuation of drug-induced suppression in subjects with increased sensitivity for reward may be, at least in part, explained by transition from a controlled state of drug use to a more compulsive state.

Facilitation of Suppression by Differential Sensitivity to Rewards

Initial predictions regarding the effect of increased sensitivity to rewards on the drug-induced suppression of a natural reward cue stated: The greater the perceived value of the drug, the greater the drug-induced suppression. Chapter 2 confirmed this prediction in rats. That is, relatively reward preferring Lewis rats demonstrated greater suppression of intake of a saccharin cue when paired with cocaine compared with the relatively reward insensitive Fischer 344 rats (Experiment 2.1). A similar facilitation in suppression of CS intake was observed when a known rewarding US such as sucrose was used in the anticipatory contrast study (Experiment 2.3), but not when an aversive US such as LiCl was used (Experiment 2.2). Furthermore, Sprague-Dawley rats with a history of chronic exposure to morphine also demonstrate greater cocaine-induced suppression of intake of a saccharin cue compared with drug naïve rats (Grigson et al., 2001). In addition to anticipatory or anterograde comparisons, this facilitation of suppression also extended in Lewis rats to a retrograde model of comparison between natural rewards, successive negative contrast. Lewis rats demonstrate greater and more prolonged contrast effects compared with Fischer 344 rats in response to a perceived loss of reward (Experiment 3.1).

The data from Chapters 2 and 3 are consistent with the hypothesis that increases in sensitivity to reward work to increase the perceived value of the drug and, via comparison, further devalue associated natural rewards. Consequently, in terms of human drug addiction, addicts that are relatively sensitive to rewards would experience a greater devaluation of the natural rewards around them as a consequence of the perceived increase in the value of the drug (i.e., increased

sensitivity to rewards should result in greater devaluation of natural rewards due to exposure to drugs of abuse). The data in Chapters 4 and 5, however, indicate that increases in reward preference can attenuate rather than facilitate drug-induced suppression of intake of a non-drug cue suggesting that increases in reward sensitivity, under other conditions, may produce a protective effect against the devaluation of natural rewards by drugs of abuse.

Attenuation of Suppression by Differential Sensitivity to Rewards

Although an increase in reward sensitivity facilitated drug-induced suppression of intake of a natural reward cue in rats in Chapters 2 and 3, current data indicate that female rats and female mice show attenuated suppression of CS intake compared to males in this paradigm. Female Sprague Dawley rats demonstrate greater intake of a saccharin cue (i.e., attenuated effect) when it is paired with the opportunity to self-administer cocaine compared with male rats. In addition, female rats also self-administer less drug in the presence of the saccharin cue than in its absence (Cason and Grigson, *manuscript in prep*). In general, it has been demonstrated that female rats, compared with male rats, demonstrate greater preference for drug as evidenced by faster acquisition of drug self-administration (Lynch and Carroll, 1999; Jackson et al., 2006), greater conditioned place preference (Torres et al., 2009; Zakharova et al., 2009), and greater motivation during progressive ratio testing following abstinence (Roberts et al., 1989). Thus, our finding that female rats attenuate suppression of CS intake compared with male rats of the same strain runs counter to the hypothesis presented in Chapters 2 and 3. Similarly, preliminary data shows that, while GABA_A γ 2 knockout mice do not show differences in drug-induced suppression, female wild types demonstrate attenuated suppression compared with male wild types in this paradigm (unpublished findings). The attenuated suppression associated with increased preference for rewards was also observed in male mice in Chapters 4 and 5. The

relatively reward preferring C57BL/6 mice demonstrated attenuated suppression of intake of a saccharin cue that was paired with cocaine compared to the relatively reward insensitive DBA/2J mice (Experiment 4.1). Similarly, NSE-tTA x TetOp- Δ FosB mice with elevated Δ FosB in the striatum (resulting in increased sensitivity to reward) demonstrated a similar attenuated effect compared with transgenic controls (Experiment 5.1).

