

The Pennsylvania State University
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**FACTORS THAT CONTRIBUTE TO INDIVIDUAL DIFFERENCES
IN RESPONSIVENESS TO COCAINE AND NATURAL REWARDS IN A
REWARD COMPARISON PARADIGM**

A Thesis in

Neuroscience

by

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Abstract

In rats, intake of an otherwise normally preferred saccharin solution is reduced when presentation of the saccharin solution is repeatedly followed by the administration of a drug of abuse, such as morphine, heroin, or cocaine. Recent data suggest that avoidance of the taste cue occurs because the rewarding properties of the saccharin are devalued in anticipation of the availability of the more rewarding drug of abuse. This 'reward comparison' paradigm serves as the first and only model of devaluation of natural rewards by drugs of abuse. Interestingly, while drug-induced suppression of saccharin intake is a robust phenomenon, clear individual differences have emerged. Indeed, approximately half of the Sprague-Dawley rats greatly avoid intake of the saccharin cue following taste-drug pairings (referred to as the large suppressers), while the other half of the rats (referred to as the small suppressers) do not. In addition, greater avoidance of the saccharin cue by the large suppressers is associated with greater saccharin cue induced elevations in circulating corticosterone (CORT) at test and, importantly, with greater cocaine self-administration behavior, as well. Although it is clear that humans also exhibit individual differences in the susceptibility to drug-taking behavior, it is not clear what mediates these differences in behavior or what factors contribute to the transition from recreational use to addiction. It is possible that individual vulnerability to addiction is mediated, in part, by greater drug craving or withdrawal when presented with conditioned drug cues. In the present paradigm, it is hypothesized that greater avoidance of the saccharin cue reflects better

learning of the taste cue-drug association and this learning is facilitated by increased circulating levels of CORT. The present set of experiments addressed this hypothesis by employing behavioral and genetic methods to examine the nature of the differences observed in the large and small suppressers. The first few sets of experiments were designed not only to evaluate the nature of the CORT response in the phenomenon but also to determine whether the unconditioned CORT response to cocaine can predict which rats will go on to become large or small suppressers. The results of these experiments support the conclusion that greater elevations in both the conditioned and unconditioned CORT response are associated not only with greater cocaine self-administration behavior but also with greater suppression in saccharin intake. Thus, higher levels of circulating CORT result in being more prone to drug-taking behavior and subsequent devaluation of a naturally rewarding saccharin solution. In a separate experiment, the general preference for sweets was tested for its predictive value in our paradigm. Greater intake of sucrose, however, was not associated with greater cocaine-induced avoidance of saccharin intake. The second set of experiments was designed to determine the extent to which genetic background would influence responsiveness to cocaine and subsequent devaluation of the saccharin reward. The results of a three generation selective breeding study revealed significant differences in the large and small suppresser lines by the second generation, suggesting that genetic makeup alone can influence behavior in the reward comparison paradigm. This conclusion was supported by the results of the final experiment that tested several inbred mouse strains

(C57BL/6J, DBA/2J, and Sv129J) in the reward comparison model that revealed significant strain differences in saccharin intake behavior. Taken together, these findings support that individual differences in cocaine-induced avoidance of saccharin intake are mediated by differences in genetic makeup, CORT, and learning (i.e., conditioning). Implications of these findings for drug addiction are discussed.

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LIST OF ABBREVIATIONS

ACE	anticipatory contrast effect
ACTH	adrenocorticotropin hormone
ANOVA	analysis of variance of the means
cm	centimeter
CORT	corticosterone
CRF	corticotrophin releasing factor
CS	conditioned stimulus
CTA	conditioned taste aversion
DA	dopamine
dH ₂ O	distilled water
FR	fixed ratio
HPA	hypothalamus-pituitary-adrenal
hr	hours
I.D.	inner diameter
ip	intraperitoneal
iv	intravenous
ISI	interstimulus interval
kg	kilogram
LiCl	lithium chloride
M	molar
min	minutes
mg	milligram
ml	milliliter
O.D.	outer diameter
RC	reward comparison
RCP	reward comparison paradigm
S1	first selectively bred generation
S2	second selectively bred generation
S3	third selectively bred generation
SA	self-administration
sc	subcutaneous

SEM	standard error of the mean
sec	seconds
US	unconditioned stimulus
VPMpc	parvicellular region of ventral posteromedial nucleus of the thalamus
vs	versus

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“What if these guys in white coats who bring us food are, like, studying us and we’re part of some kind of big experiment?”

- unknown source

Part 1

INDIVIDUAL DIFFERENCES IN REWARD COMPARISON

Chapter 1

Introduction

Contrast:

Contrast occurs when, just as the word implies, there is a striking difference in responding as a result of things that are being compared. All organisms, at some level, are programmed to compare the relative value of one stimulus over another to maximize their chance of survival and improve their quality of life. One of the clearest examples of the role that contrast plays in survival is easily observed in ingestive behavior. Animals in the wild are constantly forced to make choices for one kind of food over another. To do so, the organism must evaluate the relative value of one kind of food reward compared with a different kind of food reward (i.e., grain vs. fruit, vegetable vs. meat, a low vs. a high concentration of sugar). This form of reward comparison is commonplace and well documented (for a review see Flaherty 1996).

Indeed, several paradigms for contrast behavior have been established in the laboratory (Flaherty, 1996). Three major types of consummatory contrast behavior are successive negative contrast, simultaneous contrast, and

anticipatory contrast. In successive negative contrast, rats with a history of access to a highly preferred 32% sucrose solution are unexpectedly downshifted to a lesser reward, such as 4% sucrose. These rats consume far less of the 4% sucrose compared with rats that have only experienced the lesser 4% sucrose reward (Flaherty and Checke 1982; Flaherty 1996). A simultaneous contrast effect occurs when rats are given the opportunity to compare two disparate levels of reward within a single daily session (Flaherty and Rowan 1986; Flaherty 1996). Finally, in anticipatory contrast, most relevant to the present set of studies, rats are given daily access to a sweet tasting reward such as saccharin followed by access to a more preferred food reward such as sucrose. An anticipatory contrast effect occurs when intake of the otherwise rewarding saccharin solution is suppressed in anticipation of the more preferred sucrose reward (Flaherty and Checke 1982; Flaherty and Rowan 1986; Flaherty and Grigson 1988).

Although in the examples above, stimuli are being compared within the same modality (gustatory), disparate rewards can also be compared across different modalities. Examples of contrast across different modalities can easily be seen with naturally motivated or goal directed behaviors (e.g., food intake, fluid intake, mating, exercise). Since, usually, only one goal directed behavior can be executed at a time, the most appropriate behavior for any given time must be selected over others. There are many factors (i.e., hunger, satiety, thirst, hormones, etc.) that can and will influence decisions about which behavior is most important. Naturally, priority is often given to the behavior that is perceived to be most rewarding or beneficial to survival. The relative value placed on one

reward compared with another is a dynamic function and depends on the state of the organism and also on experiences with other rewards. As such, animals must compare rewards over time and responding for one reward can change as a result of having experience with another reward.

There is now evidence that similar mechanisms mediate the rewarding properties of drugs of abuse and natural rewards such as food, water, and sweets (for review see Grigson 2002). Indeed, studies have shown that experience with a rewarding drug of abuse can result in devaluation of a natural reward and, although rare, experience with natural rewards can, in turn, result in devaluation of a drug of abuse (Carroll, Lac et al. 1989; Cosgrove, Hunter et al. 2002; Grigson and Twining 2002; Liu and Grigson 2005). The present set of investigations utilizes a paradigm in which a rodent, specifically, either a rat or a mouse, is placed into a situation where two rewards are presented, evaluated, and compared over time. In this model, the first reward is a sweet tasting saccharin solution and a drug of abuse serves as the second reward.

The Model: A Reward Comparison Paradigm.

The model used for all of the experiments presented in this dissertation revolves around a very specific behavior observed when pairing access to a naturally rewarding saccharin solution with administration of a drug of abuse. Specifically, rats will avoid intake of a normally preferred saccharin solution when paired with a drug of abuse such as morphine, cocaine, amphetamine, nicotine, or heroin (Le Magnen 1969; Cappell and LeBlanc 1971; Cappell and LeBlanc

1977; Grigson, Twining et al. 2000). This avoidance behavior takes place during classical conditioning where the saccharin solution serves as a gustatory conditioned stimulus (CS) (Sarnyai, Biro et al.) that is repeatedly paired with a drug of abuse that serves as an unconditioned stimulus (US). As pairings go on, the saccharin CS becomes a cue that is not only associated with but also is predictive of the coming of the drug US. Although the resulting behavior, avoidance of the saccharin solution, has been described for many decades, exactly what mediates this avoidance has yet to be established.

This phenomenon has long been interpreted as a conditioned taste aversion (Nachman, Lester et al. 1970; Cappell and LeBlanc 1971; Goudie, Dickins et al. 1978). A major reason for this interpretation is that the same behavior, avoidance of a gustatory CS, is observed when a tastant is paired with aversive stimuli such as lithium chloride (LiCl) or gamma radiation (Garcia, Kimeldorf et al. 1955; Nachman and Ashe 1973; Carroll and Smith 1974). As a result, it is thought by many that, despite the well known rewarding properties of drugs of abuse (see van Ree 1979 for review), aversive drug properties are responsible for avoidance of the gustatory CS.

Nevertheless, there is growing evidence to suggest that this phenomenon is mediated by the drug's rewarding, rather than aversive, properties (Parker 1995; Grigson 1997; Grigson and Freet 2000; Grigson, Lyuboslavsky et al. 2000). In light of such findings, Grigson (1997) has proposed the reward comparison hypothesis that states that rats avoid intake of an otherwise palatable saccharin solution following taste-drug pairings because the value of

the saccharin CS pales in anticipation of the availability of the highly rewarding drug of abuse. The reinterpretation underlying the reward comparison hypothesis stems largely from another phenomenon referred to as anticipatory contrast, mentioned above. This phenomenon also involves classical conditioning where a saccharin solution serves as the CS and is paired with a preferred sucrose solution that serves as the US. Thus, the anticipatory contrast design is set up much like the standard taste-drug design except that sucrose, rather than a drug of abuse, serves as the US. As the saccharin CS comes to predict access to the sucrose solution over repeated daily pairings, an anticipatory contrast effect (ACE) occurs when intake of the otherwise palatable saccharin CS is suppressed in anticipation of the highly preferred sucrose US (Flaherty and Checke 1982; Flaherty and Grigson 1988). The phenomenon is thought to involve a mechanism that detects and compares the relative hedonic value of the CS with the memory of the US (Flaherty, Turovsky et al. 1994) and occurs because the perceived value of the saccharin CS pales in comparison with the highly rewarding sucrose US.

To summarize, all three agents (morphine, LiCl, and sucrose) similarly suppress intake of an otherwise palatable tastant such as saccharin. Figure 1.1 depicts the results of 3 experiments in which the suppressive effects of an aversive agent, a rewarding agent, and a drug of abuse appear indistinguishable. Thirty-six rats were deprived to 82% of their free-feeding body weight. All rats were given 3 or 5 min access to the CS (0.15% saccharin) and then given one of the 3 USs: Rats in the left panel were injected i.p. with either saline or 0.009 M

LiCl, rats in the center panel were given a second 3 min access period to either 0.15% saccharin or 32% sucrose, and rats in the right panel were injected i.p. with either saline or 15 mg/kg morphine. Pairings occurred either every day or every other day. The data were analyzed using a repeated measures analysis of variance (ANOVA) and post hoc comparisons for each experiment were made using the Newman Keuls tests where appropriate. As is apparent, all USs caused a decrease in intake of the saccharin CS relative to controls. Intake of the saccharin CS was reduced by LiCl (aversive conditioning), by sucrose (appetitive conditioning), and by morphine. As described above, for over three decades it was believed that the suppression resulting from morphine administration resulted from a CTA. Recent evidence, however, indicates that this suppression is mediated by appetitive, rather than aversive, US properties (akin to that induced by a rewarding sucrose US).

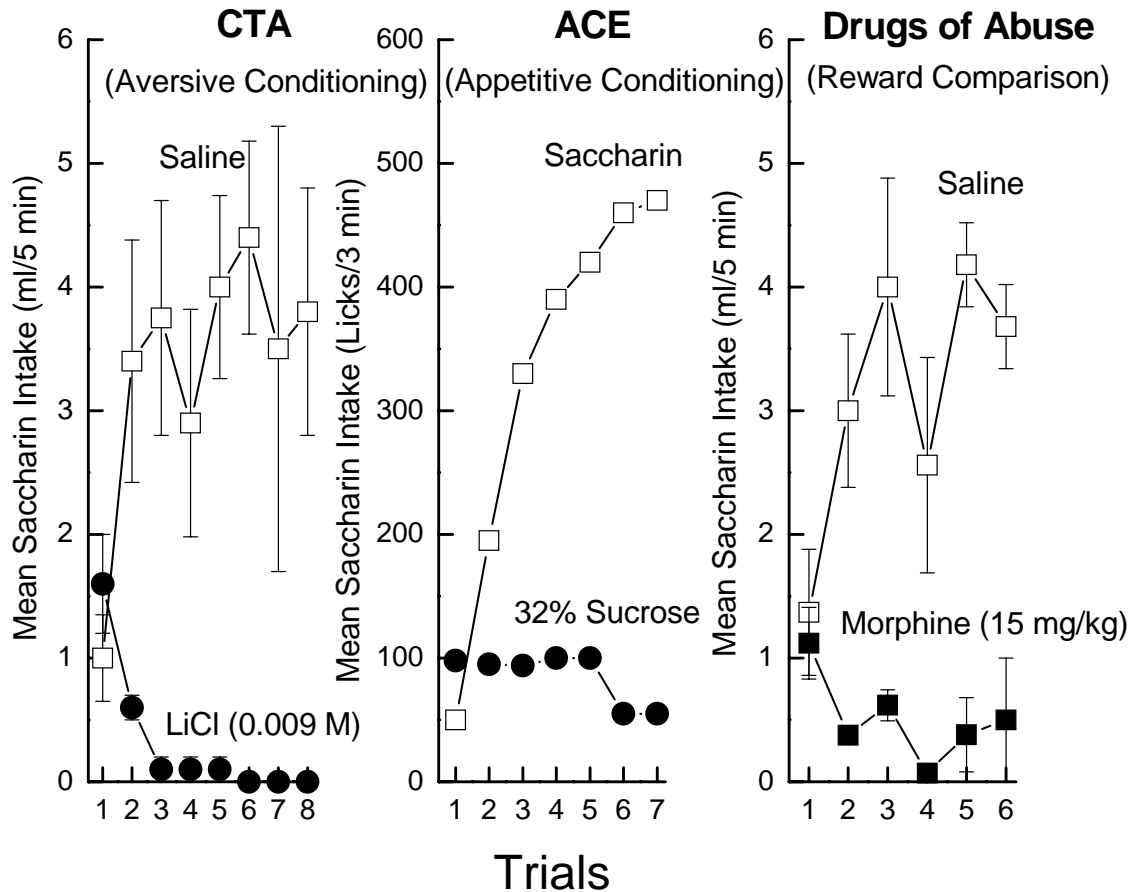


Figure 1.1: Conditioned taste aversions, anticipatory contrast effects, and reward comparison effects yield similar data. The left panel illustrates LiCl-induced suppression of saccharin intake. The middle panel shows sucrose-induced suppression of bottle 1 saccharin intake and the right panel shows the suppressive effects of morphine. In all cases the saccharin cue is avoided as it predicts the US after only one pairing.

A growing body of evidence in support of the reward comparison hypothesis comes from studies designed to investigate the role of the gustatory thalamus in reward comparison behavior. The gustatory thalamus, contained in the parvicellular component of the ventral posteromedial nucleus (VPMpc) of the thalamus, relays taste information from the pontine parabrachial nuclei to the gustatory cortex (Norgren 1984; Kosar, Grill et al. 1986; Norgren 1995). Although very little is known about the neural substrates of reward comparison behavior, it has been shown that bilateral lesions of the gustatory thalamus disrupt the suppressive effects of sucrose and morphine but not of LiCl (Flynn, Grill et al. 1991; Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005). These data reveal that the neural mechanisms underlying sucrose and drug-induced suppression of saccharin intake differ from those underlying a LiCl-induced CTA.

Just as gustatory thalamus lesions differentiate the mechanisms underlying sucrose, drug- and LiCl-induced suppression in saccharin intake, there are several other factors that similarly support the reward comparison hypothesis. For example, the suppressive effects of sucrose and drugs of abuse, but not LiCl, are affected by the nature of the CS, the caloric value of the CS, and the deprivation state of the animal (Flaherty, Grigson et al. 1991; Gomez and Grigson 1999; Grigson, Lyuboslavsky et al. 1999). Furthermore, the suppressive effects of sucrose and cocaine, but not LiCl, are exaggerated in reward-preferring Lewis rats and in Sprague-Dawley rats that have been treated with chronic morphine (Glowa, Shaw et al. 1994; Grigson and Freet 2000; Grigson, Wheeler

et al. 2001). Taken together, these data support that drug-induced suppression of saccharin intake is different from a LiCl CTA and more similar to a sucrose-induced ACE. While evidence to support the reward comparison hypothesis continues to grow, utilization of the experimental design as a potential model for drug abuse also continues to be explored.

Clinical and Social Implications of the Reward Comparison Paradigm:

The reward comparison paradigm serves as a model for two important features of drug addiction in humans. The first is cue-induced craving, where the tastant serves as a cue to predict the availability of the drug of abuse. This is important because addiction is a disease of chronic relapse. Addiction is often characterized by a vicious cycle in which periods of compulsive drug use are followed by periods of drug avoidance, or abstinence, which ultimately come to an end with relapse, where the individual reinstates drug-seeking behavior (Gawin and Kleber 1986). Several paradigms exist to model drug craving influenced by factors such as exposure to stress, the drug itself, and contextual stimuli associated with the drug-taking behavior (de Wit and Stewart 1981; Childress, Ehrman et al. 1992; Ehrman, Robbins et al. 1992; Erb, Shaham et al. 1996; Ahmed and Koob 1997; Shaham, Erb et al. 2000). The present paradigm, however, has been the first to model cue-induced craving and relapse using a gustatory cue (Grigson and Twining 2002). Indeed, our laboratory has recently discovered that use of the saccharin cue is highly effective in eliciting

reinstatement of drug seeking behavior in rats even after 6 months of abstinence in rats.

The reward comparison paradigm also serves as a model for drug-induced devaluation of natural rewards. In fact, the present paradigm is the first and only animal model available to study the mechanisms by which anticipation of the availability of a drug of abuse can quickly result in the devaluation of natural rewards. Many individuals who suffer from drug addiction will neglect otherwise naturally rewarding activities because the drug experience is perceived to be more rewarding than these other activities (Jones, Casswell et al. 1995; see Santolaria-Fernandez, Gomez-Sirvent et al. 1995; Nair, Black et al. 1997). Indeed, human cocaine addicts report that nearly all of their thoughts are focused on cocaine during binges while nourishment, money, loved ones, responsibility and even survival lose all significance (Gawin and Kleber 1986; Gawin and Ellinwood 1988). This feature of drug addiction is very important because the consequences related to neglecting such naturally rewarding activities are devastating to the individual and to society as a whole.

Individual Differences in Responsiveness to Drugs of Abuse:

While drug addiction can be a life-threatening disease, it is also a complex disease that demands attention at the individual level. Only about ten to fifteen percent of humans who initially try alcohol or cocaine become addicted according to estimates made by the National Institute for Drug Abuse. Once addicted, there are large individual differences in successfully abstaining from the drug without

eventual relapse. Like humans, rats also exhibit clear individual differences in responsiveness to drugs of abuse. While, in our laboratory, drug-induced suppression of saccharin intake in rats is a robust phenomenon, clear individual differences in suppression of saccharin intake have emerged. A specific example of differential responsiveness in the reward comparison paradigm comes from a study conducted by Gomez, Leo and Grigson (2000). The results from this study, in fact, provide the preliminary data leading to the work of this thesis.

In the Gomez, Leo, and Grigson (2000) study, the standard taste-drug pairing design was employed in which male Sprague-Dawley rats were given five min access to a saccharin solution and five min later were injected with either morphine or saline. There was one taste-drug pairing a day, occurring every other day, for a total of seven trials. The suppressive effects of morphine were evident by the second trial and the morphine-treated group significantly reduced their intake of saccharin over trials when compared with saline-injected controls. Upon closer inspection of the data, however, it became clear that morphine-treated rats exhibited large individual differences in intake. In fact, the morphine-treated rats actually fell into two distinct populations. This led to the division of the morphine-treated animals into two separate groups (the large suppressers and the small suppressers) based on the median split of saccharin intake data on the final trial. When the data were analyzed under the new criteria, it became evident that the large suppressers consumed significantly less of the saccharin cue than the saline group, while intake of the saccharin cue by the small suppressers did not differ from that of the saline-injected controls. Thus, half of

the rats were sensitive to the suppressive effects of morphine while the other half were not.

Interestingly, there also were individual differences in glucocorticoid hormone levels observed at the end of the experiment. Plasma corticosterone (CORT) levels were assessed to determine whether the saccharin cue would induce conditioned elevations in hormone levels following taste-drug pairings. The results showed that while small suppressers exhibited only a slight elevation in CORT from pre- to post-conditioning, the large suppressers exhibited a robust elevation in CORT. Results of a correlation analysis revealed a significant negative relationship between CS intake and plasma CORT at test. Thus, individual differences in morphine-induced suppression of saccharin intake were associated with individual differences in elevated CORT levels at test such that greater avoidance of the saccharin CS was significantly correlated with higher levels of circulating CORT following presentation of the saccharin cue.

The first experiment of this dissertation (presented in Chapter 2) was designed, in part, to test whether significant individual differences in reward comparison behavior also would occur when cocaine is administered as the US instead of morphine.

Factors that Contribute to Individual Differences in Responsiveness to Drugs of Abuse:

The data presented above show that rats, like humans, exhibit individual differences in responsiveness to drugs of abuse. Although it is not clear why some individuals are more vulnerable than others to drug-taking behavior, there are two types of factors known to contribute to individual variability in any behavior or phenotype. The first set of factors can be described as external or environmental and include variables such as being exposed to the drug itself, peer pressure, stress, and lack of emotional support from family and friends. The second set of factors is internal, determined by each individual's genetic composition. Differences in genetic composition ultimately govern differences in how an organism responds to changes in the environment. In turn, changes in the environment differentially influence and modify gene expression within an organism. It is, therefore, very difficult to study the issue of individual differences in behavior without addressing both environmental and genetic factors that are constantly working to affect each other.

One factor that is under the influence of both genetic and environmental factors and is known to affect drug sensitivity is the hormone, corticosterone (CORT). CORT is the rat equivalent of cortisol in humans and is a steroid glucocorticoid hormone produced by the adrenal cortex. The CORT response, which is just one part of the prototypical stress response, depends on both central and peripheral limbs of the stress system (see Munck, Guyre et al. 1984; Stratakis and Chrousos 1995; Sapolsky, Romero et al. 2000, for reviews).

Neurons in the paraventricular nuclei of the hypothalamus aid in the central coordination of the stress system while the hypothalamic-pituitary-adrenal (HPA) axis is central to the peripheral limb of the endocrine stress system. Briefly, the stress response is triggered by any changes in the external or the internal environment of the organism (e.g., internal/external physical bodily injury, emotional triggers such as anxiety, fear, anger, etc.). These changes lead to the release of corticotropin releasing factor (CRF) along with other hormones by the paraventricular nuclei of the hypothalamus into the portal circulation where they travel to the pituitary. A few seconds later, CRF triggers the release of adrenocorticotropin hormone (ACTH) by the anterior pituitary gland. ACTH then enters the circulatory system and becomes the key regulator of glucocorticoid secretion by the adrenal cortex. Once released, CORT produces several effects in the organism such as modifying glucose utilization (Exton 1987; Bryan 1990; Dimitriadis, Leighton et al. 1997), acting as anti-inflammatory agents, and modifying the immune response (Reichlin 1993; McEwen, Biron et al. 1997). Moreover, increases in CORT levels following acute stress works in the brain to enhance learning and memory through its actions in both the hippocampus and the mesolimbic dopamine system (McEwen and Sapolsky 1995; Barrot, Abrous et al. 2001; Shors 2001). After the hormone completes these various tasks, the CORT response is turned down through a negative feedback mechanism involving the hippocampus (Sapolsky, Krey et al. 1984).

Indeed, several findings provide evidence to suggest an important role for glucocorticoids in drug sensitivity. First, the acute administration of cocaine alone

is known to produce elevations in ACTH and CORT by activating the HPA axis (Sarnyai, Biro et al. 1992; Teoh, Sarnyai et al. 1994; Mantsch, Schlussman et al. 2000). Second, when morphine or cocaine is repeatedly paired with a stimulus, such as saccharin as described above (Gomez, Leo et al. 2000) or a peppermint scented chamber (DeVries, Taymans et al. 1998), presentation of the drug-associated stimulus (or cue) also will come to produce a conditioned rise in CORT. Third, not only will drugs of abuse and associated cues increase CORT, but the exogenous administration of CORT, in the absence of stress, has been shown to facilitate acquisition and maintenance of drug (i.e., cocaine) self-administration behavior (Goeders and Guerin 1996; Goeders and Guerin 1996; Deroche, Marinelli et al. 1997). Taken together, these data implicate a mediating role for CORT in contributing to responsiveness to drugs of abuse, thus, encouraging us to investigate the role of CORT in the reward comparison paradigm.

First, it is important to determine to what extent CORT and differences in HPA axis sensitivity contribute to individual vulnerability for drug-taking behavior. One study that investigates the relationship between CORT and drug self-administration behavior was conducted by Piazza and colleagues (1991). Piazza et al. (1991) found that CORT levels in response to stress can determine individual vulnerability to amphetamine self-administration. Specifically, rats that exhibited enhanced locomotor activity and prolonged elevations in CORT after exposure to a novel environment (referred to as High Responders) acquired drug self-administration behavior more readily than rats that exhibited a weaker

behavioral and hormonal response (referred to as Low Responders). In a separate study, repeated administration of CORT was found, furthermore, to sensitize the locomotor response to amphetamine (Deroche, Piazza et al. 1992). It was later discovered that greater individual reactivity to novelty also can predict greater vulnerability to the psychomotor effects of morphine (Deroche, Piazza et al. 1993). These findings provide further evidence that the propensity to self-administer drugs of abuse is linked to activity of the HPA-axis.

The experiments presented in Chapters 2 and 3 were designed to investigate the role of CORT in contributing to individual differences in reward comparison behavior specific to the drug, cocaine. The first objective in Chapter 2 determines whether CORT is elevated following pairings with cocaine, as it was with morphine, and whether similar individual differences in saccharin intake and CORT also would occur. Both chapters attempt to determine the nature of CORT elevations following taste-drug pairings and begin to consider the predictive value of a particular hormonal profile for rats that are more sensitive to the reinforcing properties of cocaine. To this end, the reward comparison paradigm is utilized in an active (i.e., self-administration) design rather than a passive (i.e., injection) design to better model the human condition. In so doing, these experiments were designed to explore the role for CORT not only in the individual propensity to self-administer cocaine but also in the devaluation of natural rewards in anticipation of the availability of the drug.

Another index that is correlated with individual differences in drug self-administration behavior is preference for sweet substances. In rats, greater

consumption of sucrose is associated not only with greater locomotor response to amphetamine but also with greater self-administration of both amphetamine (Sills and Vaccarino 1994; DeSousa, Bush et al. 2000) and cocaine (Gosnell 2000). In addition, preference for saccharin can predict greater self-administration of both ethanol (Gosnell and Krahn 1992) and morphine (Gosnell, Lane et al. 1995). Interestingly, the presentation of a mild stressor prior to sucrose presentation was necessary to expose significant individual differences in consumption of the sugar. Preference for sweets is considered to be a very important risk factor for drug abuse also in humans. Both cocaine and alcohol abuse are associated with greater preferences for sweets (Hirsch 2002; Janowsky, Pucilowski et al. 2003; Kampov-Polevoy, Garbutt et al. 2003). In fact, even human newborn infants that have had prenatal exposure to cocaine demonstrate an exaggerated sucking response to sucrose (Maone, Mattes et al. 1992). The authors suggest that this heightened response to sweet stimulation might reflect a coping mechanism for the perinatal distress of intrauterine cocaine exposure. This heightened response to sweet stimulation also may reflect altered dopaminergic activity in a shared neuronal pathway that mediates the rewarding properties of both cocaine and sweet taste. Taken together, these data implicate a mediating role for CORT and suggest that preference for granulated sugar and the behavioral and hormonal response to a mild stressor may predict which rats will most greatly avoid the saccharin CS and 'consume' the drug of abuse in the reward comparison paradigm. This hypothesis is addressed in the experiment presented in Chapter 3.

Although it is not clear how CORT influences sensitivity to drugs of abuse, there are data to suggest that this occurs through direct glucocorticoid action in the mesolimbic dopamine system. This system is thought to act as one of the brain's major motivation and reward pathways and is best understood with regard to how it mediates the rewarding properties of drugs of abuse (Wise and Rompre 1989; Koob 1992; Wise 1998). Activation of the mesolimbic dopamine system, in general, begins with increased firing of dopamine neurons in the ventral tegmental area (VTA) of the midbrain which triggers increased dopamine release into the nucleus accumbens (NAc). Cocaine acts to increase dopaminergic neurotransmission of this reward pathway by inhibiting reuptake of dopamine so that the neurotransmitter remains available in the synaptic cleft causing increased postsynaptic stimulation of that pathway (reviewed in Gawin 1991). Glucocorticoids, like cocaine, exert a strong stimulatory effect on mesolimbic dopaminergic transmission (Piazza, Barrot et al. 1996; Piazza, Rouge-Pont et al. 1996). For this reason, one can begin to understand why, at a molecular and physiological level, repeated exposure to stress can lead to an increase in the propensity to develop drug-taking behavior (reviewed in Piazza and Le Moal 1998). Repeated exposure to stress induces a long-term enhancement of the dopamine response to psychostimulants, such as cocaine and amphetamine, that is thought to mediate the often observed behavioral cross-sensitization between psychostimulants and stress (Wilcox, Robinson et al. 1986; Kalivas and Duffy 1989; Sorg and Kalivas 1991). These findings suggest that one major difference between those individuals who are more vulnerable to drug-taking

behavior and those who are not, is how they respond to stress (i.e., differences in HPA axis sensitivity and prolonged elevations in CORT). In support for this conclusion, data show that rats that are vulnerable to drug self-administration behavior not only have higher and longer CORT release but also have higher and longer dopamine release in response to stress than rats that are resistant to drug self-administration behavior (Rouge-Pont, Deroche et al. 1998). Moreover, when stress-induced CORT secretion was blocked in these subjects (i.e., High and Low Responder rats), a selective reduction in the dopamine response occurred only in the addiction-prone rats, causing their dopamine response to become identical to the addiction-resistant rats. The relationships between these three factors (drugs of abuse, mesolimbic dopamine transmission, and CORT) become even more significant in light of the finding that rats will self-administer CORT suggesting that CORT, by itself, is rewarding (Deroche, Piazza et al. 1993). Taken together, these data suggest that the rewarding properties (e.g., euphoria) of a drug of abuse such as cocaine are enhanced in individuals who have greater elevations and prolonged circulation of CORT in response to stress.

Much less is known, however, about how CORT and HPA-axis activity affect devaluation of naturally rewarding stimuli. CORT and 'stress' are factors that heavily influence many complex processes that involve even the highest levels of cognition. Indeed, most cognitive dysfunction and various forms of psychiatric conditions such as depression, anxiety, psychosis, and addiction, are often either triggered or exacerbated by stress and their pathologies likely involve glucocorticoids on some level (Brunson, Kramar et al. 2005; Mason and Beavan-

Pearson 2005; van Praag 2005). By the same token, other complex thought processes such as learning and memory also are facilitated by glucocorticoids. Thus, it seems highly improbable that a complex behavior such as drug-induced devaluation of natural rewards would not be mediated, at some level, by glucocorticoid actions in the brain. The experiments presented in this dissertation are the first and only to explore this unique relationship between CORT and devaluation of a naturally rewarding saccharin solution as it is influenced by a drug of abuse such as cocaine.

Genetic Influences on Individual Differences:

Genetic factors account for 40-60% of the risk of becoming alcoholic (Goodwin 1975; Erwin and McClearn 1981; Kendler, Neale et al. 1994). Although there are less data concerning the genetic basis of addiction to other substances, recent studies reveal even higher estimates of heritability (60-80%) apply to opiate and cocaine abuse (Tsuang, Lyons et al. 1998; Kendler, Karkowski et al. 2000). Data from human genetic studies that are of particular relevance to reward comparison behavior show that not only can preference for sweet substances predict drug taking behavior (esp. with alcohol), but that this preference turns out to be a powerful risk factor for addiction (Kampov-Polevoy, Garbutt et al. 2003; Kampov-Polevoy, Ziedonis et al. 2003). In a patient population admitted for the treatment of alcoholism, drug dependence, or psychiatric conditions, those with a family history of alcoholism were five times more likely to prefer stronger sweet solutions than those without the same history

(Kampov-Polevoy, Ziedonis et al. 2003). These statistics emphasize how powerful an influence one's makeup has over how likely it is to develop drug addiction. Although human twin and pedigree studies have been essential in providing evidence for a strong genetic influence on drug addiction, these studies do not provide information related to mechanisms underlying the gene to behavior pathway. The use of animal models can fill such mechanistic holes by allowing identification of specific genes and exploration of how their functions mediate the development of addiction.

Selective breeding is considered one of the most powerful techniques available for studying the pharmacogenetic basis of drug responses in animal models (Crabbe, Phillips et al. 1990; Crabbe, Belknap et al. 1994). Selective breeding for maximal differences with respect to a specific trait is a very useful strategy for testing specific hypotheses regarding heritability and estimating genetic correlations between different traits. It is also useful for gene mapping efforts in conjunction with quantitative trait loci analyses or for establishing specific lines that can be tested for other indices. The logic behind selective breeding is based on the theoretical expectation that all relevant and available alleles will eventually segregate into each selected line and only those alleles with some influence on the selected trait will be segregated far beyond what would naturally occur from random genetic drift (Henderson 1997). Alleles that do not contribute to variation in the behavior should be randomly distributed between the lines. Therefore, any differences between the lines can theoretically be attributed to a causal network among genes and phenotypes (Plomin 1990).

Rodents have been selectively bred for many drug and taste related traits. Examples include high and low intake of saccharin (Carroll, Morgan et al. 2002), successive negative contrast in consummatory behavior (Flaherty, Krauss et al. 1994), various responses to ethanol (Eriksson 1969; Crabbe, Kosobud et al. 1987; Crabbe, Young et al. 1987), opiates (Belknap, Danielson et al. 1987), nicotine (Smolen and Marks 1991; Smolen, Marks et al. 1994) and cocaine (Smolen and Marks 1991; Marley, Arros et al. 1998). Interestingly, rats selectively bred for higher saccharin intake show more rapid and successful acquisition of cocaine self-administration than those bred for low saccharin intake (Carroll, Morgan et al. 2002). To evaluate the genetic component of reward comparison behavior, our laboratory has selectively bred three generations of the most extreme large and small suppressers tested in the passive reward comparison design using cocaine. Each generation was subsequently tested for LiCl CTA responses and cocaine self-administration. The results of these experiments are presented in Chapter 6.

The inbred mouse serves as another highly useful model system for the identification and characterization of inherited traits affecting vulnerability to drug addiction (for review, see Seale and Carney 1991). Inbred mouse strains are produced through sibling or parent-offspring matings. It takes at least 20 generations of such a breeding regimen for a strain to be considered inbred. The advantage of inbreeding is that genetic homozygosity occurs at virtually every gene locus, allowing preservation of the genotype from one generation to the next. In turn, it is easier to determine the mode of transmission and chromosomal

assignment of inherited traits. Genetic homozygosity also makes it easier to identify polymorphisms which are genes that exist in different allelic forms and are the fundamental source of individual differences in phenotype. When attempting to identify inherited gene variants (i.e., polymorphisms) that affect a specific behavior, it is important to choose a battery of strains based on their genetic diversity. Two inbred mouse strains often compared are the C57BL/6J (B6) and DBA/2J (D2) mice because they are genetically divergent (Taylor 1972). These two strains have actually become well recognized as the two progenitor strains of the C57BL/6 x DBA/2 (aka B x D) recombinant inbred strains and highly utilized for mapping chromosomal loci influencing drug-related phenotypes (Crabbe, Belknap et al. 1994). B6 and D2 mice have been shown to differ greatly in their responsiveness to drugs of abuse, especially to cocaine, with B6 mice showing greater sensitivity to cocaine's reinforcing and locomotor effects (Grahame, Phillips et al. 1995; Kuzmin and Johansson 2000). Based on these findings, experiments were designed to test B6 and D2 strains in the passive reward comparison design using cocaine and the results of these experiments are presented in Chapter 7, the final data chapter of this dissertation.

In sum, the primary goal of the present set of investigations was to determine what factors mediate individual differences in responsiveness to cocaine in the reward comparison paradigm. This goal was pursued by investigating how behavioral, physiological, environmental, and genetic factors contribute to these individual differences. The first few sets of experiments (presented in Chapters 2 and 3) evaluate the nature of the CORT response in the

phenomenon and determine whether the unconditioned CORT response to cocaine can predict which rats will go on to become large or small suppressers. The results of these experiments provide evidence that greater elevations in CORT following the first exposure to cocaine are associated not only with greater cocaine self-administration behavior but also with greater suppression in saccharin intake. The consumption of sweets also was measured (in Chapter 4) for its ability to predict which rats would be more sensitive to the suppressive effects of cocaine. While this measure was not found to be associated with cocaine-induced avoidance of saccharin intake, the experience with the highly rewarding sucrose, together with extra handling, appears to modify subsequent reward comparison behavior. The last few experiments (presented in Chapters 5, 6 and 7) were designed to determine the extent to which not only genetic background but also sex would influence reward comparison behavior. The results of the selective breeding study, together with the results from testing different inbred mouse strains, reveal that genetic make-up alone can influence behavior in the reward comparison paradigm. Collectively, these data demonstrate that not just one, but all sets of factors play important roles in influencing responsiveness to cocaine in a reward comparison paradigm. These data also provide evidence that greater CORT responses and genetic background can serve as predictive tools for determining which drug naïve individuals will go on to become a small or a large suppresser. Indeed, the results of these studies begin to build an important behavioral/hormonal profile that currently does not exist for the large and small suppressers. One of the most

important findings is that simple measures such as extra handling and experience with other highly rewarding stimuli (e.g., sucrose) can prevent the transition from drug use to abuse.

Chapter 2

THE NATURE OF THE CORTICOSTERONE ELEVATION OBSERVED ON TEST DAY: AN ANALYSIS OF INTERSTIMULUS INTERVAL

As described above, rats will avoid intake of a saccharin CS when paired with a drug of abuse. It has been shown, however, that large individual differences emerge following saccharin-morphine pairings (Gomez, Leo et al. 2000) whereby approximately half of the Sprague-Dawley rats will greatly avoid intake of the saccharin cue while the other half will not. Furthermore, greater avoidance of the saccharin cue is correlated with greater saccharin cue-induced elevations in CORT at test (Gomez, Leo et al. 2000). These elevations in CORT are likely conditioned effects induced by presentation of the saccharin CS. The interpretation of these findings, however, is limited by the procedure used on the test day. During conditioning, a 5 min interstimulus interval (ISI) was used where the drug US was administered exactly 5 min following the CS access period. On the test day, no drug was administered in order to assess the conditioned effect of the saccharin cue and blood was sampled 15 min after CS access, which was 10 min after the drug would have been injected. As a consequence, the elevation in CORT may have been due to cue-induced conditioned anticipation of the availability of the drug of abuse. The elevation in CORT also could be due to stress and 'withdrawal' that may ensue when the expected drug is withheld. The experiments in this chapter were designed not only to test this hypothesis, but

also to extend the findings described with morphine to another drug of abuse, cocaine.

Exposure to drug-associated stimuli can precipitate craving and withdrawal. As a consequence, exposure to drug cues is a leading cause of relapse in humans or reinstatement of cocaine-seeking behavior in rats following long periods of abstinence (de Wit and Stewart 1981; Ehrman, Robbins et al. 1992; Meil and See 1996; Foltin and Fischman 1997; Weiss, Maldonado-Vlaar et al. 2000; Grimm, Hope et al. 2001; Grigson and Twining 2002). Presentation of drug-associated cues induces behavioral and physiological responses that are thought to be classically conditioned effects of drug anticipation, craving, withdrawal, or the drug itself (Pavlov 1927/1960; Wikler and Pescor 1967; Childress, Ehrman et al. 1992; Ehrman, Robbins et al. 1992; O'Brien, Childress et al. 1992; DeVries, Taymans et al. 1998; Gomez, Leo et al. 2000). Relapse occurs in up to 90% of cocaine addicts even after extended periods of abstinence (DeJong 1994). It is therefore important to understand how craving and withdrawal are precipitated following presentation of conditioned cues, as it is a leading cause of relapse.

In rats, several findings suggest an important role for the HPA axis not only in acquisition and maintenance, but also in reinstatement of cocaine-seeking behavior following periods of abstinence. Because the acute administration of cocaine is known to produce an unconditioned rise in CORT levels (Sarnyai, Biro et al. 1992; Mantsch, Schlussman et al. 2000), it is no surprise that through classical conditioning, stimuli associated with the drug also can produce a rise in

CORT levels (DeVries, Taymans et al. 1998; Gomez, Leo et al. 2000). In fact, the rise in CORT following exposure to conditioned stimuli occurs specifically during reinstatement of cocaine-seeking behavior in rats (Goeders and Clampitt 2002). Other factors that increase levels of CORT and cause reinstatement of cocaine-seeking behavior include priming injections of the drug itself (de Wit and Stewart 1981), stressors such as electric footshock (Erb, Shaham et al. 1996; Ahmed and Koob 1997), and the exogenous administration of CORT in the absence of stress (Deroche, Marinelli et al. 1997). Reinstatement of cocaine-seeking behavior by these factors likely occurs by triggering, or mimicking, the activity of the HPA-axis.

Several findings suggest that activity of the HPA-axis increases during drug craving and withdrawal. In rats, withdrawal from cocaine will produce intense anxiogenic-like behavior characterized by decreased open-arm exploration on an elevated-plus maze (Sarnyai, Biro et al. 1995). The same study showed that pretreatment with a CRF-antiserum completely prevented the development of anxiety induced by cocaine withdrawal. In another study using the same model, exposure to conditioned cocaine stimuli produced the same anxiogenic-like behavioral response, which also was attenuated with CRF-antagonist pretreatment (DeVries, Taymans et al. 1998). Similarly, cocaine-abstinent humans report intense anxiety and cocaine craving when exposed to both conditioned cues and stress (Gawin and Kleber 1986; Sinha, Catapano et al. 1999). Furthermore, like rats, humans experience increased HPA-axis activity following the acute administration of cocaine (Teoh, Sarnyai et al. 1994) and

during stress- and cue-induced cocaine craving (Berger, Hall et al. 1996; Sinha, Talih et al. 2003; Fox, Talih et al. 2005). Reports of high anxiety often were associated with drug craving in these clinical studies as well. It appears, then, that increased activity of the HPA-axis contributes to drug withdrawal and craving as a result of both conditioned (cue-induced) and unconditioned (stress-induced) responses.

The following experiments evaluate whether the elevation in CORT observed following presentation of a saccharin cue reflects a conditioned or an unconditioned response. Experiment 1 tests the hypothesis that the elevation in CORT observed at test reflects a conditioned response to the presentation of a saccharin cue (Sarnyai, Biro et al. 1995) after pairings with cocaine (US). Experiment 2, on the other hand, addresses the hypothesis that the elevation in CORT observed at test reflects a stress/withdrawal response associated with not getting drug when it was expected (i.e., cocaine was expected 5 min after CS access and never delivered). In Experiment 1, the ISI was extended from 5 to 30 min for a second group of rats while still sampling CORT at 15 min. By extending the ISI to 30 min for a second group of rats, CORT could be sampled 15 min before the drug is expected to test the conditioned effect of the saccharin cue.

Experiment 1

Experiment 1 replicated the parameters described above (i.e., the use of the 5 min ISI and the 15 min CORT test) with the addition of a separate group in a 30 min ISI condition. CORT was, therefore, measured at the same time (e.g., 15 min following CS access), but under two different circumstances. The first condition used the 5 min ISI and, consequently, measured CORT when the saccharin CS predicted the availability of the drug and the drug was overdue. The second condition, however, used the 30 min ISI and, in so doing, measured CORT when the saccharin CS predicted drug availability while the drug was not yet expected. Thus, in the 30 min ISI condition, the test fell at the same time point following CS access as in the 5 min ISI condition, but 15 min before, rather than 10 min after, the drug was expected. We hypothesized that if the elevation in CORT is a conditioned effect associated with cue-induced anticipation of the availability of the drug, then the elevation should be evident for rats in both the 5 and 30 min ISI conditions. On the other hand, if the elevation is due to unconditioned withdrawal in the absence of the availability of the expected drug of abuse, then CORT levels should be elevated only for rats in the 5 min ISI condition.

Materials and Methods

Subjects. This experiment was conducted in two complete replications. There were a total of 96 naïve, male, Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing 300 – 400 g at the beginning of the experiment (N = 48/replication). They were housed individually in stainless steel hanging cages in a temperature and humidity controlled animal care facility with a 12 hr light-dark cycle (lights on at 7 am). Food and water were available ad libitum, except where noted otherwise. The experiment was conducted in the home cage from 9:30 - 11:30 am.

Apparatus, tastant, and drug. The solutions were presented in inverted, graduated, Nalgene cylinders with silicone stoppers and stainless steel spouts attached to the front of the cage with springs. Intake was measured to the nearest 0.5 ml. Sodium saccharin (Fischer Scientific, Malvera, PA) was dissolved in distilled water overnight and presented at room temperature. Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse and was prepared on the morning of each injection day. In this, and all subsequent experiments, to avoid the necrosis that typically accompanies a sc injection of cocaine (Durazzo, Gauvin et al. 1994), the drug was prepared as a 1.5 mg/ml stock solution and the volume injected was adjusted by weight to obtain a 10 mg/kg dose.

Procedure. All rats were handled (i.e., gently held for approx 1 min at the beginning of the experiment and for shorter periods as the rats became habituated to the experimenter) and weighed once a day throughout the

experiment. Deprivation state. Following several days of handling, all rats were placed on a water deprivation regimen in which they were given 5 min access to dH₂O on the front of the cage each morning and 1 h access each afternoon to maintain proper hydration. All rats were kept on this daily regimen for 6 days when morning intake stabilized. Saccharin pre-exposure and pre-conditioning corticosterone. Twenty-four h following the last 5 min access period to water and 48 hours prior to the first taste-drug pairing, all rats were pre-exposed to the 0.15% saccharin CS for 5 min. Blood samples (approximately 0.4 ml) were collected by means of a tailcut 15 min after saccharin pre-exposure and plasma was stored at -80 degrees Celsius for later evaluation. This was designed to control for any possible unconditioned effect of saccharin on baseline CORT. The 15 min time point replicates the procedure used previously (Gomez et al., 2000) and is approximately how long it takes to detect a rise in circulating levels of CORT (Fluttert, Dalm et al. 2000). Conditioning. Rats were matched on the basis of 5 min water intake on the last day of water training (day 6) and divided into 2 ISI conditions: 5 min ISI (n=48) or 30 min ISI (n=48). They were then further subdivided into 2 drug conditions: saline (n=16) or cocaine (n=32). More subjects were placed in the cocaine group to allow for an assessment of individual differences. During conditioning, the rats were given 5 min access to 0.15% saccharin and, after a 5 or 30 min ISI, injected subcutaneously (sc) with either saline or 10 mg/kg cocaine. There was one such CS – US pairing every other day for a total of 7 pairings, followed by one CS only test. All rats were given 5 min access to water each morning on the days between conditioning trials and 1

h every afternoon to re-hydrate. Post-conditioning corticosterone. A second blood sample (approximately 0.4 ml) was collected from the tail 15 min after access to the saccharin CS on the final CS-only test trial. No injections were given on this day.

CORT Radioimmunoassay. In this, and all subsequent experiments, blood samples were kept on ice and then centrifuged at 11,000 rpm for 10 min. The serum obtained from each sample was stored at –80 degrees Celsius until the CORT radioimmunoassay was performed. Pre- and post-conditioning CORT concentrations were then determined using a standard radioimmunoassay kit (ICN Biomedicals). All samples were run in duplicate.

Results: Experiment 1

All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc comparisons were conducted, when appropriate, using the Newman Keuls test, with alpha set at 0.05.

Saccharin (CS) Intake. The main effect of replication was not significant, $F < 1$, following a multifactorial $2 \times 2 \times 2 \times 8$ ANOVA varying drug group (saline and cocaine), ISI group (5' and 30'), replication (2), and trial (1-8). Therefore, the data from both studies were combined and are presented accordingly. The saccharin intake data (shown in Figure 2.1a) were analyzed using a $2 \times 2 \times 8$ ANOVA varying drug group (saline and cocaine), ISI group (5' and 30'), and trials (1-8). The results of this analysis revealed a significant main effect of Drug, $F(1, 92) = 34.14$, $p < 0.01$, and Drug x Trial interaction, $F(7, 644) = 17.01$, $p < 0.01$, indicating that rats treated with cocaine significantly reduced their intake of saccharin over trials when compared with saline-injected controls. A significant Drug x ISI interaction, $F(1, 92) = 3.97$, $p < 0.05$, indicated that the cocaine-treated group given the shorter, 5' ISI, during conditioning exhibited a greater reduction in intake than the group given the longer, 30' ISI. This effect may be explained by the strength of the CS-US association being weakened as a result of the longer ISI. Although this difference is present, the ISI x Drug x Trials interaction was not significant, $p > 0.05$, indicating that the main effect of the Drug over trials was not affected by the ISI.