The attenuated suppression may reflect a generalized increase in reward sensitivity that encompasses both the CS and the US as opposed to a more selective increase in reward sensitivity that would selectively increase the hedonic value of the US. Selective increases in the value of the US (e.g., the drug) would increase the relative difference between the rewards and would facilitate suppression. Conversely, a perceived increase in the hedonic value of the CS, compared with the US, would decrease the relative difference between the rewards and would attenuate suppression. There is evidence for increased preference for the CS in Chapters 4 and 5. Figure 4.1.2 shows that, following saccharin-cocaine pairings in Experiment 4.1, C57BL/6 mice demonstrated a preference for saccharin (compared with dH₂O) in a two-bottle intake test while DBA/2J mice did not. In fact, C57BL/6 mice demonstrated an increased preference for saccharin in the saline and all cocaine groups with the exception of the highest 30 mg/kg cocaine group (there was no significant difference between dH₂O and saccharin in this group). DBA/2J mice did not show preference for saccharin in any of the groups and the 20 mg/kg and 30 mg/kg cocaine groups demonstrated a preference for dH₂O. Similarly, NSE-tTA x TetOp- Δ FosB mice in Experiment 5.3 with elevated Δ FosB demonstrated a preference for a natural reward (.01 M sucrose) whereas control mice did not.

Selective Increase in Reward Sensitivity and Suppression

As the majority of the experiments in this dissertation involve comparisons between different strains of rat or mouse, the issue of whether differences between strains, other than reward sensitivity, impact the obtained data must be considered. For example, in the case of C57BL/6 and DBA/2J mice, there is a considerable amount of literature detailing differences between these strains across a wide range of topics including learning and memory, taste perception, and impulsivity (Forgie et al., 1988; Cabib et al., 2002; Passino et al., 2002; Helms et al., 2006; Sung et al., 2008). The possibility exists that the attenuated suppression observed in the C57BL/6 mice, relative to DBA/2J mice, reflects other effects that these phenotypic differences may have on behavior. In the case of impulsivity, Experiment 4.2 was conducted specifically to address this concern. The results demonstrated that C57BL/6 mice are less impulsive compared with DBA/2J mice and that an inability of C57BL/6 to delay intake likely did not contribute to the attenuated suppression observed in Experiment 4.1. Since considering all possible confounding differences and experimentally testing them was not possible here, Experiment 5.1 was conducted to evaluate suppression in NSE-tTA x TetOP- Δ FosB transgenic mice with a selective increase in reward sensitivity. As such, control and experimental groups in Experiment 5.1 were identical with the exception of this experimental manipulation (elevated striatal Δ FosB) and the attenuated suppression observed in the experimental group could be attributed with greater confidence to the increased sensitivity to reward.

Transition from Controlled to Compulsive Drug Use

Differences in the effect of reward sensitivity on drug-induced suppression of intake of a non-drug cue (i.e., attenuation versus facilitation) may, in part, reflect differences in the state of

drug use (controlled as opposed to compulsive) in these experiments. Recently, differences in drug availability in laboratory animals (e.g. short access versus long access) have been shown to produce different states of drug use. For example, short access (< 3 hours/daily) produces stable, controlled drug use whereas extended, longer access (> 3 hours/daily) results in a pattern of escalating, compulsive drug use more characteristic of drug addiction (for review, see Ahmed, 2005). Extended access to drugs in rats has been shown to produce greater self-administration, motivation for drug, drug seeking behavior during extinction, and drug-induced reinstatement compared with rats that received short access to the drug (Ahmed and Koob, 1998, 1999; Ahmed et al., 2000; Paterson and Markou, 2003; Vanderschuren and Everitt, 2004). In addition, rats with extended access to cocaine are less sensitive to cues associated with footshock during drug extinction (Vanderschuren and Everitt, 2004).