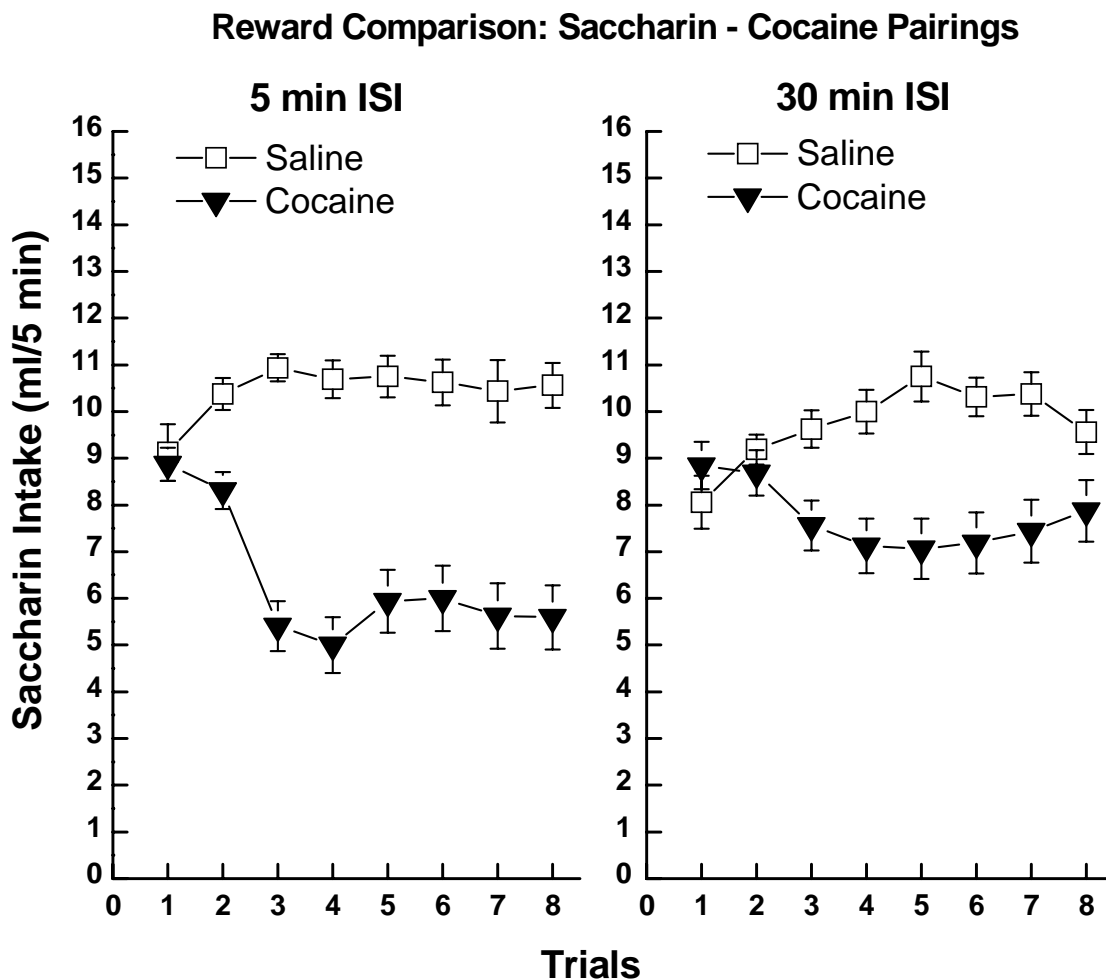


Figure 2.1a: Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) across seven pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the 5 min interstimulus interval (ISI) group (N=48) are shown in the left panel and data for the 30 min ISI group (N=48) are shown in the right panel.

Upon closer inspection of the data, it became clear that cocaine-treated rats, like morphine-treated rats (Gomez, Leo et al. 2000), exhibited large individual differences in intake. Indeed, there appeared to be a bimodal distribution of intake data on Trial 8 for both the 5' and 30' ISI groups (see Figures 2.1b and 2.1c).

Consequently, the cocaine-treated animals were divided into two separate groups (large suppressers and small suppressers) based on the median split of trial 8 intake (see Figure 2.2). The data were then re-analyzed using a mixed factorial 3 x 2 x 8 ANOVA varying group (saline, small suppressers, and large suppressers), ISI (5' and 30'), and trials (1-8). The results of this analysis revealed a significant Group x ISI x Trials interaction, $F(14,630) = 1.90$, $p < 0.05$. Post hoc tests (Newman Keuls) of this interaction showed that for the 5' ISI group, the large suppressers consumed significantly less of the saccharin CS than the saline group on Trials 2 – 8, $p_s < 0.05$, while the small suppressers exhibited a smaller reduction in intake that attained significance only on Trials 3 and 4, $p_s < 0.05$. For the 30' ISI group, reduction of saccharin intake was again significant for the large suppressers when compared with the saline group on Trials 2 – 8, $p_s < 0.05$. Saccharin intake by the small suppressers, on the other hand, did not differ from intake by the saline-injected controls, see Figure 2.2, right panel. Post hoc comparisons also revealed that avoidance of the saccharin cue was greater in the 5 min ISI group compared with the 30 min ISI group. Large suppressers in the 5 min ISI group demonstrated greater avoidance than large suppressers in the 30 min ISI group on Trials 3-8 while small suppressers

demonstrated greater avoidance Trials 3 and 4, $p < 0.05$. These results show that the suppressive effects of cocaine were attenuated when using a longer ISI.

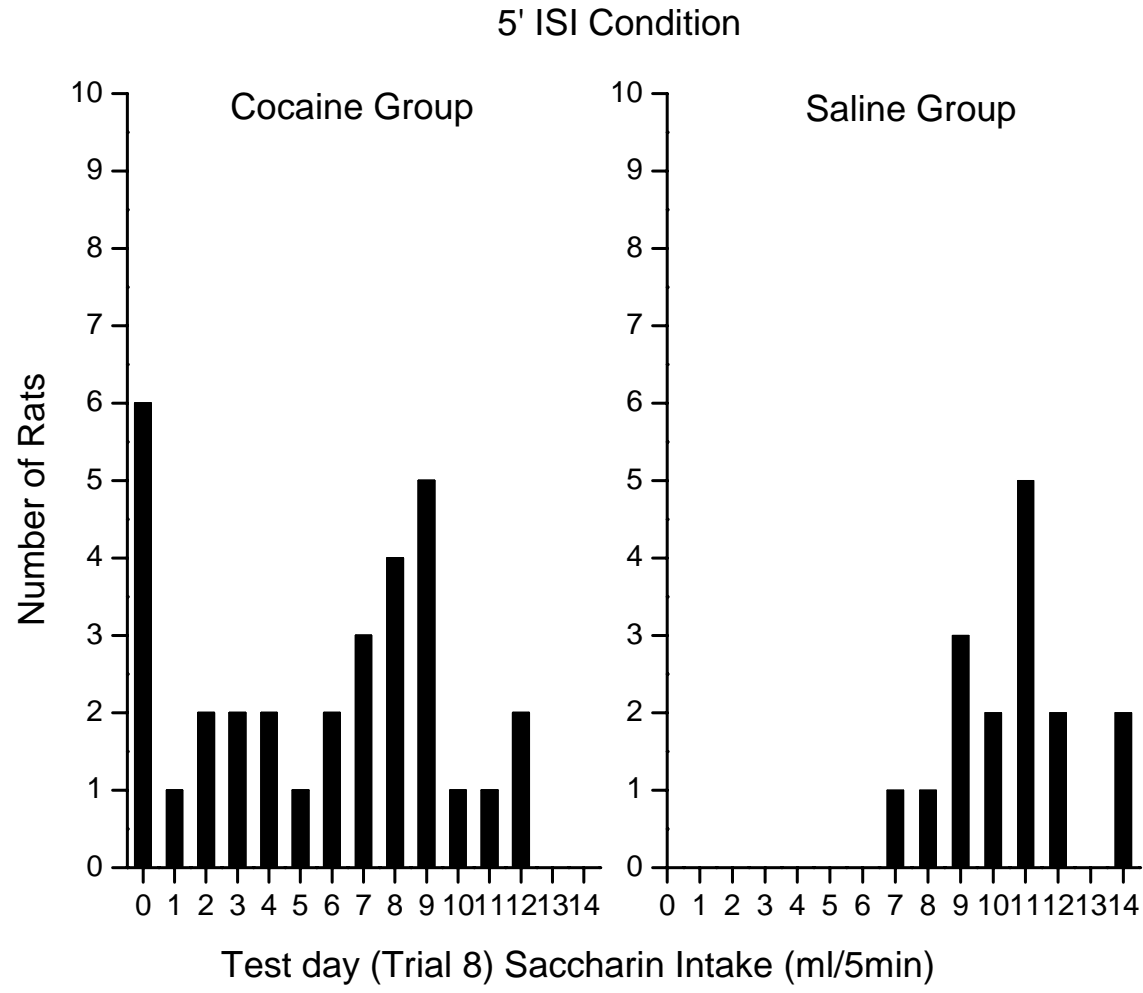


Figure 2.1 b: Distribution of subjects in the 5' ISI condition. Absolute frequencies of test day (Trial 8) saccharin intake (ml) for cocaine-treated rats (left panel) and saline-treated rats (right panel).

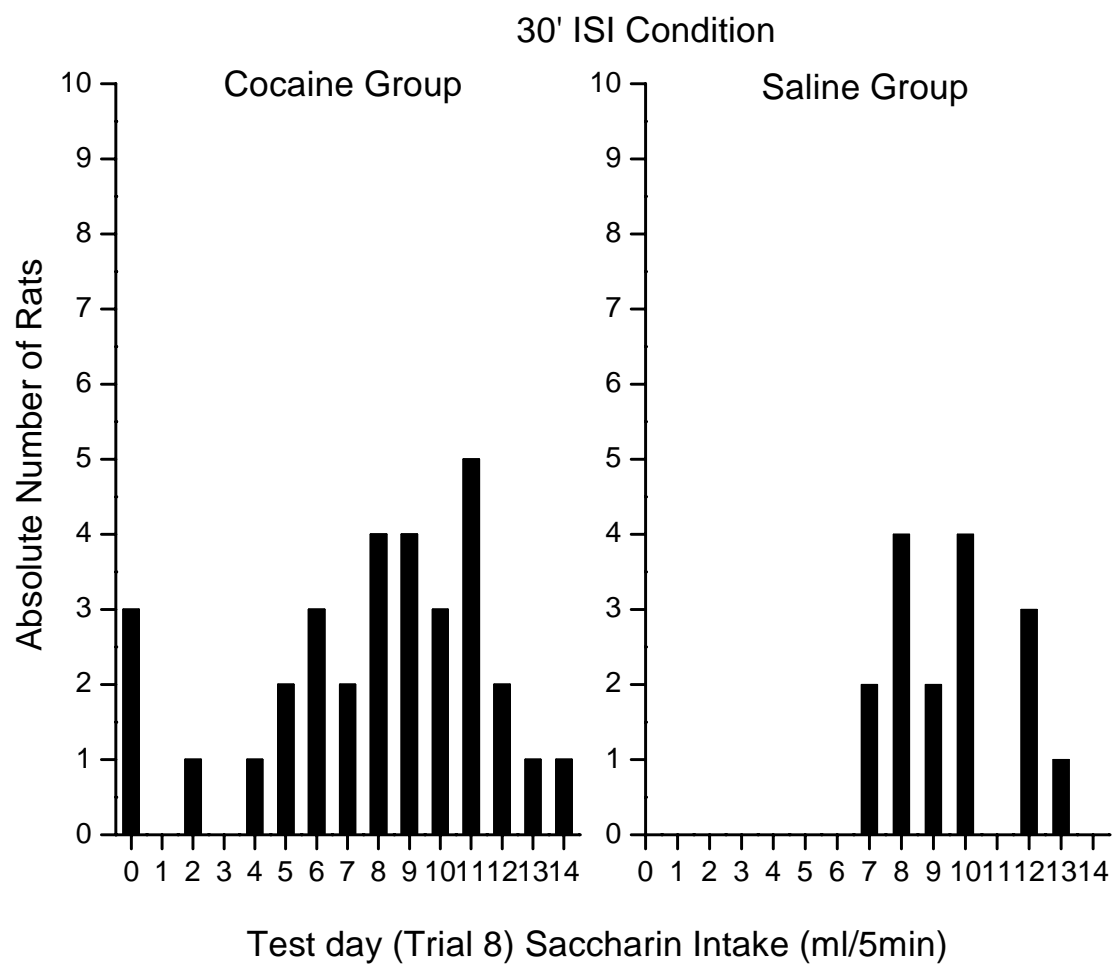


Figure 2.1 c: Distribution of subjects in the 30' ISI condition. Absolute frequencies of Test day (Trial 8) saccharin intake (ml) for cocaine-treated rats (left panel) and saline-treated controls (right panel).

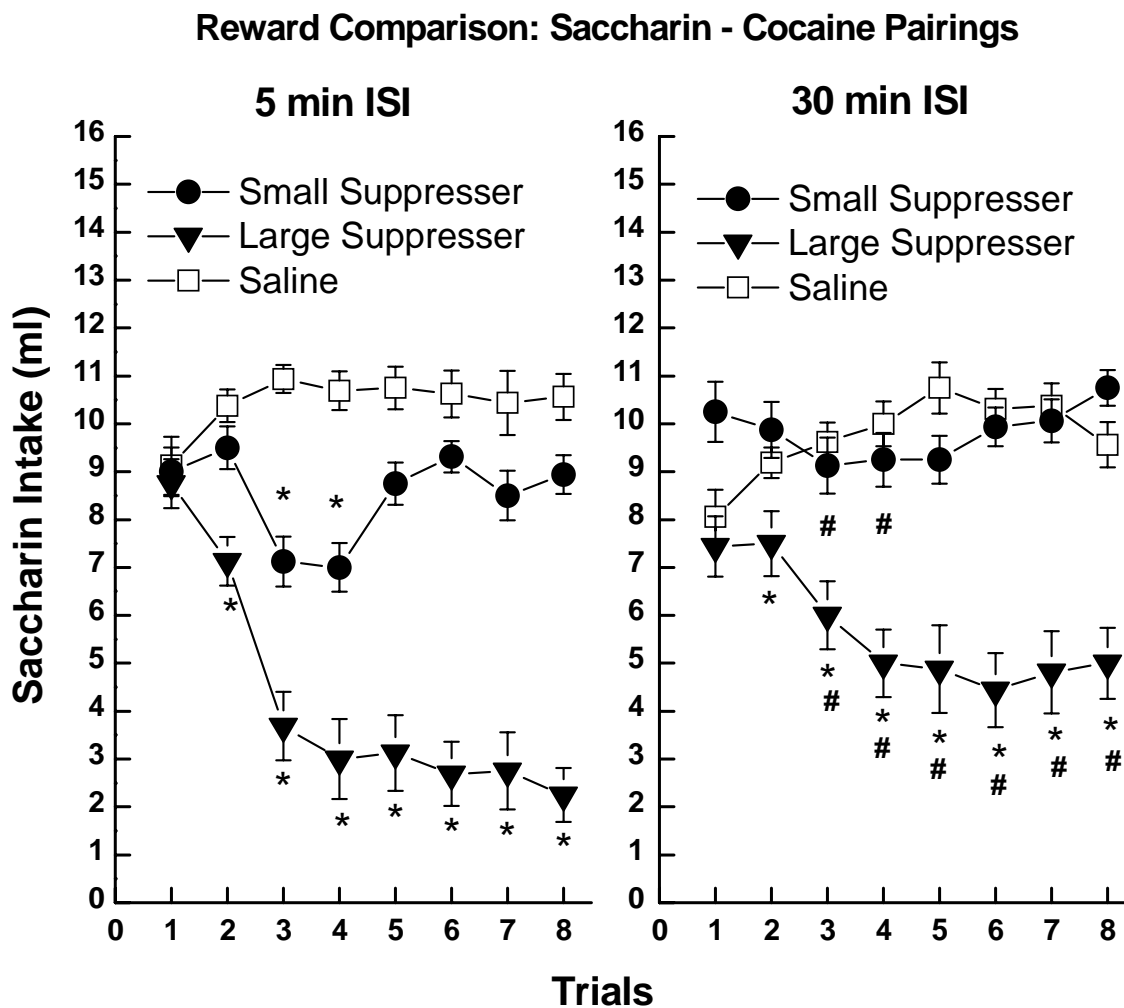


Figure 2.2: Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) for saline controls, small suppressors, and large suppressors across 7 pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the 5 min interstimulus interval (ISI) group are shown in the left panel and data for the 30 min ISI group are shown in the right panel (N=16/group).

*Significantly different from saline-injected controls, # different from 5' ISI group, $p < 0.05$.

Corticosterone. Blood was collected 15 min after presentation of the saccharin CS 48 hr before the start of taste-drug pairings (pre-conditioning) and then 48 hr following taste-drug pairings (post-conditioning). As described earlier, CORT was assessed both before and after conditioning to test whether presentation of the saccharin cue would cause conditioned elevations in CORT following pairings with cocaine. The CORT data (shown in Figure 2.3) were analyzed using a mixed factorial 3 x 2 x 2 ANOVA varying group (saline, small suppressers, and large suppressers), ISI (5' and 30'), and trials (pre- and post-conditioning). A significant main effect of Group, $F(2,90)=9.54$, $p<0.05$, indicated that large suppresser rats demonstrated higher CORT levels than the small suppressers and than the saline-treated controls overall. A significant main effect of Trial, $F(1,90)=21.1$, $p<0.05$, showed that post-conditioning CORT levels were higher than pre-conditioning CORT levels, overall. The Group x ISI x Trials interaction was not significant, $p>0.05$. Post hoc comparisons of a significant Group x Trials interaction, $F(2,90)=16.9$, $p<0.05$, however, showed that post-conditioning CORT levels were significantly elevated from conditioning to test for the large suppressers, $p<0.05$, but not for the small suppressers or the saline control group, $p_s>0.05$.

Although results from the above analysis of the CORT data did not indicate any differences between ISI conditions, inspection of the behavioral (i.e., saccharin intake) data suggest that the two ISI conditions differed. Thus, the CORT data for the 5' and 30' ISI groups were analyzed separately using a 3 x 2 mixed factorial ANOVA varying group (saline, small suppressers, large

suppressers) and trial (pre- and post-conditioning). The results for the 5' ISI group revealed a significant main effect of Group, $F(2,45)=5.69$, $p<0.05$, Trial, $F(1,45)=23.8$, $p<0.05$, and Group x Trial interaction, $F(2,45)=13.1$, $p<0.05$, see Figure 2.3, left panel. Post hoc comparisons of this interaction showed that there were no differences in levels of pre-conditioning CORT between groups. At Test, however, the small suppressers demonstrated a strong, but non-significant, elevation in CORT from pre- to post-conditioning, while the large suppressers exhibited a robust and highly significant elevation in CORT, $p<0.05$ (left panel). The results for the 30' ISI group revealed that although the main effect of Trial was not significant, $p>0.05$, both the main effect of Group, $F(2,45)=4.96$, $p<0.05$ and the Group x Trial interaction, $F(2,45)=4.75$, $p<0.05$, were significant. Post hoc comparisons of this interaction showed that while pre-conditioning CORT levels did not differ across groups, post-conditioning CORT levels were significantly elevated for the large suppressers, $p<0.05$ (right panel). Results of a 2 sample t-test (in Origin), however, revealed that this elevation was not as great as it was for the large suppressers in the 5' ISI group, $p<0.05$.

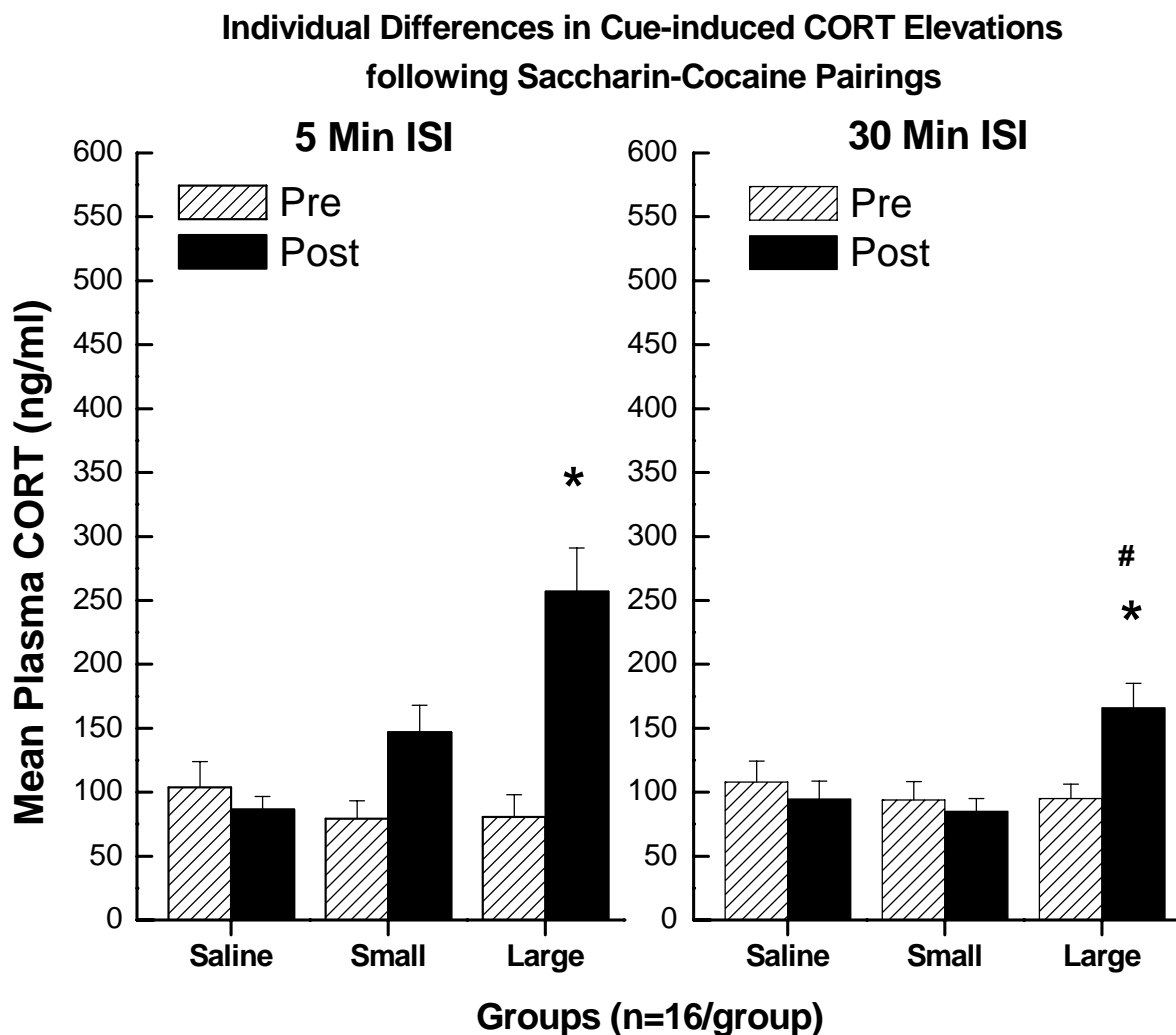


Figure 2.3: Mean (\pm SEM) pre- and post-conditioning CORT levels (ng/ml) for saline controls, small suppressors, and large suppressors in the 5 min ISI group (left panel) and the 30 min ISI group (right panel). * Significantly different from saline, # different from 5' ISI, $p_s < 0.05$.

Taken together, these data suggest that small and large suppressers differ not only in their consumption of the saccharin CS, but also in their endocrine response (plasma CORT) to the presentation of the saccharin cue at test. To assess the strength of this relationship, correlation analyses were conducted for each ISI condition, with terminal CS intake and post-conditioning CORT as factors for all rats in the cocaine groups (N=32 per ISI condition; see Figure 2.4). The results of these analyses revealed a significant negative relationship between terminal CS intake and post-conditioning plasma CORT for both the 5' ($r = -0.47$, $p < 0.05$, see left panel), and 30' ($r = -0.64$, $p < 0.05$, see right panel) ISI conditions. Thus, greater avoidance of the saccharin CS (i.e., lower CS intake) was significantly correlated with greater levels of circulating CORT for both ISI conditions at test.

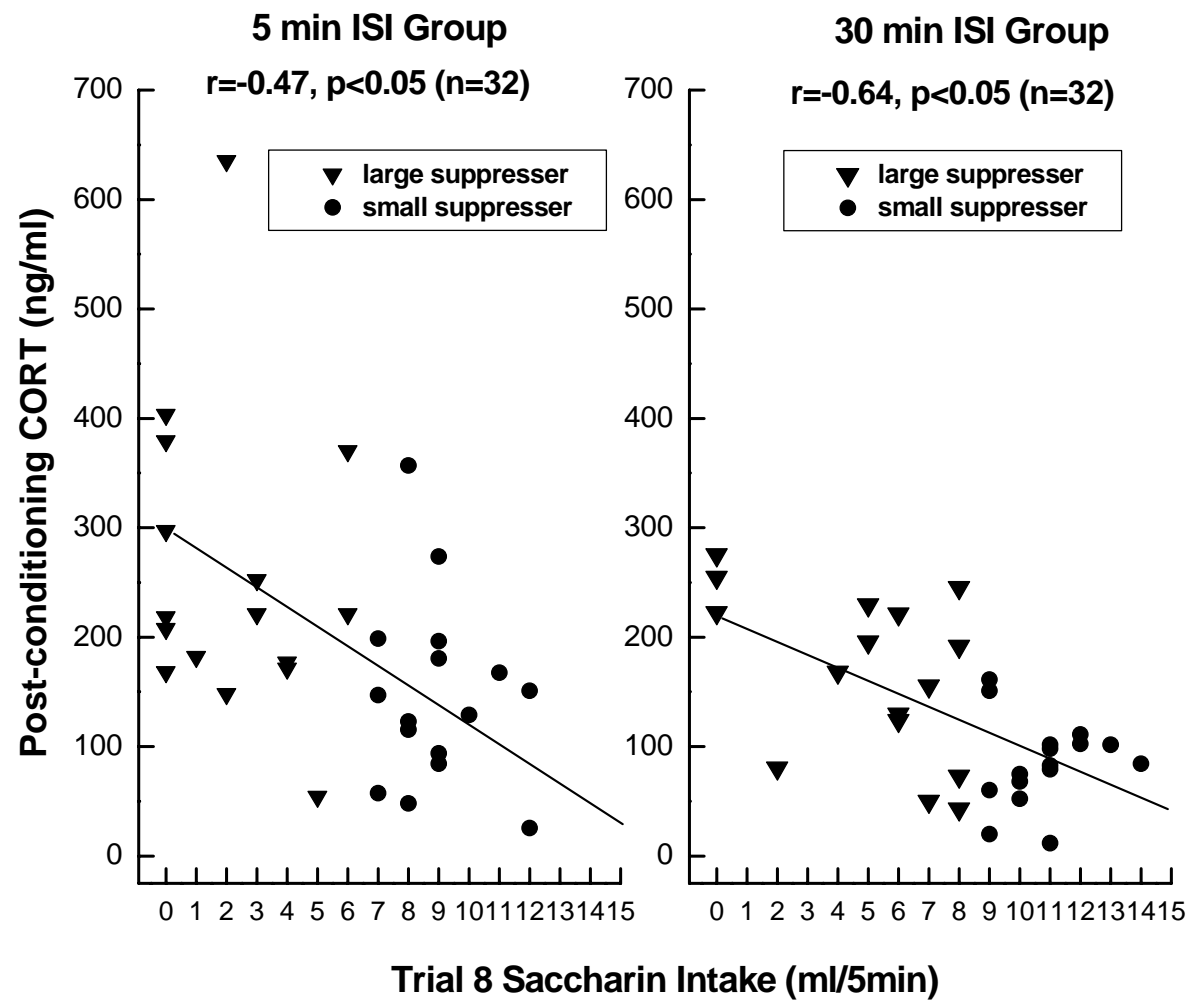


Figure 2.4: Intake of the saccharin conditioned stimulus as a function of post-conditioning corticosterone levels at test for small and large suppressers in the 5 min (left panel) and the 30 min ISI group (right panel).

These data demonstrate that rats will suppress intake of a sweet tasting saccharin solution when it is paired with the administration of cocaine. These results also show that, like morphine, cocaine reveals individual differences in suppression whereby approximately half of the Sprague-Dawley rats (the large suppressers) avoid intake of the saccharin cue, while the other half of the rats (the small suppressers) do not. Individual differences were present in both the 5 and 30 min ISI conditions. Furthermore, these individual differences in intake were associated with individual differences in cue-induced elevations in CORT such that greater avoidance by the large suppressers was associated with greater elevations in CORT at test. These patterns in behavior and CORT were evident when using either a 5 or a 30 min ISI.

Although similar patterns in behavior and CORT were present when using either a 5 or 30 min ISI, the suppressive effects of cocaine and elevations in CORT were greater in the 5 min ISI condition. One possible reason for this is that shorter ISIs support better learning (i.e., greater suppression). In general, overall performance, in associative learning paradigms, decreases as the ISI increases (Prokasy, Clark et al. 1975; Holland 1980; Misanin, Kaufhold et al. 2005). Recently, this pattern has been demonstrated specifically within the cocaine-induced conditioned taste avoidance paradigm (Freeman and Riley 2005). The results of this study showed that although learning will occur using progressively longer interstimulus intervals (10, 60, 120, 180, 240, 420, and 540 min), the magnitude of the effect (i.e., suppression) decreased as the ISI increased. Although it seems likely that the strength of the association is weaker with

greater ISIs, it is not clear whether performance decreases as a result of less learning, less memory retention, or a combination of both.

Experiment 2

The results from Experiment 1 support the conclusion that the drug need only be anticipated (e.g., in the 30' ISI condition), but not over due (e.g., in the 5' ISI condition), at the time that CORT is assessed (i.e., at 15'), to cause an elevation in circulating CORT at Test. This conclusion is supported by the finding that large suppressers in the 30 min ISI group exhibited a cue-induced elevation in CORT 15 min before the drug was expected to be administered. The CORT elevation observed in these large suppressers, however, was not as great as the elevation seen in the large suppressers tested with the 5 min ISI. This observation may suggest that, while unconditioned withdrawal from the drug of abuse is not necessary for an elevation in circulating CORT, it may still be a contributing factor. The second experiment, therefore, was designed to test this hypothesis. In Experiment 2, all rats were trained with a 30 min ISI during conditioning. On the test day, CORT was evaluated either 15 min before (with a 15 min tailcut) or 15 min after (with a 45 min tailcut) the drug was expected. We predicted that if CORT is elevated, in part, because of unconditioned withdrawal associated with the absence of the expected drug of abuse, then CORT levels should be higher for rats in the 45 min tailcut group than for rats in the 15 min tailcut group. If on the other hand, it is more of a conditioned effect, then CORT levels should be higher in the 15 min test condition when assessed in closer temporal proximity to the presentation of the saccharin cue.

Methods

Subjects. This experiment was conducted with naïve male Sprague-Dawley rats in two complete replications as described for Experiment 1 (n = 48 rats/replication for a total of 96). The rats were obtained and maintained as described for Experiment 1 and weighed between 300 – 500 g at the start of testing.

Apparatus, tastant, and drug. The experiment was conducted in the home cages from 9:30 – 11:30 am as described for Experiment 1. The saccharin solution and cocaine also were prepared as described for Experiment 1.

Procedure. The procedure used for Experiment 2 was the same as that for Experiment 1, with two exceptions. First, all rats were given a 30' ISI during conditioning. Second, at test, half of the rats had their blood sampled 15' after CS access (referred to as group 15' Tailcut) while the other half had their blood sampled 45' after CS access (referred to as group 45' Tailcut). The first group, then, was a replication of the 30' ISI condition described for Experiment 1.

CORT assay. CORT was analyzed as described above for Experiment 1.

Results: Experiment 2

All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc comparisons were conducted, when appropriate, using the Newman Keuls test, with alpha set at 0.05.

Saccharin (CS) intake. The cocaine-treated rats in both replications exhibited large individual differences in intake (data not shown), just as observed in Experiment 1, and were divided into large and small suppressers using the median split of trial 8 intake. The saccharin intake data were then analyzed using a mixed factorial 3 x 2 x 2 x 8 ANOVA varying drug group (saline, small suppressers, and large suppressers), tailcut group (15' and 45'), replication (2), and trials (1-8). The results of this analysis revealed a significant main effect of Replication, $F(1,83)=6.24$, $p<0.05$, and Replication x Group interaction, $F(2,83)=5.13$, $p < 0.05$, showing that cocaine-treated rats reduced their intake of the saccharin CS more in the second replication than in the first. Although this was a significant interaction, the patterns in intake behavior did not vary across replication as a function of Tailcut Group. In support of this conclusion, the main effect of Tailcut Group was not found to be significant, $p>0.05$, nor were there any significant interactions thereof, $p_s>0.05$. As such, the data from both replications were combined and are presented accordingly.

The saccharin intake data (shown in Figure 2.5) were then reanalyzed using a 3 x 2 x 8 ANOVA varying drug group (saline, small suppressers, and large suppressers), tailcut group (15' and 45'), and trials (1-8). The results of this analysis showed that large suppressers demonstrated greater avoidance of the

saccharin intake than both the small suppressers and the saline treated rats, who consumed approximately the same amount of saccharin. This conclusion was supported by a significant main effect of drug Group, $F(2,89) = 53.14$, $p < 0.05$, and Group x Trial interaction, $F(14,623) = 15.0$, $p < 0.05$. Once again, intake behavior did not differ whether blood was sampled either before or after the drug was expected to be on board, $ps > 0.05$.

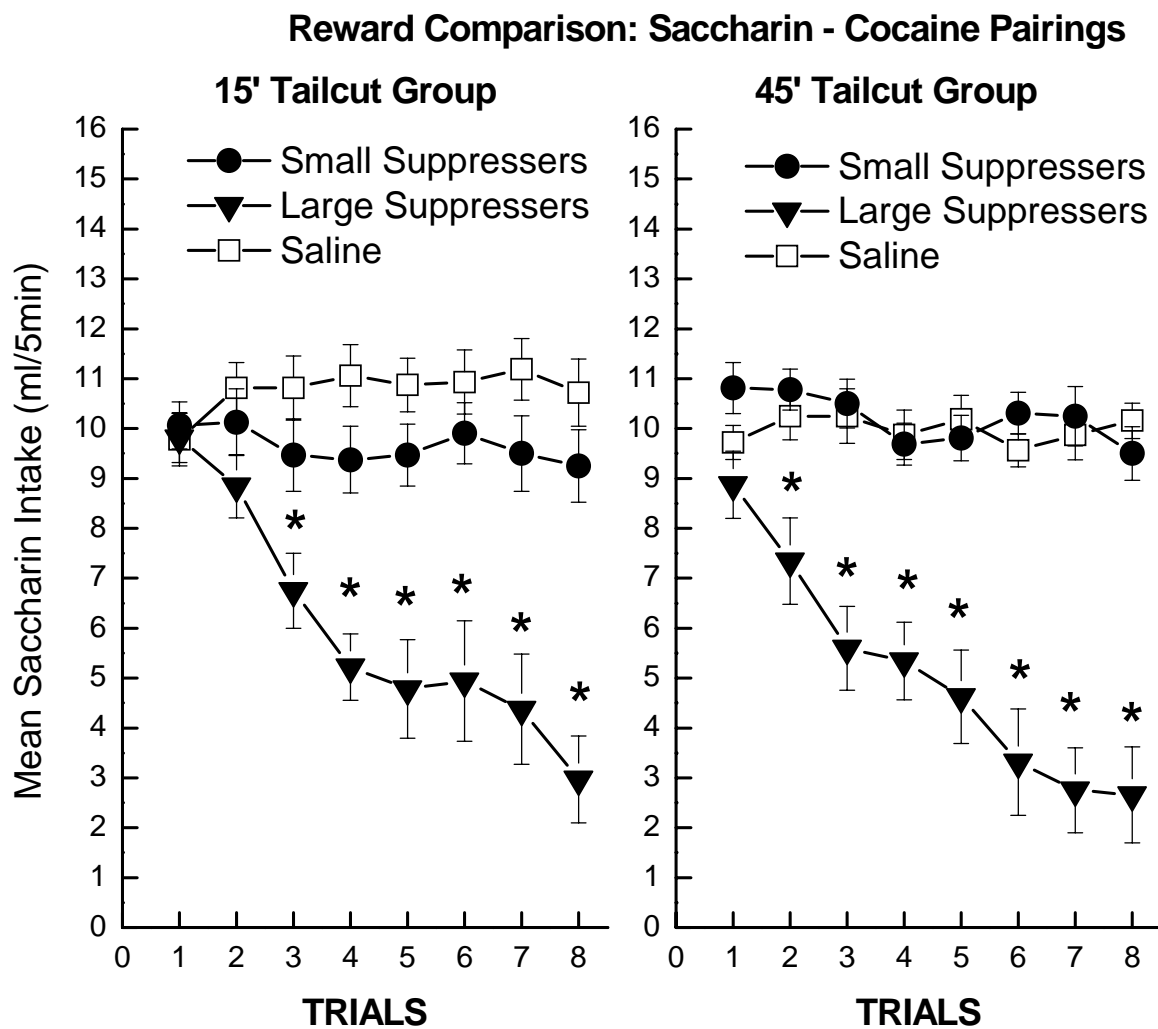


Figure 2.5: Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) for saline controls, small suppressers, and large suppressers across 7 pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the 15 min tailcut group are shown in the left panel and data for the 45 min tailcut group are shown in the right panel. All animals were given a 30 min ISI.

Corticosterone. The reduced magnitude of the CORT response in the 30' ISI condition, the overall 3 x 2 x 2 ANOVA, varying drug group (saline, small suppressers, and large suppressers), tailcut group (15 and 45 min), and trials (pre- and post-conditioning), failed to attain statistical significance, $F(2,90)=1.55$, $p>0.05$. Given the prediction, however, that these two tailcut groups would differ, the CORT data (shown in Figure 2.6) were then analyzed separately for each tailcut group using a mixed factorial 3 x 2 ANOVA varying drug group (saline, small suppresser, and large suppresser) and trial (pre- and post-conditioning). For the 15' tailcut group (see left panel), Newman Keuls post hoc tests of a significant Group x Trial interaction, $F(2,45)=6.68$, $p<0.001$, showed that the CORT elevation demonstrated by the large suppressers at test was significantly higher than that of both the small suppressers and the saline-injected controls. Neither the saline-injected control group nor the small suppressers showed an elevation in CORT at test. These data replicate the findings obtained in Experiment 1 when CORT was measured 15 min before drug was expected to be on board. For the 45' tailcut group (see right panel), on the other hand, there was no significant interaction between Drug Group and Trial to allow for post hoc comparisons. There was a significant main effect of Trial, pre to post, suggesting that CORT levels were higher at test, overall, than they were prior to conditioning.

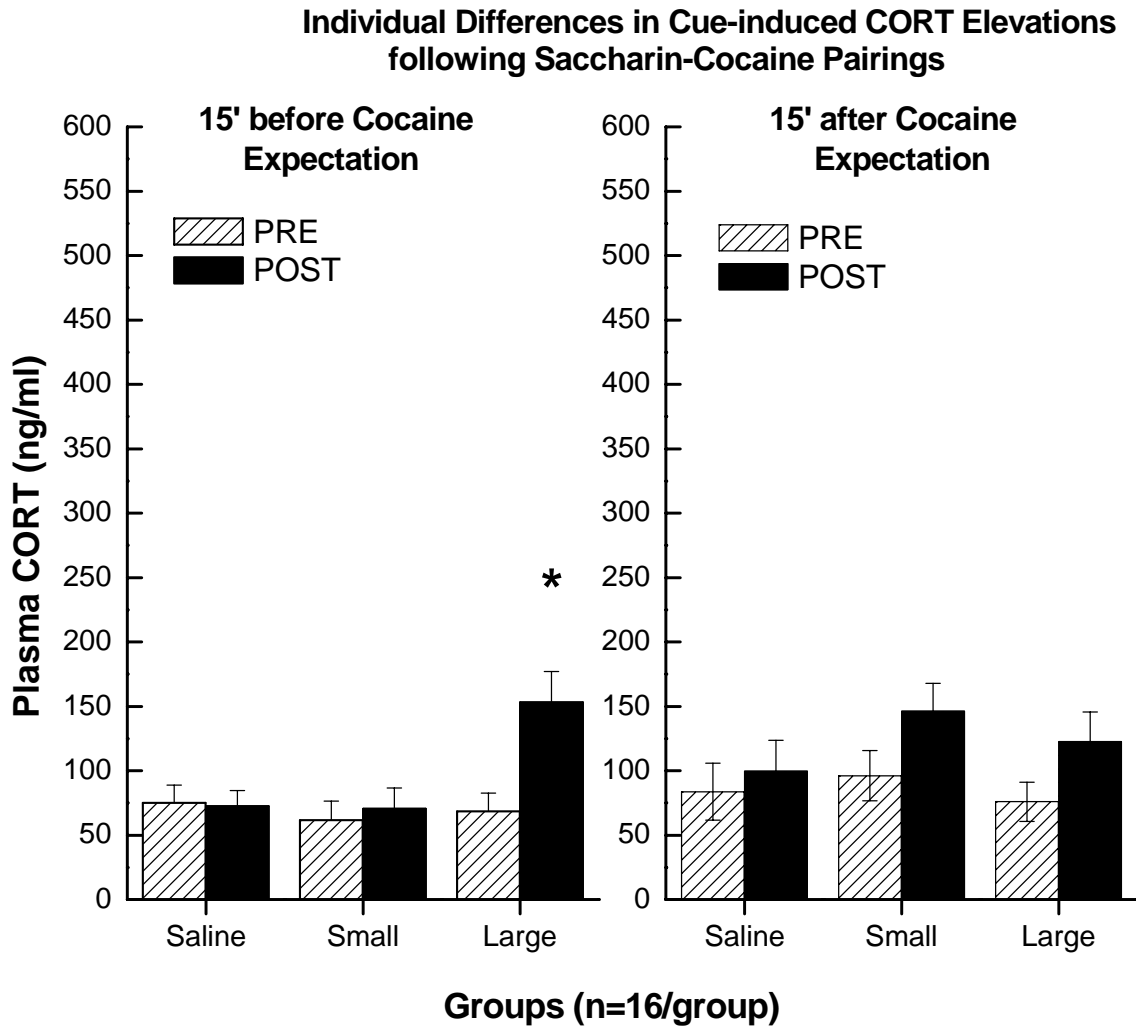


Figure 2.6: Mean (\pm SEM) pre- and post-conditioning CORT levels (ng/ml) across saline controls, small suppressors, and large suppressors in the 15 min tailcut group (left panel) and the 45 min tailcut group (right panel).

To assess the strength of the relationship between saccharin intake and CORT at test, correlation analyses were conducted for each tailcut group, with terminal CS intake and post-conditioning CORT as factors, for all rats in the cocaine groups (N=32 per tailcut group; see Figure 2.7). The results of these analyses revealed a significant negative relationship between terminal CS intake and post-conditioning CORT for the 15 min tailcut group, $r=-0.71$, $p<0.001$ (see left panel), but not for the 45 min tailcut group, $p > 0.05$, (see right panel). Thus, greater avoidance of the saccharin CS was significantly correlated with greater levels of circulating CORT only when CORT was sampled 15, but not 45, min after presentation of the saccharin CS.

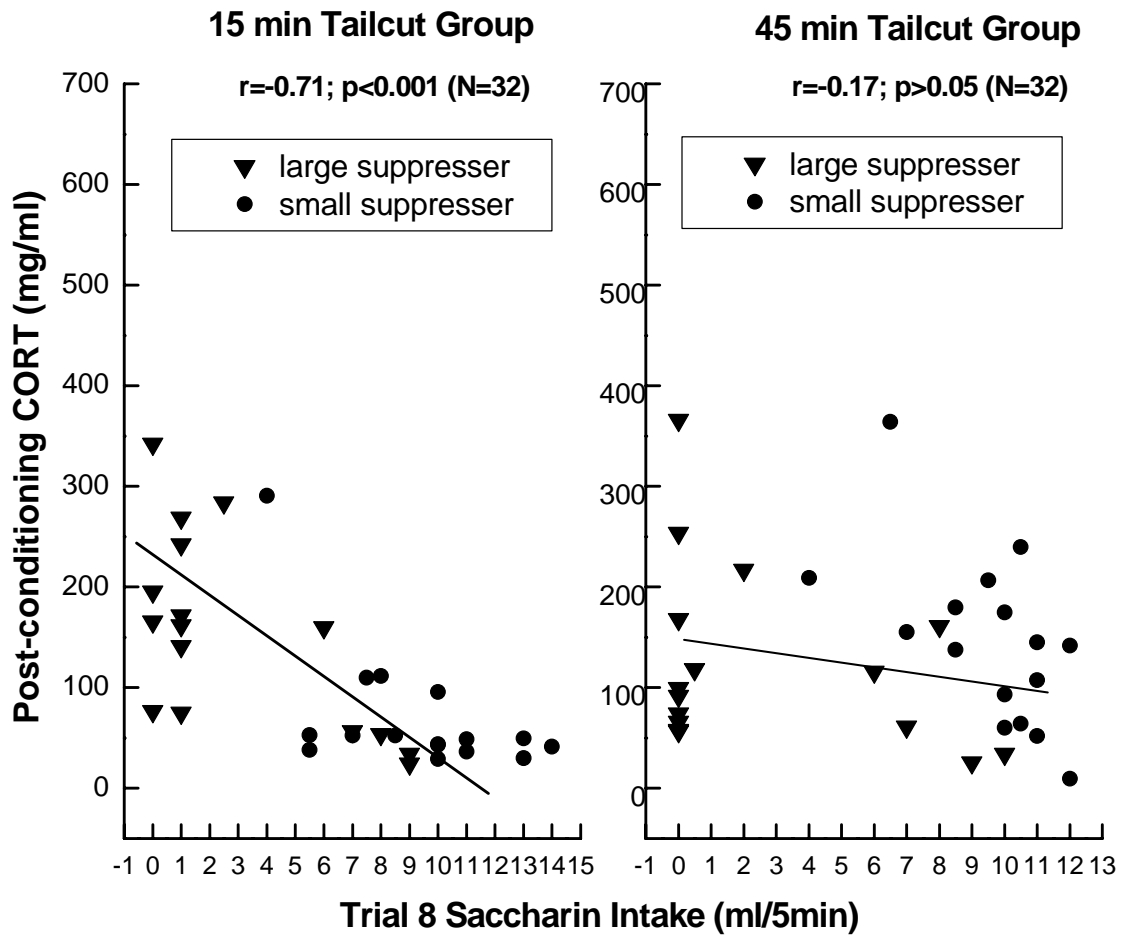


Figure 2.7: Intake of the saccharin conditioned stimulus as a function of corticosterone levels at test for small and large suppressors in the 15 min (left panel) and the 45 min tailcut group (right panel).

The results of Experiment 2, like Experiment 1, showed that patterns in saccharin intake behavior were similar to patterns described previously such that the cocaine treated group demonstrated greater suppression of saccharin intake across trials compared to saline injected controls. Furthermore, large individual differences in intake also were present. Cocaine-treated rats in the present experiment, however, tended to exhibit greater suppression in intake, overall, than cocaine treated rats in Experiment 1 that also were given a 30 min ISI. One possible explanation for this is that the rats in the present study, on average, weighed more than the rats used in the first study. Since the cocaine is prepared using a mg/kg dose, the larger rats received more drug than the smaller rats. Although this dosage scheme is used to control for naturally occurring differences in weights, it is possible that certain effects of the drug may be greater when the absolute quantity of the administered drug is larger. A second possible explanation is that rats in the second experiment were not on the water training/deprivation regimen for as long as rats in the first experiment (Experiment 1: 14 days vs Experiment 2: 6 days). Stabilization of water intake took longer during the first experiment because several rats became temporarily ill. Regardless of this difference, robust individual differences in saccharin intake still were present and cocaine-treated rats still were divided into large and small suppressers in the present experiment. Moreover, there were no differences in CS intake between the 15 and 45' tailcut groups.

Saccharin cue induced elevations in CORT were, again, smaller when using the 30 min ISI in the present experiment. In fact, the same pattern in CORT

elevations observed in Experiment 1 for the 30 min ISI group (15 min tailcut) was replicated in the present experiment. Specifically, the large suppressers in the 15 min tailcut group demonstrated a significant elevation in CORT at test, while the small suppressers did not. This finding reaffirms the hypothesis that the CS-US association is weaker when using a 30 min ISI, resulting in a conditioned elevation of CORT only in the large suppressers. The 45 min tailcut group was implemented in order to test the hypothesis that CORT elevations seen using the 5 min ISI were greater than elevations seen when using the 30 min ISI because of the stress and/or unconditioned withdrawal associated with not getting the drug when it was expected (i.e., 5 min after access to the saccharin cue). If this hypothesis were true, then CORT should have been elevated for the large suppressers in the 45 min tailcut group and the magnitude of the effect should have been greater than that obtained for the 15 min tailcut group in the same ISI condition. This, however, proved not to be the case. Indeed, the elevations in CORT for the rats in the 45 min tailcut group proved not to be statistically significant relative to the saline treated controls. This finding argues strongly that the cue induced elevation in CORT does not depend upon the drug being overdue. Use of the 30 min ISI degrades the association and this disruptive effect is exaggerated when CORT is measured at 45, rather than 15, min after CS access. This conclusion is further supported by results from the correlation analysis which revealed that the association between terminal saccharin intake and CORT no longer exists when evaluated at 45 min. The elevation in CORT, then, is “time-locked” to the presentation of the CS, augmented when using

shorter CS-US interstimulus intervals, but little affected, apparently, by withholding the drug at test.

Discussion

The results from Experiment 1 support and extend earlier findings attained with morphine (Gomez, Leo et al. 2000) to another drug of abuse, cocaine. To summarize, these data show that, although rats will avoid intake of saccharin when it is paired with the administration of cocaine, there are large individual differences in saccharin intake. Moreover, these individual differences in intake were associated with individual differences in CORT such that greater avoidance of the saccharin cue was associated with greater cue-induced elevations in CORT at test. To determine whether these elevations in CORT were due, in part, to unconditioned stress/withdrawal associated with the drug being overdue (i.e., CORT was initially evaluated 15 min following access to saccharin which was 10 min after the drug was expected), the ISI was extended to 30 min. The results for Experiment 1 showed that CORT levels were elevated when tested 15 min after CS access whether using a 5 min or a 30 min ISI. These data show that presentation of the saccharin cue is sufficient to elicit the elevation in circulating CORT whether or not the drug is overdue (e.g., 5 min vs 30 min ISI). This conclusion was confirmed in Experiment 2 when rats in the 30 min ISI group failed to exhibit a significant elevation in circulating CORT when levels were evaluated at 45 min (i.e., after the drug was overdue). Thus, the cue induced

elevation in CORT is, in part, a conditioned effect of the drug and not an effect caused by the unconditioned stress and/or withdrawal associated with the drug simply being overdue. Rather, it is proposed here that the saccharin cue induces a conditioned elevation in CORT that reflects both conditioned craving and conditioned withdrawal in anticipation of the impending availability of the drug of abuse.

As mentioned in the Introduction, drug-associated cues are known to elicit craving for the drug, withdrawal symptoms, and anxiety-like behaviors. Furthermore, these behavioral and physiological responses are thought to evolve through classical conditioning and often take on a close resemblance to the effects of the drug itself. (Pavlov 1927/1960; Wikler and Pescor 1967; Childress, Ehrman et al. 1992; Ehrman, Robbins et al. 1992; O'Brien, Childress et al. 1992; DeVries, Taymans et al. 1998; Gomez, Leo et al. 2000). Specifically, in humans, exposure to cocaine-associated cues will result in reports of intense anxiety, cocaine craving, and withdrawal that are associated with decreases in skin temperature and skin resistance, increases in heart rate and HPA axis activity (i.e., increased ACTH and cortisol levels) (Gawin and Kleber, 1986; Ehrman, Robbins et al. 1992; Sinha, Talih et al. 2003). Although craving and withdrawal are often induced by exposure to drug-associated cues through classical conditioning, exposure to stress also has been shown not only to induce reports of craving, but also to increase circulating CORT (Sinha, Catapano et al. 1999; Sinha, Talih et al. 2003). Thus, increases in HPA axis activity are induced by both conditioned (i.e., drug associated cues) and unconditioned (i.e., stress)

stimuli and appear to reflect both craving and withdrawal simultaneously. Taken together, these findings implicate a mediating role for CORT, not only in contributing to individual differences in initial responsiveness to drugs of abuse and relapse during periods of abstinence, but also in drug-induced devaluation of the naturally rewarding saccharin cue.