Based on the extended access data cited above, a hypothesis regarding the differential effect of increased reward sensitivity observed in the current dissertation emerges. It is possible that increases in sensitivity to reward may be able to attenuate drug-induced suppression of CS intake (and drug-taking, as shown in female rats) in the controlled state of drug use but this effect can be overridden and instead produces facilitation in the compulsive drug state. The reward comparison experiments conducted in C57BL/6 and DBA/2J mice (Experiment 4.1) and in the NSE-tTA x TetOp- Δ FosB mice (Experiment 5.1), as well as the cited examples of attenuation in female rats (Cason and Grigson, *manuscript in prep*) and mice (unpublished data), used a short access period to the drug (passive i.p. injection or 1 hour self-administration). As such, the subjects in these experiments are presumably in the controlled state of drug use and may be influenced by the presence of a non-drug reward (i.e., attenuated suppression). Conversely, although the Lewis rats, as well as the chronic morphine treated Sprague-Dawley rats cited as an example of facilitation (Grigson et al., 2001), received similar short access to drug during testing, there is reason to believe that these rats may have transitioned from controlled drug use to

compulsive drug use. Specifically, rats in the Grigson et al. (2001) study received chronic morphine treatment via subcutaneous pellet implantation for 5 days prior to saccharin-cocaine pairings. This route of administration would have provided continuous, cumulative exposure to morphine (75 mg/day) over 5 days and could have produced a compulsive state of drug use in which the rats then were tested. Similarly, Lewis rats have been shown to have numerous neurochemical and neuroanatomical adaptations in the drug-naïve state that parallel those in animals with chronic drug exposure (see Introduction, Chapter 2). As such, Lewis rats likely serve as a model of rats in the compulsive drug state. This hypothesis is consistent with the observed facilitation of suppression of intake of a CS paired with cocaine (Experiment 2.1) and sucrose (Experiment 2.3), as well as the tendency to express greater successive negative contrast effects in Experiment 3.1. Of course, such a hypothesis is testable. Future directions of study could focus on the testing of C57BL/6 and DBA2 mice, NSE-tTA x TetOp- Δ FosB mice, and female Sprague-Dawley rats following chronic exposure to drug with the prediction that the attenuated effects would be reversed.

Conditioned Taste Aversion, Cue-induced Withdrawal, and the Devaluation of Natural Rewards by Drugs of Abuse

As discussed in Chapter 1, the hedonic properties that mediate drug-induced suppression of intake of a CS are still under debate. Original interpretation of drug-induced suppression labeled it conditioned taste aversion; that is, the acute aversive properties of the drug were thought to transfer to the CS and subsequently decreased intake of the associated taste cue. The reward comparison model, on the other hand, states that the highly rewarding properties of the drug are responsible for suppression of intake. In addition to resolving paradoxical findings that arose in the literature from the CTA interpretation, the reward comparison hypothesis also

explains a growing body of evidence that aligns drug-induced suppression with the suppressive effects of known rewarding, as opposed to aversive, unconditioned stimuli (see Introduction, Chapter 1). We believe that the data in the current dissertation adds to that body of literature. Experiments in the current thesis that evaluate sensitivity to reward and drug-induced suppression of intake of a CS have been accompanied by parallel studies using a known aversive agent, LiCl, as the US for comparison. Differential sensitivity to rewards did not significantly affect suppression in these experiments. Lewis and Fischer rats did not differ in LiCl-induced suppression in Experiment 2.2 and C57BL/6 and DBA/2J mice did not differ in LiCl-induced suppression in Experiment 6.3. Similarly, NSE-tTA x TetOp- Δ FosB mice with elevated striatal Δ FosB and their controls did not differ in LiCl-induced suppression in Experiment 5.2. The reward comparison hypothesis predicts this. There is no reason to believe that sensitivity to reward would necessarily affect an aversive, associative process. The experiments in this dissertation involving drug-induced suppression, on the other hand, were affected by reward sensitivity, regardless of whether it was in the form of attenuation or facilitation. If an aversive property of the drug was driving suppression, the CTA theory would predict parallel findings with drugs and LiCl. The reward comparison theory, however, predicts that drug suppression, but not a LiCl-induced CTA, would be affected by strain differences in sensitivity to reward. While the hedonic properties mediating drug-induced suppression has proven complex and there has yet to be irrefutable data to end the debate, we believe the current literature and the data in this dissertation supports the position that the intensely rewarding properties of the drug are primarily responsible for drug-induced suppression.