It is not clear why individual differences consistently emerge not only in drug-induced suppression of saccharin intake but also in post-conditioning CORT levels. To interpret these data, one must first consider reasons for why rats suppress intake of the saccharin CS following taste-drug pairings, why circulating CORT levels are elevated at test, and whether or not these two measures reflect a similar process. The two responses are not likely mediated by the same biological mechanisms, but rather, separate mechanisms that mutually affect each other. The mechanisms responsible for reducing saccharin intake most likely are involved with perception and ultimately measuring and comparing the perceived value of one reward with another. The circuit in which CORT likely plays an important role is one that modifies perception so that greater CORT levels might directly change the perception of how valuable and desirable a saccharin solution is at that time. This modification would likely occur through actions in the dopamine 'reward' pathway and possibly the prefrontal cortex as well. In sum, although these elevations in CORT appear to be conditioned effects of the drug, the possibility remains that elevations in CORT simultaneously enhance not only learning, but also the rewarding effects of cocaine.

As discussed in the Introduction, there is growing evidence to support the reward comparison hypothesis which states that rats avoid intake of a naturally rewarding saccharin CS in anticipation of a highly rewarding and preferred drug of abuse. One explanation for why some rats are more sensitive to the suppressive effects of cocaine than others is that some rats (i.e., large suppressers) are more sensitive to cocaine's rewarding properties than others (i.e., small suppressers). If this were the case, then one would predict that large suppresser rats would be more likely to demonstrate greater cocaine self-administration behavior. Indeed, we have shown that when saccharin is paired with the opportunity to self-administer cocaine, individual differences occur and greater suppression of intake of the saccharin cue is associated with greater self-administration of the drug (Grigson and Twining 2002).

The second possibility for individual differences in this paradigm relates to learning and memory. Under this premise, large suppresser rats perform better in this paradigm because they learn faster and better retain the memory of the association. One possible underlying mechanism mediating such a learning difference comes from the differences in CORT that we have observed. Greater CORT elevations were observed not only in large suppressers but also in rats given a shorter ISI (i.e. 5 vs. 30 min). Overall suppression was greater for large suppressers and for rats in the 5 min ISI condition than for those in the 30 min ISI condition. This increased performance may have been facilitated by greater CORT elevations during conditioning.

Heightened emotional states such as those brought on by acute stress also are known to enhance learning and memory (McEwen and Sapolsky 1995; Cahill and McGaugh 1998; Shors 2001). A finding of particular relevance to drug conditioning is that brief exposure to a mild stressor can enhance morphine conditioned place preference learning (Ferguson, Patton et al. 2004). Furthermore, conditioned taste aversion learning using LiCl also has been shown to be enhanced (i.e., greater suppression of CS intake) by the acute administration of CORT (Kent, Cross-Mellor et al. 2000; Gorzalka, Hanson et al. 2003). As mentioned earlier, glucocorticoids are thought to facilitate learning and memory through their actions in the hippocampus and the mesolimbic dopamine system (McEwen and Sapolsky 1995; Barrot, Abrous et al. 2001; Shors 2001). There is also some evidence for the role of glucocorticoids in memory modulation in the basolateral amygdaloid complex (Cahill and McGaugh 1998). Not only is dopamine released within the mesolimbic system in response to stress and drugs of abuse but release also occurs during associative learning of neutral stimuli (Imperato, Angelucci et al. 1992; Young, Ahier et al. 1998). The finding that functional CORT receptors have been identified in the striatum and have been found to be associated with at least half of the nigrostriatal and mesolimbocortical dopaminergic neurons imply direct actions by glucocorticoids on dopamine neurons (Defiore and Turner 1983; Fuxe, Cintra et al. 1987; Fuxe, Cintra et al. 1987). Taken together, these findings suggest that greater CORT elevations could enhance drug-induced suppression of saccharin intake through actions in the mesolimbic dopamine system.

Another possible way in which differences in observed CORT levels might influence individual differences in reward comparison behavior is through the rate at which the HPA-axis is activated. That is, the small suppressers may have an HPA-axis that responds slower than that of the large suppressers. If this were the case, then perhaps the small suppressers do experience a conditioned elevation in CORT that is of equal or greater magnitude to that of the large suppressers. This elevation in CORT would not be observed, however, if the response is delayed in the small suppressers. In support of this idea, there are studies that show differences in HPA-axis activity predict drug-taking behavior. As mentioned previously, the CORT response to novelty predicts psychostimulant self-administration. Specifically, prolonged elevation in CORT by Piazza's high responder rats was associated with faster acquisition of amphetamine self-administration (Piazza, Maccari et al. 1991). These differences in hormonal responses have been attributed to a dysfunctional negative feedback system that is responsible for shutting off the CORT response (Maccari, Piazza et al. 1991). It is thought that corticosteroid receptors in the hippocampus are important for regulating (i.e., shutting down) the activity of the HPA-axis and damage to these receptors can lead to prolonged adrenocortical activation (Sapolsky, Krey et al. 1984; Magarinos, Somoza et al. 1987). These findings raise the possibility that HPA-axis functioning also is different for small and large suppresser rats. If there are differences, they could be the result of dysfunctional negative feedback or as mentioned above, delayed activation of the CORT response in the small suppresser rats.

In conclusion, there are individual differences in sensitivity to the suppressive effects of cocaine that are correlated with individual differences in saccharin cue-induced elevations in CORT. Specifically, the rats that are most sensitive to the suppressive effects of cocaine also demonstrate the highest elevations in CORT following presentation of the saccharin cue at test. These higher levels of CORT in the large suppressers may reflect a more active HPA-axis (i.e., greater production of CORT) or an HPA-axis that is activated more quickly than in small suppressers, or in other words, delayed in the small suppressers. Although it is still not clear what the underlying causes for these differences are, these findings help to build a profile for individual sensitivity to cocaine that could predict which individuals are more vulnerable not only to drug-taking behavior but also to devaluation of natural rewards. The next two chapters specifically address the search for a predictive tool using a self-administration design.

Chapter 3

SEARCHING FOR A PREDICTIVE TOOL: SELF-ADMINISTRATION AND THE EFFECT OF COCAINE ON CORTICOSTERONE

If the reward comparison hypothesis is correct and rats avoid intake of saccharin because its rewarding properties pale in comparison to the rewarding properties of the drug, then the paradigm may serve as a model of drug-induced devaluation of natural rewards. Moreover, if presentation of the saccharin CS creates anticipation of the drug, then the saccharin can serve as a cue to elicit craving and that would make our paradigm a model for cue-induced craving as well. A more useful model for drug-taking behavior, however, would be to allow rats to actively self-administer the drug rather than receive it passively by the experimenter. The experiments in Chapter 2 addressed individual differences in reward comparison behavior using a passive drug administration design. Recently, Grigson and Twining (2002) have shown that individual differences in reward comparison behavior also occur when using a self-administration design. These data show that, as in the passive design, rats will reduce intake of the saccharin cue as it comes to predict the opportunity to self-administer cocaine. Furthermore, greater avoidance of the saccharin cue is associated with greater cocaine self-administration. The results from that study further support the

reward comparison hypothesis by showing that avoidance of the saccharin cue after taste-drug pairings is associated with an increase, rather than a decrease, in instrumental responding for the drug of abuse. If rats avoid intake of saccharin because of the aversive properties of the drug, then avoidance would be associated with a reduction in instrumental responding for the drug, as observed for LiCl-induced CTAs (White, Sklar et al. 1977). Our self-administration data are consistent with an earlier study showing that increased responding for apomorphine was associated with increased avoidance of a saccharin cue (Wise, Yokel et al. 1976).

The experiment presented in Chapter 3 was designed to further investigate individual differences in reward comparison behavior using the active drug self-administration design. One goal was to determine how to predict which drug-naïve rats would most greatly avoid intake of the natural saccharin reward and 'consume' the most cocaine. Establishing such a predictive tool requires the establishment of a behavioral and hormonal profile that currently does not exist for the large and small suppresser rats. The ability to predict which rats will be prone to drug taking behavior and to avoidance of the natural reward cue would help to elucidate factors that contribute to these individual differences in behavior and ultimately, factors that contribute to the transition from use to abuse. Inroads, then, might be made not only in the treatment of the disease, but in prevention as well.

The results presented in Chapter 2 show that the elevation in CORT observed at test is, in part, a conditioned response to presentation of the

saccharin cue following taste-drug pairings. In classical conditioning, the conditioned response parallels the unconditioned response (Pavlov 1927/1960). Given this knowledge, we hypothesized that the magnitude of the conditioned CORT response to the saccharin CS seen at test parallels the magnitude of the unconditioned CORT response to the cocaine US. If so, then the magnitude of the unconditioned CORT response to cocaine should predict the magnitude of avoidance of the saccharin cue and cocaine self-administration. Ultimately, the rats demonstrating the greatest unconditioned CORT elevations in response to their first exposure to cocaine should become large suppressers and self-administer the most cocaine.

Although evaluating CORT as a predictive tool was one objective, there were three additional objectives for evaluating CORT throughout the experiment. One objective was to determine whether pre- and post- conditioning patterns in CORT observed at 15 min also would appear at 30 and 120 min following presentation of the saccharin CS. This would help to not only elucidate the time-course of the CORT response but also test whether a conditioned CORT elevation might be evident in the small suppressers when assessed at a later timepoint. A second objective was to test whether basal CORT levels would predict subsequent reward comparison behavior. Finally, a third objective was to test whether large suppresser rats have a dysfunctional negative feedback system for CORT. Dysfunctional negative feedback would be indicated by prolonged elevations in CORT at the 120 min time point. This is the first time blood was sampled repeatedly so that CORT data would be available for more

than one time point in the reward comparison paradigm. These measures help to establish a hormonal profile for large and small suppresser rats that currently do not exist.

Materials and Methods

Subjects. There were a total of 36, naïve, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing between 400 and 500 g at the beginning of the experiment. They were individually housed in stainless steel hanging cages in a temperature and humidity controlled animal care facility with a 12 hr light-dark cycle (lights on at 7 am). Food and water were available ad libitum, except where noted otherwise. The experiment was conducted in six operant self-administration chambers (see below for details). All rats were handled and weighed once a day throughout the experiment.

Catheter Construction and Implantation

Self-administration catheter. As described by Grigson and Twining (2002), the catheters were made in our laboratory using a modified procedure described by Koob and colleagues (1987). The catheter consists of two pieces of Silastic tubing (0.012 in I.D., 0.025 in O.D., 14 cm long, and 0.025 in I.D., 0.047 in O.D., and 2.5 cm long; Baxter Scientific) attached to a stainless steel guide cannula bent at one end at a 90 degree angle (Plastic One, Item #C3136). The cannula assembly is molded into a permanent dental cement base using a mold. A 2.5 x 2.5 cm mesh (Small Parts, Inc.) is permanently fixed to the base via dental cement and functions as a baseplate for the catheter assembly. A small silicon rubber bubble is placed appropriately 3.5 cm from the end of the small tubing. The 3.5 cm length of tubing is placed in the jugular vein and anchored with surgical silk (Ethicon, A53, General Medical Corp.). The entire catheter is flushed with and then soaked in 100% alcohol for 24 hr before implantation.

Catheter implantation. The rat was anesthetized with Ketamine (70 mg/kg)/Xylazine (15 mg/kg) im and shaved on the back between the shoulder blades and directly on top of the jugular vein on the neck. One incision was then made above the jugular vein at the neck (approximately 10 mm in length), at about a 30 degree angle away from midline. Another incision was made on the back of the rat (approximately 1 in length), horizontally positioned between, and just below, the shoulder blades. The skin was separated from the muscle in both locations using blunt dissection. A cannula was then subcutaneously pushed from the incision at the back, over the right foreleg, and through the incision on the ventrum of the rat. The catheter was inserted through the cannula, and then the cannula was removed. The rat was then placed supine and the jugular vein was exposed. Once the jugular vein was located and cleared from surrounding tissue, a stainless steel rod (3 mm diameter) was gently placed under the jugular vein. Once the rod was in place it was used to lift the vein to enable the experimenter to make a small incision (approximately 0.5 mm) in the vein. The catheter (0.025 in O.D. side) was then inserted into the vein through the incision. Verification that the catheter was in the jugular vein was completed by attaching a syringe filled with saline to the other end of the catheter (coming out the back of the rat) and drawing back blood through the syringe. The catheter was then secured into position by tying two ligatures around the vein and tubing both before and after the silicon rubber bubble. Once anchored, the skin is sutured closed and Betadine antibiotic ointment (Baxter Co.) was placed over it. The rats

were then treated with ampicillin (60,000 U, im) after surgery. Patency was verified when necessary using 0.15 cc of 1% Brevital administered iv.

Coupling assembly. Prior to the start of each self-administration session, a coupling assembly was anchored to the back of the rat to provide protected passage of the catheter tubing from the animal. The coupling assembly (a metal spring attached to a metal spacer with Tygon tubing inserted down the center) was attached to the catheter assembly. The catheter tubing was attached to a counterbalanced swivel device (Instech, Inc.) that in turn was attached to a fluid injection assembly (syringe pump) outside the sound attenuating cubicles. The fluid injection assembly enabled intravenous infusion of cocaine during self-administration sessions. In the animal's home cage, the catheter was sealed with a piece of Tygon tubing and a metal spacer was placed over the catheter assembly. General maintenance of catheter patency involved daily examination, cleaning of the coupling assembly, and flushing of the catheter with heparinized saline. With this procedure, approximately 75% of the catheters remain patent for 2 months or longer. This is a sufficient period to conduct our behavioral tests.

Apparatus: Self-administration chambers. The rats were tested in one of six modular operant chambers (MED Associates, Inc., St. Albino, VT) that measured 30.5 cm long x 24.0 cm wide x 29.0 cm high and were housed in a light- and sound-attenuating cubicle. All chambers had a clear Plexiglass top, front, and back wall. 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). Two retractable sipper tubes were located on the left wall of the

chamber and entered the chamber through 1.3-cm diameter holes spaced 16.4 cm apart (center to center). A stimulus light was positioned 6 cm above each tube. 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. Each chamber was equipped with a house light (25 W), a tone generator (Solalert Time Generator, 2,900 Hz), and a speaker for white noise (75 dB). Cocaine reinforcement was controlled by an electronic circuit that operated a syringe pump (Razel Scientific Inst., Model A). All events were monitored and controlled on-line with a PC Pentium computer using a commercially available software program (MED Associates, Inc.).

Procedure. Phase I. Baseline CORT. Following one week of recovery from surgery, blood was taken via catheters from all rats in the morning between 10 – 12 am to assess basal CORT levels. Phase II. Cocaine pre-exposure CORT. On the following day, all the rats were injected s.c. with a 10 mg/kg dose of cocaine and blood was taken from the catheter 15, 30, and 120 min following the injection to assess the unconditioned CORT response to their first exposure to cocaine. Deprivation Regimen and Habituation. Two days later, all the rats were placed on a restricted water schedule and habituated to the operant chambers simultaneously. Thus, rats were placed in the operant chamber for 5 minutes, during which time they had access to water from a retractable sipper tube. Rats also were given 1 hour access to water in the afternoon. There were 5 days of habituation. Saccharin Pre-Exposure & Pre-conditioning CORT: Twenty-four hr following the last 5 min access period to water, and 48 hours prior to the

first trial, all the rats were pre-exposed to the 0.15% saccharin solution for 5 minutes in the operant chambers. Blood samples were taken 15, 30, and 120 min following access to saccharin and rats were returned to their home cages after the 30 min sample. In so doing, CORT was assessed in response to presentation of the saccharin solution before it became associated with the cocaine. This approach controlled for any possible unconditioned effect of saccharin on baseline CORT. Phase III. Conditioning: Saccharin Intake and Self-Administration. Two days after pre-exposure to saccharin, conditioning began. A total of 8 animals were dropped from the study by this time. Two rats died and the catheters failed in the other six. The remaining 28 rats were first divided into one of two testing conditions: saccharin-saline iv (n=10) or saccharin-cocaine iv (n=18). The groups were counterbalanced for weight, saccharin intake on pre-exposure day, and water intake on training days. During testing, the rats were placed in the chambers with the house light and white noise on. The left tube was advanced to give the rats 5 min access to 0.15% saccharin. When the CS tube retracted, the empty tube was advanced on the right as well as an inactive empty tube in the center. The stimulus light was illuminated above the spout and the house light was turned off. When a rat licked the empty tube 10 times, it received an iv infusion of saline or 0.5 mg/kg cocaine over a 6 sec period. This is termed a fixed ratio 10 schedule of reward (FR10). Drug or saline delivery was signaled by offset of the stimulus cue light, retraction of the spout, and onset of the tone and the house light. The tone remained on for 20 sec and during this time further responding was not reinforced. The access period for the drug or saline was 1 h

and after this time, the empty US tube and the inactive spout were retracted. Supplemental water was provided for 1 h, no sooner than 45 min after the rats were returned to the home cage. There was one CS-US pairing a day for 13 days and all testing was conducted over an 8-hr period starting 2 hr into the light phase of the cycle (9 am). The number of contacts made to the spout, the spout, and the inactive empty tube were recorded. Test Day Post-conditioning CORT: The final trial served as a CS only test trial during which all rats were given 5 min access to the saccharin CS and blood was sampled from the catheter 15, 30 (in the testing chamber), and 120 (in the home cage) min later. No drug was administered on this day.

Tastants and Drugs. Sodium saccharin (Fischer Scientific, Malvera, PA) was dissolved in distilled water (dH₂O) overnight and presented at room temperature. Cocaine hydrochloride (HCl) was obtained from the National Institute on Drug Abuse and dissolved in sterile saline immediately before each session.

CORT Radioimmunoassay. Plasma CORT concentrations were determined as described for Chapter 2.

Results and Discussion

A total of 9 animals were dropped by the end of the study. 2 died before the experiment started, 1 during the experiment, and 6 due to catheter failure. The data from the remaining 27 rats (17 cocaine, 10 saline) were used in the analyses presented below. All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc comparisons were conducted, when appropriate, using either the Fisher Least Sig Diff or the Newman Keuls test, with alpha set at 0.05.

Due to an electrical circuit interruption, data points (licks, infusions, and latencies) were missing for one of the cocaine animals on trial 7 and for a different cocaine animal on trial 8. Scores for these two animals on those days were therefore extrapolated by averaging the data points from the trials before and after the trial for which the data were missing.

Saccharin Intake (Licks/5 min). The cocaine-infusing group tended to suppress intake of the saccharin CS compared with that of the control group. This tendency, however, did not attain significance following an analysis using a mixed factorial 2 x 14 ANOVA varying drug group (saline and cocaine) and trials (1-14), $F < 1$, $p > 0.05$ (see Figure 3.1, left panel).

As in other experiments, there were large individual differences within the cocaine group and the cocaine subjects were divided into large and small suppressers. The uneven number of animals in the drug group (N=17), however, would not allow an even division using a median split. Therefore, the mean number of licks on the final trial, which was 1118, was used to divide the animals

into two groups such that all the cocaine rats whose intake fell above the mean were labeled as small suppressers (N=9) and all the cocaine rats whose intake fell below the mean were labeled as large suppressers (N=8). The data were then regraphed (see Figure 3.1, right panel) and analyzed using a 3 x 14 mixed factorial ANOVA varying drug group (saline, small suppressers, and large suppressers) and trials (1-14). Newman Keuls post hoc comparisons of a highly significant Group x Trials interaction, $F(26,312)=2.36$, $p<0.001$, showed that large suppressers consumed less of the saccharin CS than did the saline controls on Trials 3-5 and 7-14, $p_s<0.05$. The large suppressers also made fewer licks for the saccharin CS than did the small suppressers and this effect attained statistical significance on Trials 3-14, $p_s <0.05$. The small suppressers, however, did not demonstrate suppression of the saccharin CS. If anything, intake by the small suppressers tended to be greater than that generated by the saline controls.

Although the rats suppressed intake of saccharin when paired with the opportunity to self-administer cocaine, overall suppression tended to be weaker than previously observed (Grigson and Twining 2002). One possible explanation for this attenuated avoidance behavior is that all the rats were pre-exposed to both the saccharin CS and the cocaine US. This pre-exposure may have weakened the association between the two stimuli as reflected by the weakened suppression in CS intake (Lubow and Moore 1959; Lubow 1973; Alek, Arzy et al. 1975).

Reward Comparison: Saccharin Intake

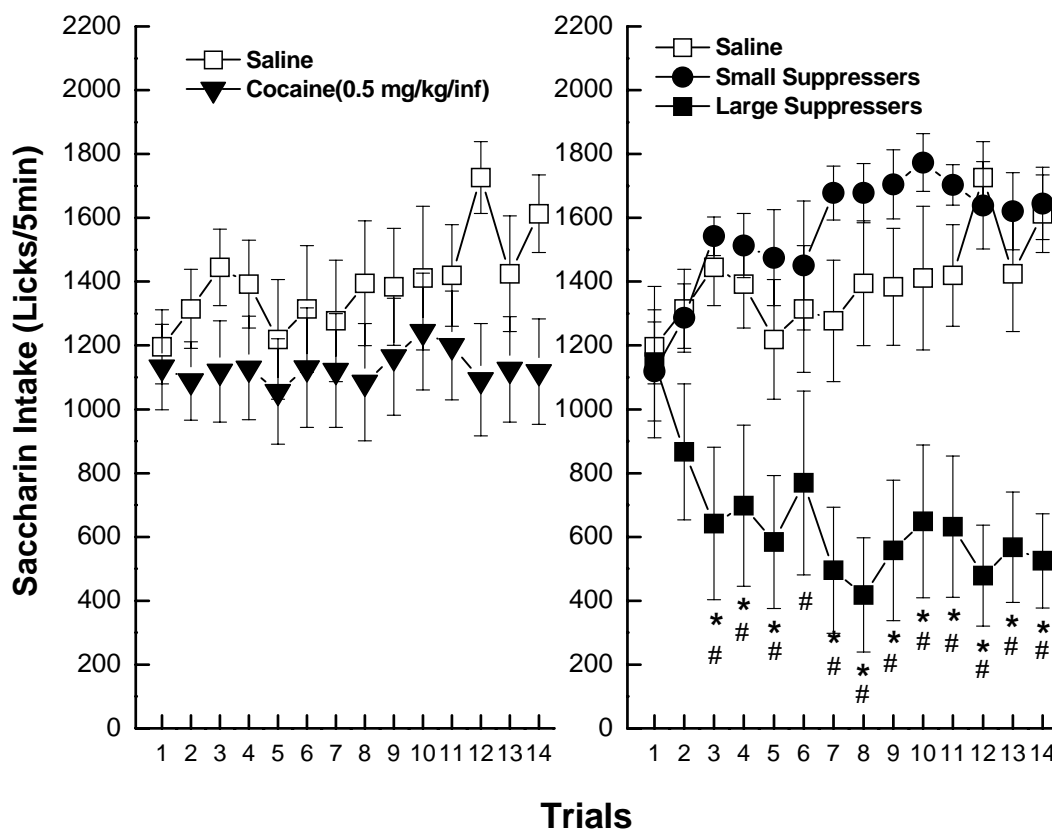


Figure 3.1: Left panel. Mean (+/- SEM) intake (licks/5 min) of 0.15% saccharin over 13 pairings with the opportunity to SA saline (N=10) or cocaine (0.5 mg/kg/infusion; N=17) for 1 hr, using a FR10 lick contingency on an empty spout. Trial 14 is a CS-only test day. Right panel. Same data re-graphed with the cocaine rats divided into small (N=9) and large (N=8) suppressers. #Significantly different from saline, *significantly different from small suppressers, $p_s < 0.05$.

Latency to Lick the CS (sec). The latency to lick the saccharin CS (data not shown) mirrored the intake behavior described above. Results of a 2 x 14 mixed factorial ANOVA varying drug group (saline and cocaine) and trials (1-14) showed that although cocaine-infusing animals tended to wait longer to make their first lick for the saccharin CS than saline-infusing animals, latency, like intake, did not statistically differ between the cocaine and the saline group, overall, $p > 0.05$. Given the large individual differences obtained in the lick data, the latency data also were analyzed on this basis (i.e., using the small and large suppresser group assignments determined for the Bottle 1 saccharin intake). The results of a 3 x 13 mixed factorial ANOVA varying drug group (saline, small suppressers, and large suppressers) and trial (1-14) revealed a significant main effect of Group, $F(2, 24) = 4.29$, $p < 0.05$, indicating that the large suppressers, not only drank less, but also waited longer than the small suppressers and the saline group to make their first lick for the saccharin cue ($M/SEM = 28.0/1.4$, $4.8/0.3$, and $3.4/0.2$ sec, respectively).

Cocaine US Infusions per Hour. Rats given the opportunity to infuse cocaine made more infusions than rats given the opportunity to infuse saline (see Figure 3.2, left panel). Support for this conclusion was provided by a significant main effect of Drug, $F(1, 25) = 6.22$, $p < 0.05$. Self-administration behavior also was evaluated on the basis of individual differences obtained in the number of licks made for the saccharin CS. The results of a 3 x 13 ANOVA varying group (saline, large and small suppressers) and trials (1-13) revealed a significant main effect of Drug, $F(2, 24) = 29.9$, $p < 0.05$, indicating that large suppressers self-

administered more cocaine than small suppressers and made more infusions than the saline group overall (see Figure 3.2, right panel). Neither the main effect of Trial, nor the Group x Trial interaction, however, was significant, $p > 0.05$.

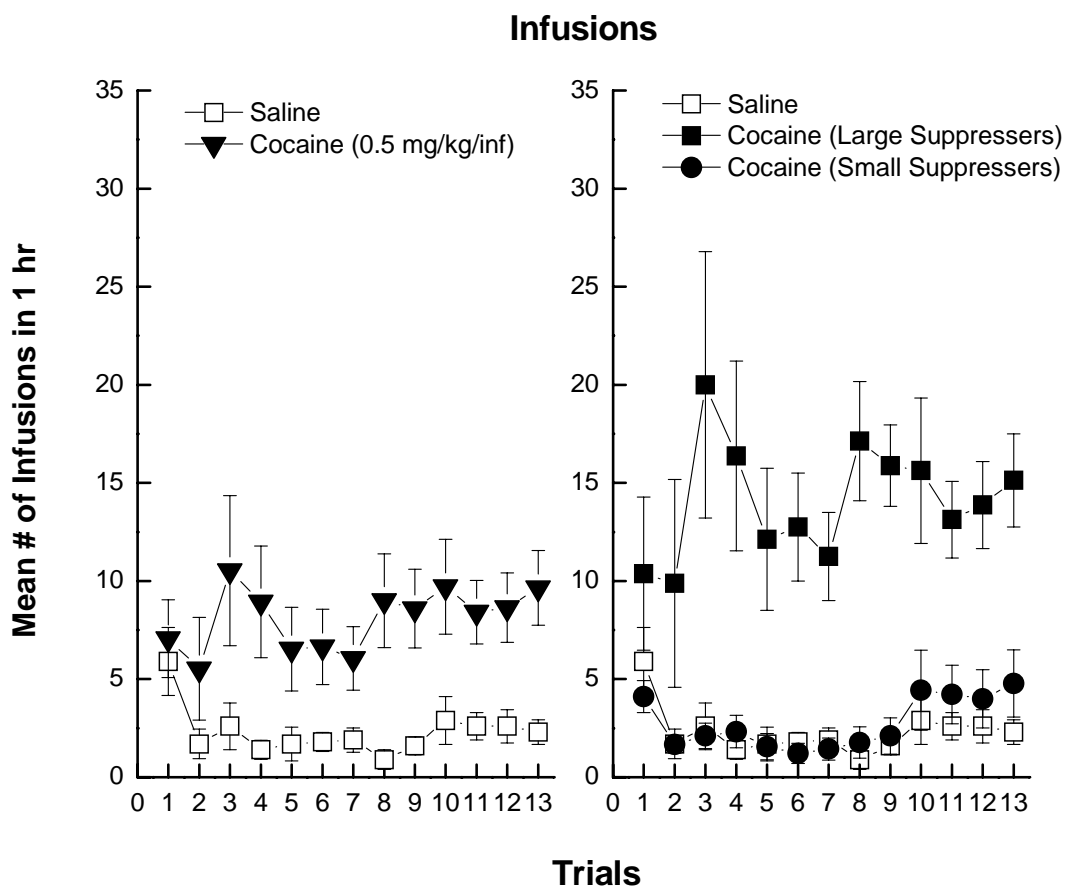


Figure 3.2. Left panel. Mean (+/- SEM) number of infusions per 1 hr made for saline (N=10) or for cocaine (0.5 mg/kg/infusion; N=17), using a fixed-ratio 10 lick contingency on an empty spout. Right panel. The same data re-drawn with the cocaine rats divided into small suppressers (N=9) and large suppressers (N=8).

Latency (in sec) to Make First Infusion. The time that it took the rats to make the first infusion (data not shown) was analyzed using a 2 x 13 mixed factorial ANOVA varying drug group (saline and cocaine) and trials (1-13). The results of this analysis showed no significant differences between the two groups. When the data were analyzed with the cocaine animals divided into small and large suppressers, there was a tendency for the large suppressers to exhibit shorter latencies to make their first infusion across trials. This effect, however, did not attain significance, $p > 0.05$.

Taken together, the data confirm that these two groups, the large and the small suppressers, behave differently on two separate measures: CS intake and cocaine self-administration. Moreover, greater avoidance of the saccharin CS was associated with greater self-administration of the cocaine. A correlation analysis was conducted to assess the strength of this relationship. The terminal number of infusions was graphed as a function of terminal saccharin intake for all rats in the cocaine group (N=17, see Figure 3.3, right panel). The results of this analysis revealed a highly significant negative correlation such that less consumption of the saccharin CS (i.e., greater avoidance of the saccharin CS) was associated with greater self-administration of cocaine ($r = -0.83$, $p < 0.001$).

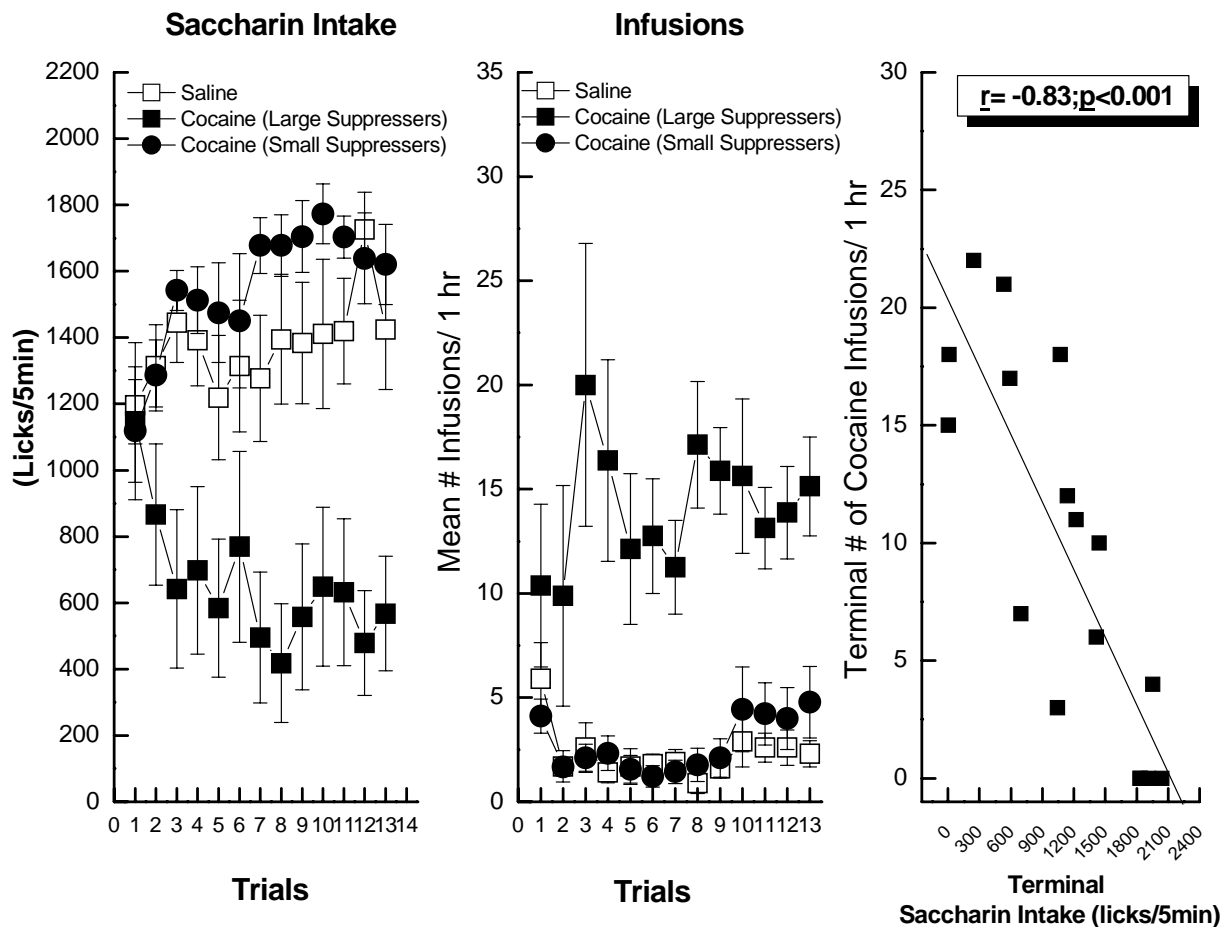


Figure 3.3: Left panel. Mean saccharin intake across trials for the saline, small, and large suppresser groups. Middle panel. Mean number of infusions made for the same groups shown in left panel. The left and middle panels are the same graphs shown in Figures 3.1 and 3.2 and are shown again for comparison. Right panel. The results of a correlation analysis show that greater terminal cocaine SA is correlated with greater avoidance of the saccharin CS ($r=-0.83, p<0.001$).

CORT. There were 33 instances (out of 270) during repeated blood sampling when the animal did not give enough blood to assay. Therefore, when necessary, the group average for a particular time point was used to provide a value that could be filled in for data points that were missing due to insufficient blood collection. This extrapolation was performed to prevent the statistical program from excluding the entire subject from a particular analysis. The group average was used so that there would be no systematic bias. Circulating CORT elicited by the saccharin cue both before (Pre) and after (Post) conditioning are presented below, followed by an analysis of the potential predictive effect of baseline CORT and the unconditioned CORT response following the first s.c. injection of cocaine (10 mg/kg).

Pre- and Post-Conditioning CORT. The CORT data were analyzed separately for each time point (15, 30, and 120 min) using 3 x 2 repeated measures ANOVAs varying group (saline, small suppresser, and large suppresser) and trial (pre- and post-conditioning). Fifteen min (Figure 3.4, left panel). Patterns in the CORT response at the 15 min time point resembled those observed previously (see Chapter 2) when using the passive design. Despite the tendency for pre-conditioning CORT levels to be higher than expected for all three groups, the large suppressers still managed to demonstrate an increase in CORT from pre- to post-conditioning. Fisher's LSD post hoc comparisons of a significant Group x Trial interaction, $F(2,23)=3.82$, $p<0.05$, revealed that post-conditioning CORT levels were significantly higher for the large suppressers than for both the saline and small suppresser groups, $ps<.05$. CORT levels for saline

animals were actually significantly lower at test than they were before conditioning, $p < 0.05$. Thirty min (Figure 3.4, middle panel). Mean circulating CORT levels rose slightly at the 30 min time point in all groups both before (pre) and after (post) conditioning. With the slight increases in mean CORT levels, however, there also were increases in variability within each group. As a result, no statistical differences were found following a Group x Trial ANOVA, $p_s > 0.05$. Two hr (Figure 3.4, right panel). By the two hr time point, mean CORT levels decreased substantially across the board. For all three groups, there was a tendency for post-conditioning CORT levels to be higher than pre-conditioning levels. Support for this conclusion was provided by a significant main effect of Trial, $F(1,24)=9.95$, $p < 0.05$. There was also a significant main effect of Group, $F(2,24)=8.13$, $p < 0.05$, indicating that CORT, overall, differed among groups. Specifically, CORT levels (pre and post combined) were significantly higher for the cocaine groups (both small and large suppressers) than for the saline group, $p_s < 0.05$. The Group x Trial interaction, however, did not attain significance, $p > 0.05$.

One major difference in CORT patterns observed in this experiment was that pre-conditioning CORT levels at the 15 min time point tended to be higher for all three groups than those described previously for the passive design. The most likely cause for such unusually high CORT levels is that the rats were not adequately habituated to the operant chambers at the time of blood sampling. During the habituation and water training phase, all the rats were placed into the operant chamber for a total of 5 min. On the CS pre-exposure day, however, all

rats were kept in the chamber for 30 min instead of the usual 5 min so that blood could be collected at the later time points (15 and at 30 min) . The high CORT levels were most likely caused by some amount of stress from being contained in the operant chamber for 30 instead of 5 min. Support for this conclusion comes from subsequent experiments where the length of habituation period in the operant chambers was extended from 5 to 15 min each day. Indeed, once this change was employed, pre-conditioning CORT levels assessed on the saccharin pre-exposure day were found to be lower than those observed here (unpublished data).

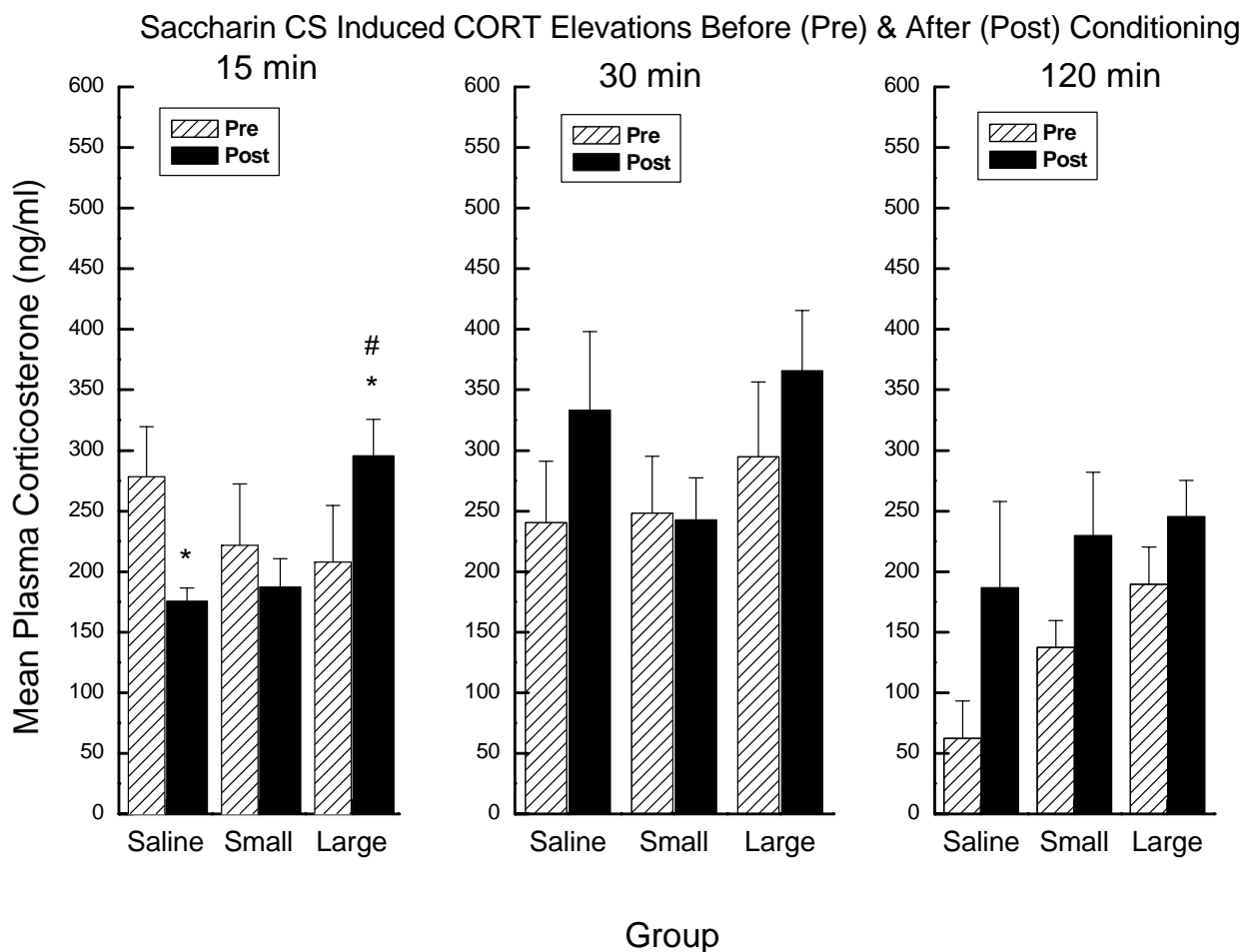


Figure 3.4: Mean plasma CORT levels following presentation of the saccharin CS pre- and post- conditioning for all three groups (saline, small, and large suppressors). CORT data are shown for the 15 (left panel), 30 (middle panel), and 120 (right panel) min time points. *significantly different from pre. # significantly different from other groups.

Despite unusually high pre-conditioning CORT levels at the 15 min time point, greater suppression of CS intake by the large suppressers was associated with greater post-conditioning CORT levels. Support for this conclusion was provided by results of a correlation analysis assessing the relationship between CS intake and CORT levels at test. The results of this analysis showed a significant negative linear relationship between terminal saccharin intake and post-conditioning CORT levels at the 15 min time point, $r=-0.48, p<0.05$, see Figure 3.5, left panel. At the 30 min time point, however, the negative linear relationship between terminal saccharin intake and post-conditioning CORT was no longer significant, $p>0.05$ (see Figure 3.5, middle panel) and disappeared completely by the 120 min time point. In fact, there was a tendency for saccharin intake to be positively, rather than negatively, associated with CORT at this later time point (see Figure 3.5, right panel).

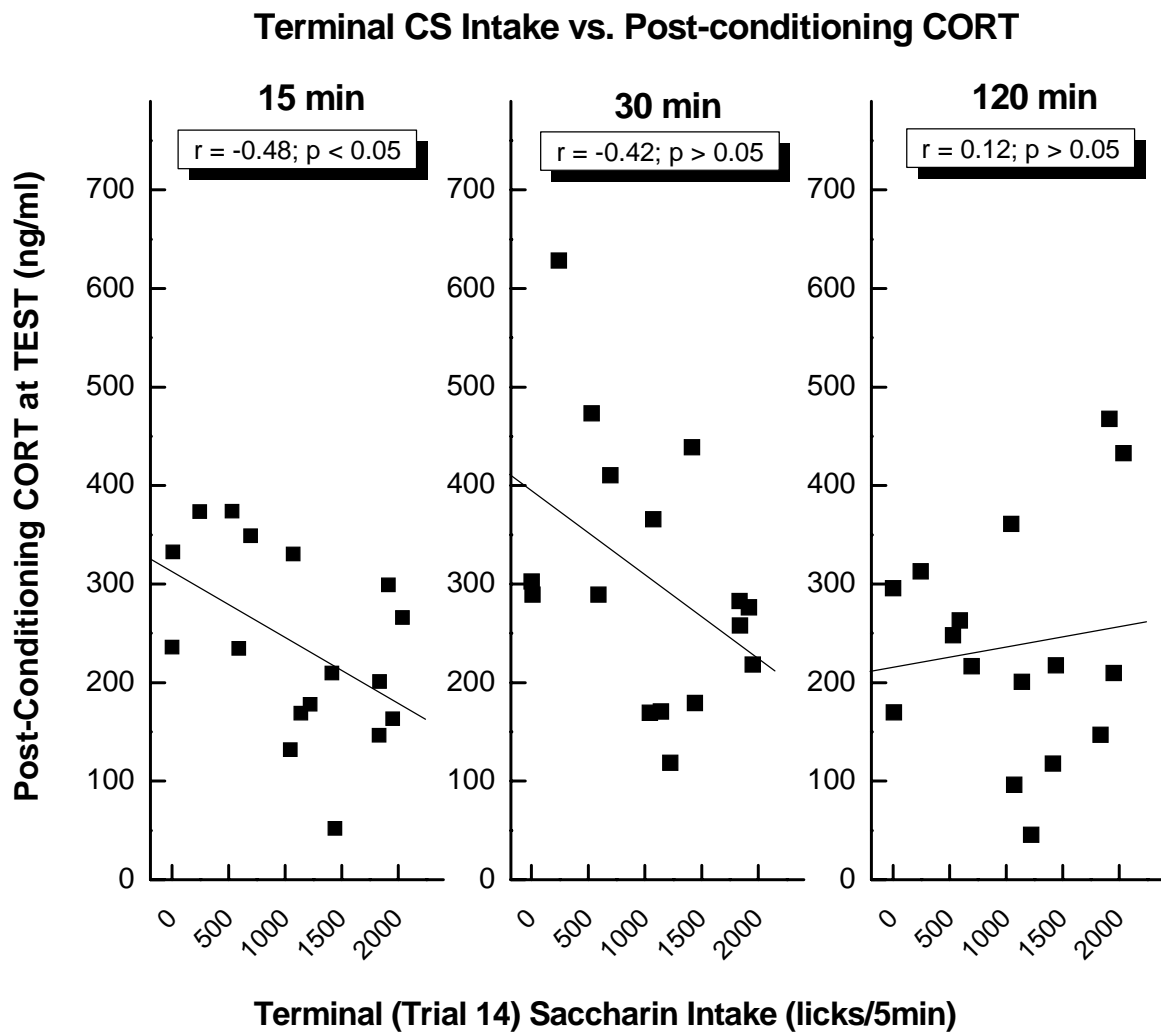


Figure 3.5: Correlation analyses assessing the relationship between terminal saccharin intake and post-conditioning CORT levels at the 15 min (left panel), 30 min (middle panel), and the 120 min (right panel) time points for cocaine infusing rats (N=17). These results show that terminal saccharin intake was significantly associated with CORT levels only at the 15 min.

A second series of correlation analyses were conducted to test whether post-conditioning CORT levels would be more closely associated with the change in saccharin intake from the first to the last trial. Although terminal saccharin intake (used above) serves as a sufficient measure of relative suppression among subjects, the difference in intake from trial 1 to trial 14 may provide a more sensitive measure of the magnitude not only of suppression, but also of the occasional increase, in CS intake by small suppressers. The results of these analyses revealed even stronger negative correlations between the change in saccharin intake over trials and post-conditioning CORT levels at both the 15 and the 30 min time points (see Figure 3.6, left and middle panels). Indeed, the negative relationship attained significance at both the 15 and 30 min time points, $p < 0.05$, whereas, significance was attained at only the 15 min time point when using terminal intake above.

Patterns in CORT at the 120 min time point were, again, not like patterns observed for the earlier time points. In fact, the positive relationship between CS intake and CORT at the 120 min time point, observed above, became even stronger in the present analysis. Although still not significant, $p > 0.05$, the trend is important in that it is the first time the relationship between suppression of CS intake and CORT has been found to go in the opposite direction (positive vs. negative). Although post-conditioning CORT levels for the large suppressers had come down from what was observed at the 30 min time point (Means=366 ng/ml at 30 min and 245 ng/ml at 120 min), post-conditioning CORT levels for the small suppressers remained elevated at the 2 hr time point (Means= 243 ng/ml at 30

min and 230 ng/ml at 120 min). Implications for the elevated CORT levels observed in small suppressers at the 120 min time point are discussed later. In general, these data show that rats in the active self-administration design, like rats in the passive design, exhibit individual differences in both saccharin intake and CORT such that greater avoidance of the saccharin cue is associated with greater cue-induced CORT elevations at test.

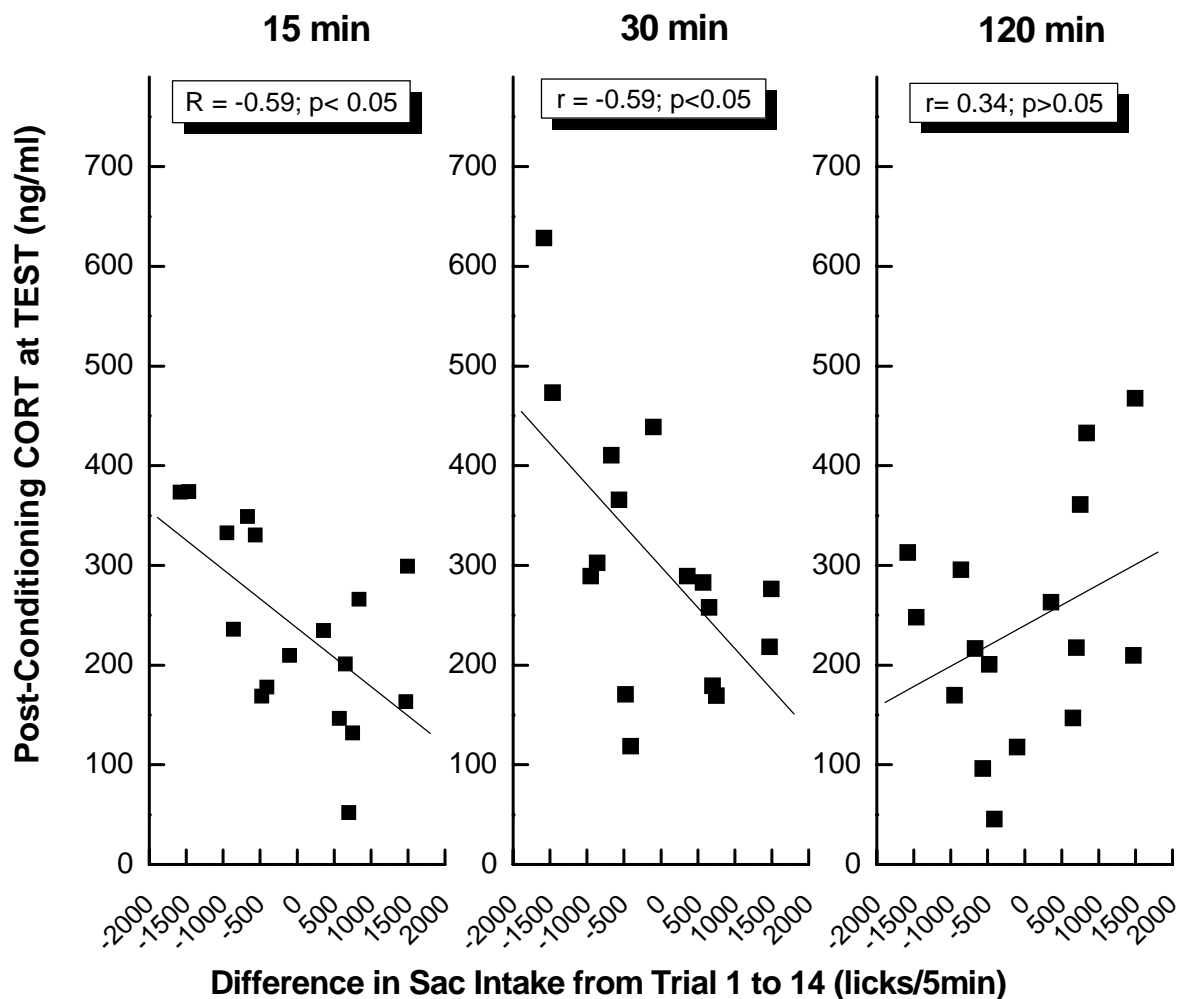


Figure 3.6: Correlation analyses assessing the relationship between the change in saccharin intake from the first to the last trial and post-conditioning CORT levels at the 15 min (left panel), 30 min (middle panel), and the 120 min (right panel) time points for cocaine infusing rats (N=17). Thus, changes in saccharin intake were significantly associated with CORT levels at 15 and 30 min, but not at 120 min.