The mediation of drug-induced suppression through rewarding properties of the drug does not, however, negate the possibility of the development of an aversive conditioned compensatory state. In fact, it is very possible that some degree of cue-induced withdrawal may develop to the non-drug CS as a counter-regulatory response to its association with the intensely

powerful drug of abuse. The signaled wait to get the more preferred drug that presentation of the CS elicits may result in an aversive affective state that behaviorally manifests as, at least part of, the observed suppression of intake (i.e., consumption of the CS becomes less important than the fact that it cues imminent access to the drug; access that is delayed for the duration of CS presentation). In fact, recent evidence shows that rats will gape to a palatable saccharin cue paired with cocaine self-administration if the CS is infused directly into the oral cavity (Wheeler et al., 2008).

In this context, the facilitated suppression observed in Chapters 2 and 3 could be viewed as an exacerbation of the conditioned compensatory response while the attenuation observed in Chapters 4 and 5 could be viewed as an alleviation of, or protection against, this negative state. In support, there is a large body of literature that demonstrates the disruptive effect of alternative, non-drug rewards on drugs of abuse. For example, access to a glucose/saccharin mixture immediately prior to access to cocaine can decrease relapse to the drug (Liu and Grigson, 2005) and a history of access to sucrose (approximately 10 days) can decrease the willingness of a rat to work for cocaine (Twining, 2007). In addition, a single exposure to saccharin has been shown to blunt the increase of dopamine in the NAc normally produced by morphine (Grigson and Hajnal, 2007b). Most strikingly, rats simultaneously presented with an intense sweet solution (saccharin or sucrose) and cocaine consume the sweet over the drug despite the fact that they had previously experienced both (Lenoir et al., 2007). This disruptive effect by alternative natural rewards is not limited to sweets, however. Enriched environments (e.g., larger cage, conspecifics, a running wheel, a small house, and several novel toys of varying colors and shapes) also prevents the establishment of preference for places associated with heroin (El Rawas et al., 2008), and can also reverse established place preferences to cocaine and interfere with acquisition and relapse to cocaine self-administration behavior (Bardo et al., 2001; Green et al., 2002; Stairs et al., 2006; Puhl et al., 2008; Solinas et al., 2008). Although sweets and environmental enrichment (EE) are

of differing modalities (i.e., gustatory vs. contextual), both have been shown to affect response to drugs of abuse at the molecular level. As mentioned previously, a single exposure to saccharin blunts dopamine elevation in the NAc normally produced by morphine (Grigson and Hajnal, 2007a). Similarly, EE decreases the number of DA cells in the striatum as well as the number of DAT per DA cell (Bezard et al., 2003). In addition, while EE increases basal levels of Δ FosB in the NAc, it decreases elevation of Δ FosB in response to cocaine and eliminates elevation of the immediate early gene zif-268 (Solinas et al., 2009). While the possibility exists that the attenuated effect observed in Chapters 4 and 5 is the result of a protective effect of alternative rewards against cue-induced withdrawal, this interpretation requires further exploration and testing to validate cue-induced withdrawal in drug-induced suppression of intake of a natural reward cue.

Regardless of the interpretation, the data in Chapter 6 demonstrated that the attenuated effect relies on the comparison of two rewards. That is, the hedonic value of the CS and the US must be rewarding for the development of the attenuated effect observed in the reward sensitive C57BL/6 mice in Experiment 4.1. The use of an aversive CS (QHCl) in Experiment 6.2 and the use of aversive US (LiCl) in Experiment 6.3 resulted in similar suppression of intake of the CS in C57BL/6 and DBA/2J mice. These findings suggest that the comparison of the CS and the US is necessary for changes in reward sensitivity to attenuate suppression of CS intake.