Basal and Unconditioned CORT Response to the Cocaine US. As

described earlier, CORT levels were assessed, not only for the standard pre- and post-conditioning phases but also prior to the beginning of the experiment to determine whether baseline or the unconditioned CORT response to cocaine (10 mg/kg/s.c.) could predict which rats would go on to become small or large suppressers. Figure 3.7 presents mean CORT levels at each time point (0, 15, 30, and 120 min) for small and large suppressers. The overall CORT response was strongest in the large suppressers. The results of a mixed factorial 2 x 4 ANOVA varying group (small and large) and time point (0, 15, 30, 120 min) revealed a significant main effect of Time, $F(3,45)=18.5$, $p<0.05$, indicating that, for all the rats, CORT levels changed significantly over time. Neither the main effect of Group, $F(1,15)=2.1$, $p>0.05$, nor the Group x Time interaction, $F(3,45)=2.5$, $p>0.05$, however, were significant. CORT levels were then analyzed separately for each time point using unpaired t-tests to determine whether mean CORT levels were significantly different between small and large suppressers at any of the time points. The results showed that mean CORT levels were significantly different for the two groups only at zero min, where large suppressers demonstrated lower basal CORT levels than small suppressers, $p<0.05$.

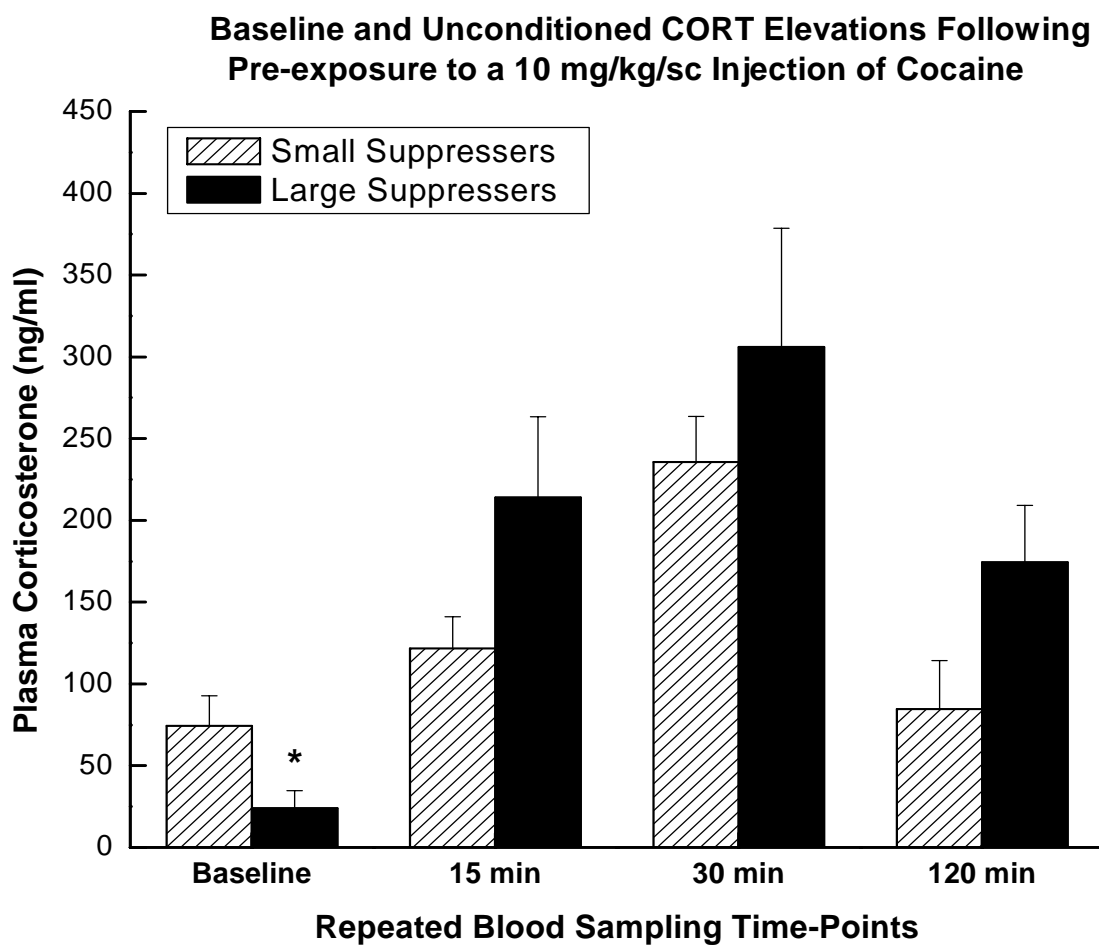


Figure 3.7: Baseline and unconditioned plasma CORT levels 15, 30 and 120 min following pre-exposure to a 10 mg/kg/sc injection of cocaine for small and large suppressers. CORT is significantly different for the two groups at Baseline where large suppressers show lower levels than small suppressers.

Unconditioned CORT response vs. suppression in CS intake:

Basal CORT. To better assess the relationship between CORT and the propensity to devalue the naturally rewarding saccharin CS, a series of correlation analyses were conducted. These analyses will focus on the change in intake over trials, rather than terminal intake, since it proved to be a more sensitive measure for the magnitude of suppression in saccharin intake in the correlation analyses conducted earlier. The first analysis tested whether there is a relationship between basal CORT levels (Time zero) and the change in individual saccharin intake from trial 1 to 14. Surprisingly, the results showed that, despite the significant difference between the two means for small and large suppressors, there was no relationship between basal CORT and the change in saccharin intake over trials, $p > 0.05$ (see Figure 3.8, panel a).

Cocaine-induced elevation in CORT. The next three panels in Figure 3.8 show individual CORT data at 15, 30, and 120 min following an injection of cocaine (10 mg/kg/sc) plotted as a function of individual saccharin intake behavior (again, the change from the first to the last trial). Based on the above and previous findings where greater avoidance of the saccharin cue has been associated with greater conditioned elevations in CORT at test, the following analyses were designed to test whether the unconditioned CORT response to cocaine also would be associated with greater avoidance of the saccharin cue. In so doing, correlation analyses were conducted to assess the relationship between the magnitude of change in saccharin intake behavior and plasma CORT levels in response to the administration of the cocaine US (10 mg/kg,

s.c.). The results of these analyses showed that greater avoidance of the saccharin cue was significantly correlated with greater unconditioned elevations in CORT when blood was sampled 15 min after the administration of cocaine, but not when sampled at the 30 or 120 min time points. Thus, both the conditioned (i.e., post-conditioning CORT) and the unconditioned CORT response to cocaine were associated with avoidance of the saccharin CS at the 15 min time point. Basal CORT, however, was not related.

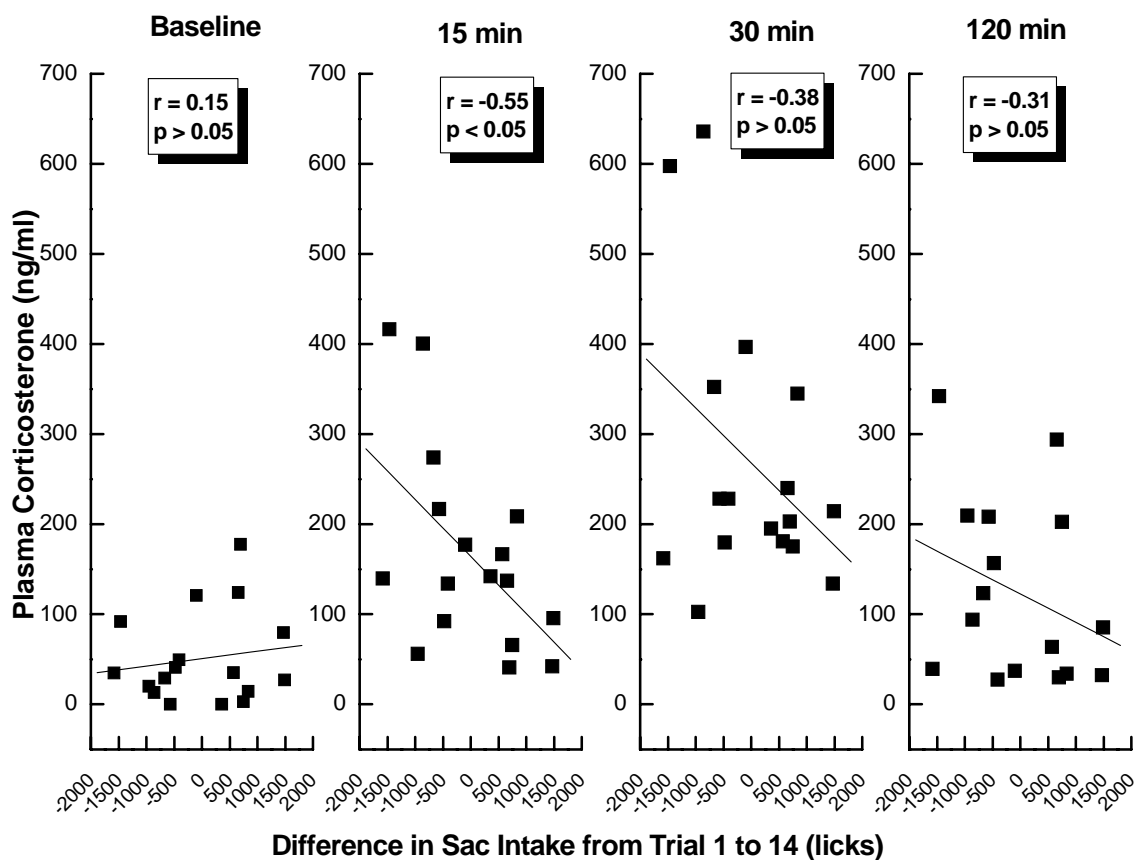


Figure 3.8: The change in saccharin intake from the first to the last trial (in licks) for each cocaine subject (N=17) as a function of plasma CORT levels (ng/ml). Samples were taken at Baseline, 15, 30, and 120 min following an injection of cocaine. The results of correlation analyses show that the change in saccharin intake was significantly associated with CORT only when blood was sampled 15 min after an injection of cocaine.

Unconditioned CORT response vs. cocaine SA:

The next set of correlation analyses was designed to assess the relationship of the unconditioned CORT response to cocaine with cocaine SA (see Figure 3.9). The total number of cocaine infusions from trial 5 through trial 13 was plotted against the unconditioned CORT response at baseline, 15, 30, and 120. The results of these analyses showed that although there was no relationship between basal CORT and cocaine SA, there was a significant relationship between the unconditioned CORT response at 15 min and cocaine SA. Specifically, greater cocaine SA was correlated with greater cocaine induced elevations in CORT.

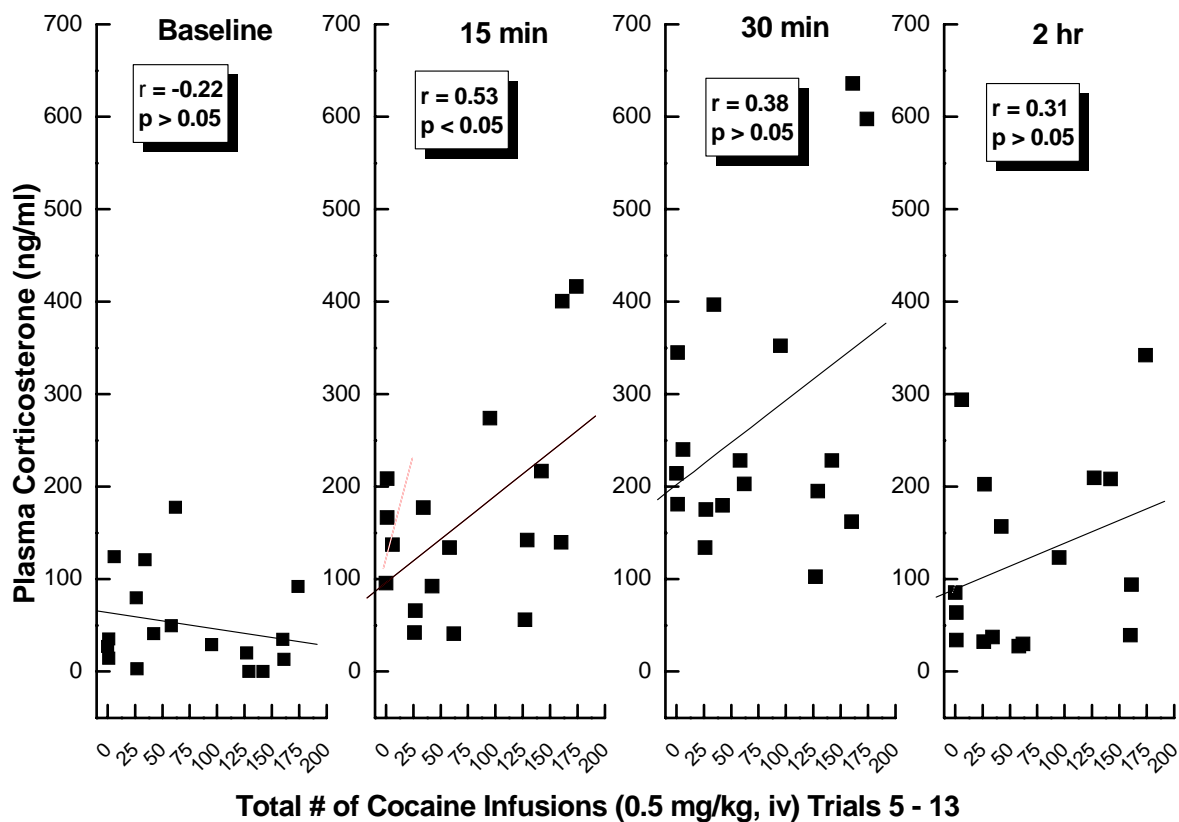


Figure 3.9: The total number of cocaine infusions from trial 5-13 for each cocaine subject (N=17), as a function of plasma CORT levels (ng/ml). Samples were taken at Baseline, 15, 30, and 120 min following an injection of cocaine (10 mg/kg, sc). The results of correlation analyses showed that the cocaine SA was significantly associated with CORT only when blood was sampled 15 min after an injection of cocaine.

Discussion

These data confirm that rats will suppress intake of a saccharin CS when paired with the opportunity to self-administer cocaine. Moreover, large individual differences in reward comparison behavior found in this study are consistent with previous findings (Grigson and Twining, 2002), as seen with the emergence of small and large suppresser rats. Individual differences occurred in not only saccharin intake, but also cocaine self-administration. Specifically, greater avoidance of the saccharin cue by the large suppresser rats was correlated with greater drug self-administration. Furthermore, greater self-administration of cocaine also was correlated with greater elevations in CORT at test and following the first exposure to cocaine (the unconditioned response to 10 mg/kg, sc). This finding supports and extends a growing body of evidence that CORT plays an important role in facilitating the acquisition of cocaine self-administration and maintaining the behavior once acquired (Goeders and Guerin 1996; Goeders and Guerin 1996; Deroche, Marinelli et al. 1997; Mantsch, Saphier et al. 1998; Mantsch, Schlussman et al. 2000).

Patterns in 15 min pre- and post-conditioning CORT observed in the present study were similar to patterns in CORT observed using the passive design. The only major difference was that, in the present the study, the pre-conditioning CORT levels were unusually high. The probable reason for high CORT, as described earlier, was inadequate habituation to the operant chambers. Despite the unusually high pre-conditioning CORT levels, the large suppressers still exhibited a significant increase from pre- to post-conditioning

CORT. Furthermore, post-conditioning CORT levels for the large suppressers were significantly higher than those for the saline group and the small suppressers. Indeed, the magnitude of the CORT response 15 min following presentation of the saccharin CS at test was correlated with the magnitude of suppression observed at test. The small suppressers, however, did not demonstrate an elevation in post-conditioning CORT levels when compared with the saline group. Although small suppressers have demonstrated elevations in post-conditioning CORT levels in past experiments, the absence of the post CORT response in this experiment mirrors the absence of suppression in saccharin intake relative to the saline group. Despite the absence of suppression in saccharin intake for the small suppressers as a group, saccharin intake on an individual basis still was significantly correlated with 15 min post-conditioning CORT levels. That is, greater avoidance of the saccharin cue was associated with greater elevations in CORT at test in both small and large suppresser rats when using a self-administration design.

Patterns in pre- to post-conditioning CORT at the 30 min time point were similar to the patterns observed at the 15 min time point. Pre-conditioning CORT levels remained unusually high for all three groups (saline, large, and small suppressers) at the 30 min time point but eventually decreased to basal levels by the 120 min time point. Post-conditioning CORT levels at the 30 min time point were highest for the large suppressers and also higher than pre-conditioning CORT levels, overall. Both pre- and post-conditioning CORT levels were higher

at 30 min than at 15 min indicating that CORT continues to rise from the 15 min mark which is the standard time point used in previous experiments.

At the 120 min time point, interestingly, the negative relationship between saccharin intake and post-conditioning CORT disappeared. Although the group means for post-conditioning CORT were nearly equal at the 120 min time point, variability in the CORT data increased within the small suppresser group. The results of correlation analyses confirmed that there was no longer a negative relationship between saccharin intake and post-conditioning CORT at 120 min. If there was any relationship, surprisingly, it was a positive linear relationship where greater suppression in saccharin intake tended to be associated with lower, rather than greater, post-conditioning CORT levels. This finding is unusual and the reasons for this observation are likely related to higher levels of post-conditioning CORT in several small suppresser rats. In any case, the relationship between suppression of CS intake and post-conditioning CORT is strongest when blood is sampled earlier (at 15 min) and closer to the CS access period.

A second objective of this experiment was to determine whether basal CORT levels could predict which drug-naïve rats would go on to become a small or a large suppresser. Interestingly, although basal CORT levels were significantly lower for the large suppressers, the results of a correlation analysis showed that there actually was no significant relationship between basal CORT levels and avoidance of saccharin intake.

A third objective was to find a predictive too by evaluating the unconditioned CORT response to the cocaine US (prior to the start of the

experiment) at 15, 30 and 120 min following the administration of a 10 mg/kg, s.c. dose of cocaine. Unlike basal CORT levels, the unconditioned CORT response to cocaine was correlated to the suppression of CS intake. The overall unconditioned CORT response to cocaine appeared to be stronger in the large suppressers than in the small suppressers at every time point (15, 30 and 120 min). Consequently, correlation analyses were conducted to test the relationship between suppression of CS intake and the unconditioned CORT response at each time point. The results of these analyses showed that this relationship was significant only at the 15 min time point. This also was the case when the relationship between the unconditioned CORT response and cocaine SA was tested. Thus, the conditioned CORT response to a cue that has been paired with cocaine parallels the unconditioned CORT response to the first cocaine exposure. Moreover, rats that experience greater elevations in CORT following their first exposure to the drug are more likely to demonstrate greater SA and to demonstrate greater avoidance of the saccharin cue.

The fourth objective for obtaining CORT data at repeated time points relates to findings from another laboratory showing that differences in drug SA are associated with differences in sensitivity of the HPA axis. Specifically, Piazza et al. (1991) have shown that rats exhibiting enhanced locomotor activity and prolonged elevations in CORT after exposure to a novel environment (referred to as the High Responders) acquired amphetamine self-administration more readily than rats that exhibited a weaker behavioral and hormonal response (referred to as the Low Responders). The prolonged adrenocortical activation demonstrated

by Piazza's High Responder rats has been attributed to dysfunctional negative feedback within the HPA axis (Maccari, Piazza et al. 1991). The 120 min time point was employed in the present experiment to see if our large suppressers, like Piazza's High Responders, would demonstrate prolonged elevations in CORT compared with the small suppressers. For this purpose, CORT was observed at 120 min for all three contexts in which it was measured, the unconditioned response following the first exposure to the drug, the pre-conditioned response, and the conditioned response at test following saccharin-cocaine pairings.

CORT data at the 120 min time point showed that our large suppressers do not demonstrate prolonged elevations in CORT, as observed for Piazza's High Responder rats. In fact, CORT levels evaluated at test (e.g., post-conditioning) remained elevated most markedly for the small rather than the large suppressers. One reason for this discrepancy may involve differences in methodology. Specifically, CORT was assessed by Piazza et al. (1991) following exposure to a novel environment that served as a mild stressor. In the present study, CORT was assessed in response to cocaine and the saccharin cue. Thus, the CORT response was triggered within different contexts in each study. Nevertheless, if the issue was related to dysfunctional negative feedback, then context should not matter. Taken together, these data suggest that differences in the physiological mechanisms underlying drug SA behavior in High and Low Responder rats are different from the mechanisms influencing reward comparison behavior involving natural rewards and drugs of abuse.

Taken together, these data suggest that the relationship between suppression of saccharin intake and conditioned CORT is strongest when blood is sampled closer to the CS access period (at 15 min). The 15 min time point also was most sensitive when assessing the unconditioned CORT response. One possible explanation for this is that with more time, other variables begin to affect the CORT response and in turn, disrupt the linear relationship. Stress, for example, associated with the first blood sampling may have confounded the CORT response at the later time points. If this were the case, then there still might be a relationship between CORT and saccharin intake at these later time points but is masked by other variables. For these reasons, it is difficult to pinpoint a specific cause for these differences in HPA-axis sensitivity.

These correlation analyses demonstrate meaningful relationships between CORT and saccharin intake, but are not necessarily causal ones. Even so, using a simple procedure such as measuring cocaine-induced CORT elevations can be valuable in helping to determine the propensity for an individual to self administer drugs of abuse and, in turn, devalue natural rewards. Although the results of this experiment are a good start, data for other physiological and behavioral indices are needed to build a more detailed profile of what makes some individuals more vulnerable than others. The experiment presented in the next chapter is designed to help build this profile.

Chapter 4

SEARCHING FOR A PREDICTIVE TOOL:

SELF-ADMINISTRATION AND PREFERENCE FOR SUCROSE

A higher preference for sweet substances has been shown to be correlated with faster and greater drug self-administration behavior. For example, rats that consume greater amounts of sucrose have been shown to demonstrate greater self-administration of amphetamine (DeSousa, Bush et al. 2000) and cocaine (Gosnell 2000). In addition, greater preference for saccharin has been shown to predict greater self-administration of ethanol in rats (Gosnell and Krahn 1992; Bell, Gosnell et al. 1994) and morphine (Gosnell, Lane et al. 1995). Similar observations have been made in human studies that have investigated the relationship between drug abuse (esp. alcohol) and taste sensitivity involving preference for sweets (Kampov-Polevoy, Garbutt et al. 1997; Kampov-Polevoy, Garbutt et al. 1999; Kampov-Polevoy, Tsoi et al. 2001). These findings suggest that mechanisms underlying sucrose preference in rats may overlap with those underlying sensitivity to the rewarding properties of drugs of abuse.

One possible explanation for how higher preferences for sweet stimuli can predict greater sensitivity to the reinforcing properties of drugs of abuse comes from a recent study conducted by Gosnell (2005). In this study, rats given access to sucrose demonstrated greater behavioral sensitization (i.e., higher locomotor activity) in response to an injection of cocaine than rats only given access to

chow. These data suggest that cross-sensitization occurred between the psychostimulant and sucrose. This raises the possibility that a taste of something sweet primed the reward center so that only a small amount of drug was able to produce the euphoric and enhanced locomotor effects normally produced by a greater amount of drug. If so, then experience with sucrose may facilitate acquisition to cocaine SA.

The present experiment is designed to test whether greater sucrose preference can predict not only greater cocaine SA but also greater cocaine-induced avoidance of a saccharin cue. If so, then it is possible that sucrose preference and reward comparison behavior are mediated by similar neurobiological mechanisms. It should be mentioned that exposure to a mild stressor (i.e., a saline injection) was necessary for individual differences to emerge in sucrose intake and subsequently predict cocaine SA (Gosnell 2000). The need for this manipulation suggests some overlap may exist for circuits mediating the preference for sweets, drug SA, and stress. Although the following experiment specifically evaluates the relationship between the intake of sucrose and the SA of cocaine, the relationship of CORT with these measures is evaluated as well.

Materials and Methods

Subjects. There were a total of 36, naïve, male, Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing between 250 and 300 g at the beginning of the experiment. They were individually housed in stainless steel hanging cages in a temperature and humidity controlled animal care facility with a 12-hr light-dark cycle (lights on at 7 am). Food and water were available ad libitum, except where noted otherwise. The experiment was conducted in four operant self-administration chambers (see below for details). All rats were handled and weighed once a day throughout the experiment.