Implications for Addiction in Humans

The data in the current dissertation indicate that greater general sensitivity to rewards may initially work to protect an addict from the devaluation of natural rewards by drugs of abuse. As seen in Chapters 4 and 5, the presence of a rewarding cue attenuates drug-induced suppression of that cue in reward preferring mice. Conversely, drug-induced suppression is unaltered if the cue is aversive (Chapter 6). Similarly, there is a large body of data demonstrating disruptive

effects of natural rewards on a wide range of drug-related behaviors in rodents (Introduction, Chapter 1) and that alternative rewards are effective in the treatment of human addiction (Higgins et al., 2002; Stitzer and Vandrey, 2008). The attenuated drug-induced suppression, and by extension devaluation of natural rewards, may be due to the presence of incentive relativity discussed previously (Introduction, Chapter 1). Prior to exposure to drugs of abuse, and likely in the early stages of drug use, an addict's brain is capable of comparison of rewards in the environment and, as a result, adjusts hedonic values of each reward accordingly. Such processing would influence the perceived value of the drug and, at least to some extent, the drug-related phenotypic behaviors dependent on this perception. Natural rewards, then, especially if one has generalized sensitivity to rewards, should be able to influence the relative value of the drug and reduce the devaluation of natural rewards in the addict. However, the data in Chapters 2 and 3 suggests that this protective effect may not be sustainable. As the addict transitions from a controlled state of drug use to the compulsive state of drug use characteristic of addiction, the ability of the natural reward to affect the drug may be eliminated. That is, in response to chronic exposure to the drug, incentive relativity processing may likely be disrupted as the brain is remodeled to most efficiently respond to the overwhelmingly powerful drug of abuse. At this stage, it is likely that greater, general sensitivity to rewards may work against an addict and facilitate the drug-induced devaluation of natural rewards. Of course, the degree and manner in which this protective effect would diminish likely depends upon numerous variables regarding the alternative reward, but the data in the current dissertation suggests that the presence of alternative, natural rewards, even if they cue imminent availability of drug, work to counter the devaluation of natural rewards observed in human addicts but that this protection may eventually be overridden.

Summary and Conclusions

Rats and mice suppress intake of a natural reward cue when it is paired with a drug of abuse. Contrary to traditional CTA interpretation, the reward comparison hypothesis states that suppression of intake of the cue occurs in response to anticipation of the rewarding properties, as opposed to association with the aversive properties, of the drug. This drug-induced suppression of intake is viewed as a model of the drug-induced devaluation of natural rewards observed in human drug addiction. The aim of the current dissertation was to evaluate the effect of increased reward sensitivity in the reward comparison paradigm using rat and mouse strains with differential preference for rewards. The results of the conducted experiments present a number of findings. First, the data in the current dissertation aligns the suppressive effects of drug with a rewarding, not aversive, US. That is, differential sensitivity to reward had differential effects when drug, but not LiCl, was used as the US. Second, differential sensitivity to reward can result in either facilitation (Chapters 2 and 3) or attenuation (Chapters 4 and 5) of drug-induced suppression of intake of a natural reward (see Table 1). The direction of this effect is hypothesized to reflect whether the subject has transitioned from a controlled state of drug use to a compulsive state. Finally, the attenuated effect observed in Chapter 4 was shown, with transgenic mice in Chapter 5, to be due to a selective increase in reward preference (opposed to possible confounding strain differences in impulsivity, for example) and this attenuated effect depended on the comparison of two rewards as it was eliminated in Chapter 6 when the hedonic valence of either the CS or the US was aversive.

Table 1. Sensitivity of non-drug and drug rewards and drug-induced suppression of non-drug cue.

<u>Species / Strain</u>	<u>Compared with</u>	<u>Non-drug Reward</u>	<u>Drug Reward</u>	<u>Suppression of CS cue</u>
Lewis rats	Fischer 344 rats	↑	↑	↑
Sprague-Dawley rats (Chronic morphine)	Sprague-Dawley rats (placebo)	↑	↑	↑
Female Sprague-Dawley rats	Male Sprague-Dawley rats	↑	↑	↓
C57BL/6 mice	DBA/2J mice	↑	↑	↓
Transgenic Δ FosB mice	Control mice	↑	↑	↓
Female WT GABA _A KO mice	Male WT GABA _A KO mice	↑	↑	↓

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Award Total: \$80,305.00

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