Catheter Construction and Implantation. The catheters were constructed and implanted as described in Chapter 3. The surgeries were performed also as described in Chapter 3.

Apparatus: Self-administration chambers. The sugar eating phase of the experiment took place in the animals' home cages. The conditioning phase of the experiment took place in the same operant chambers described above.

Procedure. Phase I. Sugar Eating. Following 4 days of handling, all rats were given 1 hr access to granulated sucrose from 10-11 am, once a day, for 7 days. During this 1 hr period, food dishes were removed from the home cage while water remained accessible. Stress-induced Sugar Eating. Consistent with the procedure described by Gosnell (2000), on the 8th sugar day, all rats were given a mild stressor in the form of an i.p. injection of saline (0.4 ml 0.9% NaCl from a 5 ml syringe with a 0.25 g needle) immediately prior to their 1 hr access period to sucrose. Surgeries. All rats were catheterized following the sugar-

eating phase. Deprivation Regimen and Habituation. Three rats were dropped from the study because of catheter failure. Following 5 days of recovery, the remaining 33 rats were placed on a water deprivation schedule and habituated to the operant chambers simultaneously, as described for Chapter 3. The only difference is that the rats were placed in the operant chamber for 15 instead of 5 min. The rats had access to water from a retractable sipper tube during the first 5 min. The rats were left in the chamber for an additional 10 min so that when 15 min blood samples were taken for pre-conditioning CORT assessment in the next phase, the animals would be habituated to the full 15 min and not expect to be returned to their homecages after only 5 min. This modification was employed to prevent unusually high pre-conditioning CORT levels, which were described in Chapter 3. The rats also were given 1 hr access to water in the afternoon. There were 6 days of habituation. Saccharin Pre-Exposure & Pre-conditioning CORT. Twenty-four hr following the last 5 min access period to water, and 48 hours prior to the first conditioning trial, all rats were pre-exposed to the 0.15% saccharin solution for 5 min in the operant chambers and blood was taken 15 min following access to the saccharin CS. Thus, CORT was assessed in response to presentation of the saccharin solution before it became associated with the cocaine. This was designed to index the unconditioned effect of saccharin intake on CORT. Phase II. Conditioning: Saccharin Intake and Self-Administration. All subjects were then divided into one of two testing conditions: saccharin-saline iv (n=11) or saccharin-cocaine iv (n=22). Groups were counterbalanced for weight, average granular sucrose intake, and average water intake on training days.

Conditioning took place exactly as described in Chapter 3 for a total of 13 taste-drug pairings. Test Day Post-conditioning CORT. The final trial served as a CS only test trial during which all rats were given 5 min access to the saccharin CS and blood was sampled from the catheter 15 min later. No drug was administered on this day.

Tastants and Drugs. Granular sucrose (Fischer Scientific, Malvera, PA) was presented in solid form at room temperature. Sodium saccharin (Fischer Scientific, Malvera, PA) was dissolved in distilled water (dH₂O) overnight and presented at room temperature. Cocaine hydrochloride (HCl) was obtained from the National Institute on Drug Abuse and dissolved in sterile saline immediately before each session.

CORT Radioimmunoassay. Plasma CORT concentrations were determined as described in Chapter 2.

Results

A total of 5 animals were dropped from the study. Three of the 5 were dropped because of catheter failure and 2 did not perform. The available data from the remaining 31 rats (21 cocaine, 10 saline) were used in the analyses presented below. All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc comparisons were conducted, when appropriate, using the Newman Keuls test, with alpha set at 0.05.

Saccharin Intake (licks/5 min). Overall intake of the saccharin CS was suppressed for rats given the opportunity to infuse cocaine relative to rats in the saline condition, see Figure 4.1, left panel. This conclusion was supported by a significant main effect of Drug, $F(1,377)=1.93$, $p<0.03$, and a significant Drug (saline vs. cocaine) x Trial (1-14) interaction, $F(13,377)=2.27$, $p<0.01$. Newman Keuls post hoc comparisons of this two way interaction revealed that rats in the cocaine condition suppressed intake of the saccharin cue on Trials 12 and 14 relative to the saline controls, $p_s<0.05$.

Once again, the presence of large individual differences within the cocaine group led to the division of the cocaine subjects into large and small suppressers. The uneven number of animals in the drug group (N=21) would not allow an even division based on a median split. Therefore, the mean number of licks on the final trial, which was 1087, was used to divide the animals into two groups such that all cocaine subjects whose intake fell above the mean were labeled as small suppressers (N=12) and those whose intake fell below the mean were labeled as large suppressers (N=9). The data were then regraphed (see Figure 4.1, right

panel) and analyzed using a 3 x 14 mixed factorial ANOVA varying group (saline, small suppressers, and large suppressers) and trial (1-14). Newman Keuls post hoc comparisons of a highly significant Group x Trials interaction, $F(26,364)=3.47$, $p < 0.001$, showed that large suppressers consumed less of the saccharin CS than did the saline controls on Trials 11-14 and than did the small suppressers on Trials 12-14, $p_s < 0.05$. The small suppressers, however, did not demonstrate suppression of the saccharin CS compared with the saline controls.

Reward Comparison: Saccharin Intake

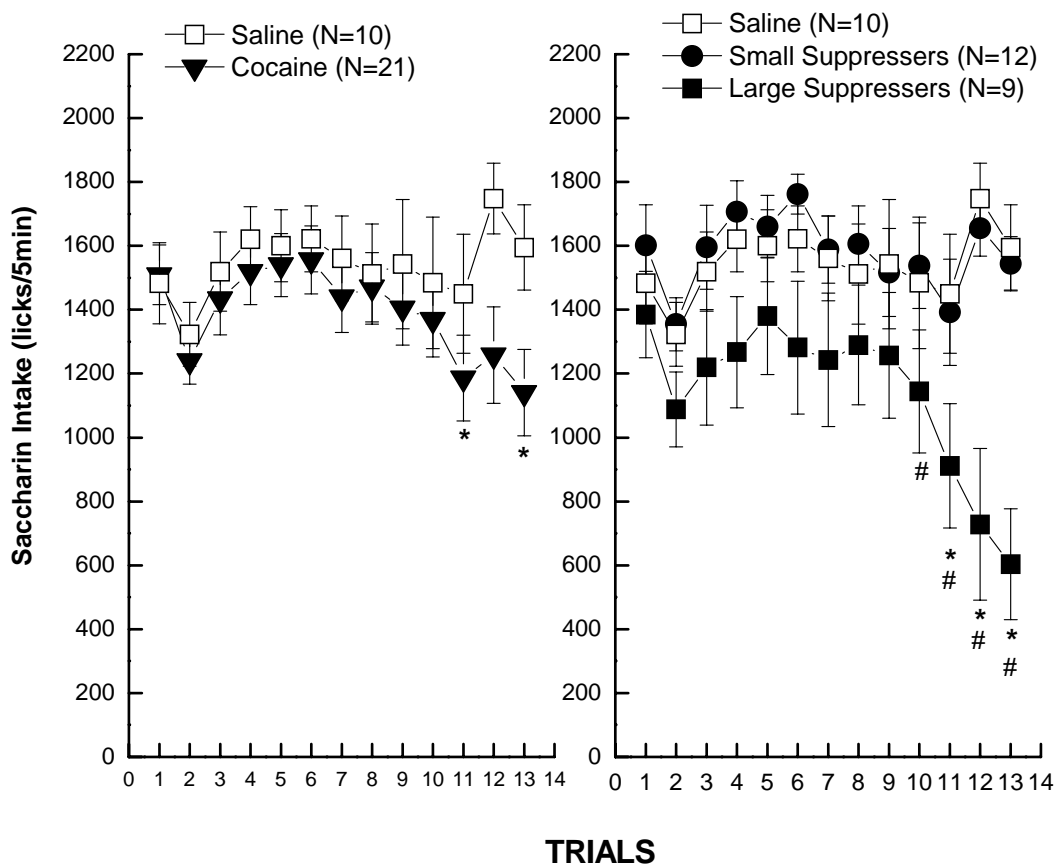


Figure 4.1: Left panel. Mean (+/- SEM) intake (licks/5 min) of 0.15% saccharin over 13 pairings with the opportunity to SA saline (N=10) or cocaine (0.5 mg/kg/infusion; N=21) for 1 hr. Trial 14 is a CS-only test day. Right panel. The same data re-drawn with the cocaine rats divided into small suppressers (N=12) and large suppressers (N=9). #Significantly different from small suppressers, *significantly different from saline, $p_s < 0.05$.

Cocaine US Infusions per Hour. The infusion data (shown in Figure 4.2) were analyzed using a 2 x13 mixed factorial ANOVA varying drug group (saline or cocaine) and trial (1-13). Newman Keuls post hoc comparisons of a significant Group x Trial interaction, $F(12,348)=4.44$, $p<0.001$, showed that rats given the opportunity to infuse cocaine made significantly more infusions than rats given the opportunity to infuse saline on Trials 10-13 (see Figure 4.2, left panel). Self-administration behavior also was evaluated on the basis of individual differences obtained in the number of licks made for the saccharin CS. The results of a 3 x 13 ANOVA varying drug group (saline, large and small suppresser) and trial (1-13) revealed a significant main effect of Drug, $p<0.05$, indicating that large suppressers self-administered more cocaine than the small suppressers and the saline controls overall, see Figure 4.2, right panel. Post hoc comparisons of a significant Group x Trial interaction, $F(24,336)=3.05$, $p<0.001$, showed that large suppressers were significantly different from small suppressers on Trials 2 and 11 and different from the saline controls on Trials 10-13, $ps<0.05$, see Figure 4.2, right panel.

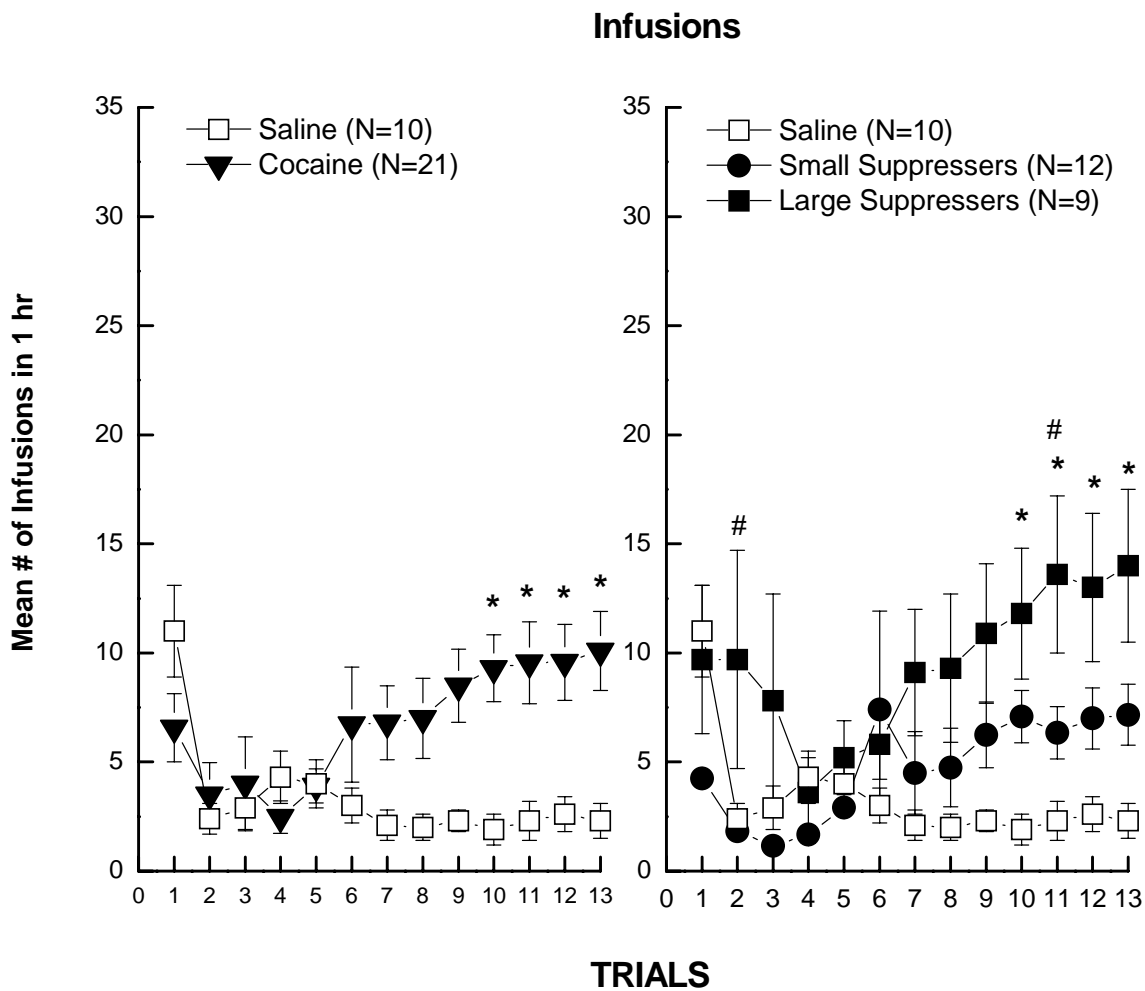


Figure 4.2. Left panel. Mean (+/- SEM) number of infusions per 1 hr made for saline (N=10) or for cocaine (0.5 mg/kg/infusion; N=21). Right panel. The same data re-graphed with the cocaine rats divided into small suppressors (N=12) and large suppressors (N=9). *Significantly different from saline, #significantly different from small suppressors, $p_s < 0.05$.

These data show that the large and the small suppressers in this experiment behaved like those in the last experiment where greater avoidance of the saccharin CS was associated with greater SA of cocaine. A correlation analysis was conducted to assess the strength of this relationship. The terminal number of infusions was graphed as a function of terminal saccharin intake for all rats in the cocaine group (N=21, see Figure 4.3, right panel). Indeed, the results of this analysis show that greater avoidance of the saccharin CS was negatively correlated with greater SA of cocaine ($r=-0.46$, $p<0.05$).

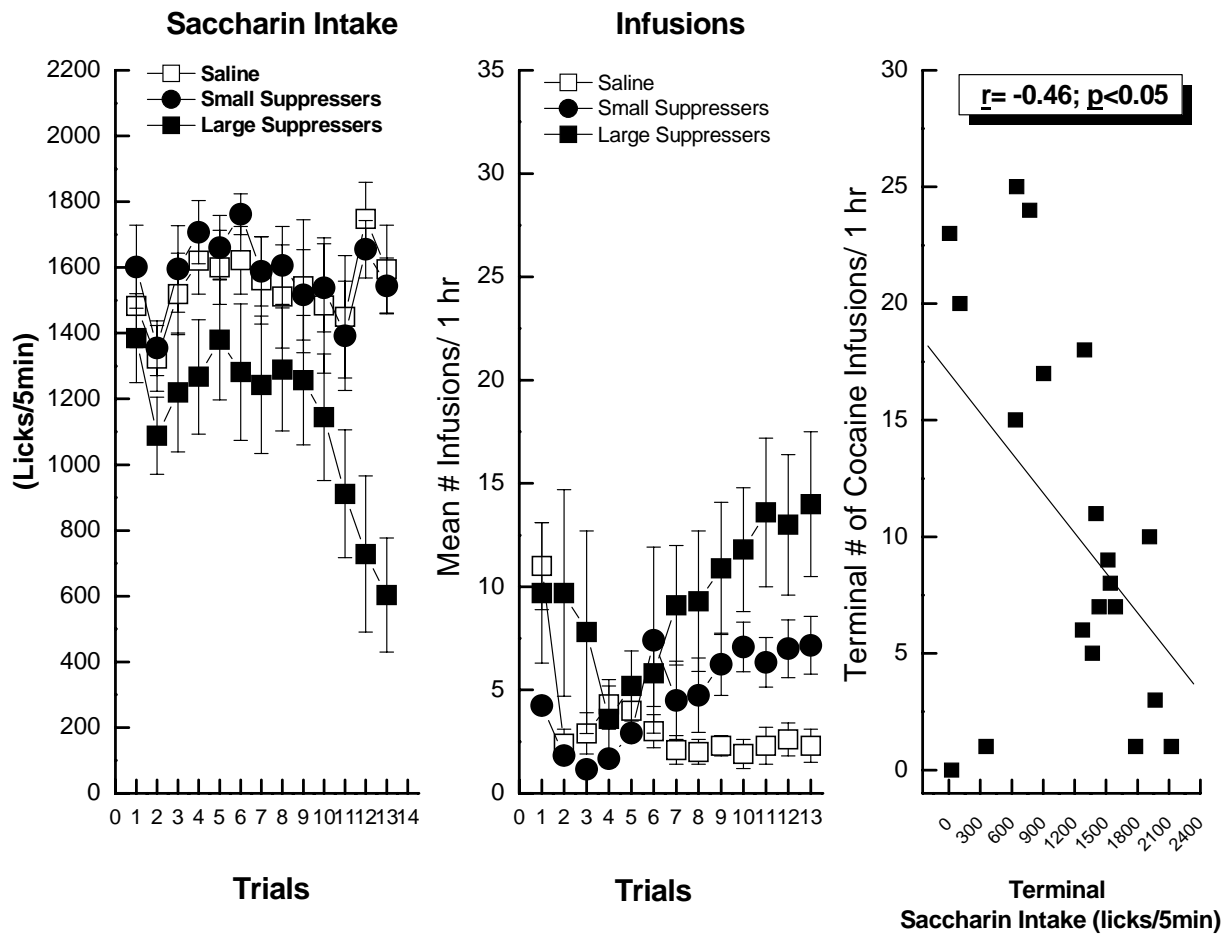


Figure 4.3: Left panel. Mean (+/- SEM) CS intake (ml/5 min) across 14 trials for the saline, small, and the large suppresser groups. Middle panel. Mean (+/- SEM) number of infusions/h made by the same groups. The left and middle panels are the same graphs shown in Figures 4.1 and 4.2 and are shown again for comparison. Right panel. Greater terminal cocaine SA is correlated with greater avoidance of the saccharin CS.

Pre- and Post-Conditioning CORT. The CORT data were analyzed using a 3 x 2 mixed factorial ANOVA varying group (saline, small suppresser, and large suppresser) by trials (pre- and post-conditioning). The results of this analysis did not show any significant differences in pre- to post-conditioning CORT elevations among the three groups, see Figure 4.4, middle panel. A significant main effect of Trial, $F(1,28)=10.55$, $p < 0.05$, showed that post-conditioning CORT levels were higher than pre-conditioning CORT levels, overall. The main effect of Group, however, was not significant, $p > 0.05$. Consequently, paired t-tests, were conducted separately for each group. The results showed that there was a significant increase from pre- to post-conditioning for both small suppressers and large suppressers, $p_s < 0.05$.

Next, a correlation analysis was conducted to assess whether there still would be a relationship between drug-induced devaluation of the saccharin CS and CORT on an individual, rather than a group, basis. The right panel of Figure 4.4 shows plasma CORT levels following presentation of the saccharin cue at test as a function of terminal saccharin intake for each rat in both groups. The results of this analysis showed that, in this experiment, unlike what has been observed for past experiments, there is no relationship between avoidance of the saccharin cue and cue-induced elevations in CORT at test. It should be noted that overall CORT levels in this experiment also were lower than CORT levels in past experiments. These lower CORT levels may have masked any otherwise apparent relationships between behavior and CORT. Nevertheless, the magnitude of the CORT response mirrored the magnitude of suppression in

saccharin intake and this finding is consistent with previous observations.

Possible reasons for these low CORT levels are discussed later.

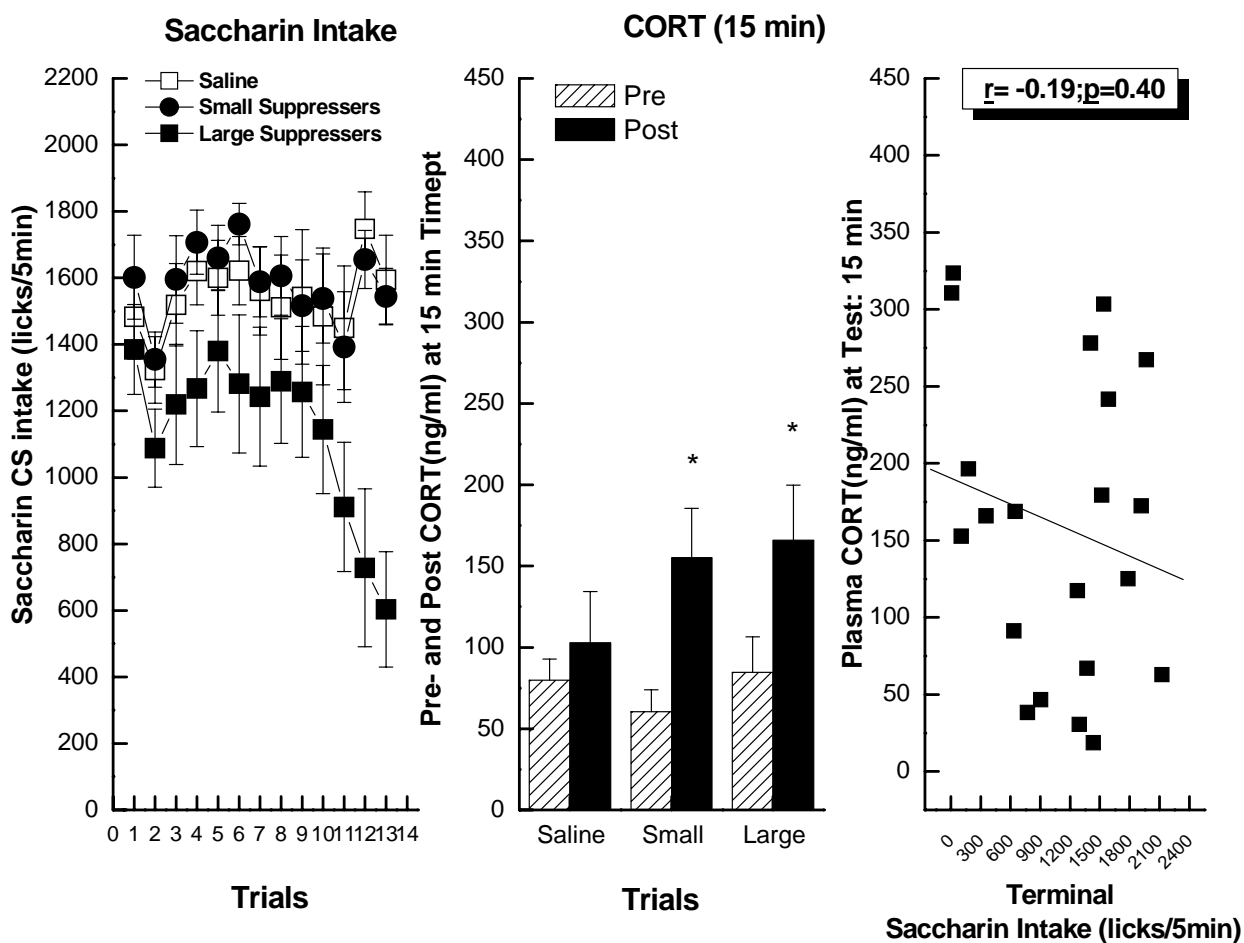


Figure 4.4: The left panel shows the same saccharin intake data shown in previous figures. The middle panel shows mean (\pm SEM) pre- and post-conditioning CORT levels 15 min after presentation of the saccharin CS for the saline, small, and large suppresser groups. The right panel shows the results of a correlation analysis for terminal saccharin intake and post-conditioning CORT levels.

Sucrose Intake (g/hr) and Saccharin CS Intake. The sucrose intake data were analyzed using a 3 x 8 mixed factorial ANOVA varying group (saline, small suppressers, large suppressers) and trial (1-8) and are shown in the left panel of Figure 4.5. The results of this analysis did not reveal any differences in sucrose intake between groups. Even so, a series of correlation analyses were performed to test whether there still was a relationship between sucrose intake and saccharin intake on an individual basis. The first correlation analysis tested the relationship between Day 8 (stress-induced) sucrose intake and terminal saccharin intake. The right panel of Figure 4.5 shows the results of this analysis with sucrose intake graphed as a function of terminal saccharin intake for every rat in the drug group. Results of this analysis confirmed that there was no relationship between stress-induced sucrose feeding and subsequent avoidance of saccharin intake following pairings with cocaine. A second correlation analysis was conducted to test whether sucrose intake on Day 7 (non-stress day) was related to terminal saccharin intake. The results of this analysis (data not shown) confirmed that, even in the absence of stress, sucrose intake was not correlated with suppression of saccharin intake at test, $r=0.34$, $p>0.05$.

Additional correlation analyses were conducted (data not shown) using change scores (i.e., the difference in saccharin intake from trial 1 to 14) rather than terminal saccharin intake. Using change scores, as shown in Chapter 3, can more accurately measure the magnitude of suppression for each rat. These analyses were performed to evaluate whether sucrose intake on any of the 8 days was associated with a different measure of suppression. The results of

these analyses confirmed that sucrose intake was not correlated with suppression of saccharin intake when measured as a difference from trial 1 to 14, Day 1: $r = 0.008$, $p = 0.97$; Day 2: $r = 0.13$, $p = 0.57$; Day 3: $r = 0.05$, $p = 0.83$; Day 4: $r = -0.005$, $p = 0.98$; Day 5: $r = -0.010$, $p = 0.65$; Day 6: $r = -0.003$, $p = 0.99$; Day 7: $r = 0.12$, $p = 0.59$; Day 8: $r = -0.14$, $p = 0.53$.

Sucrose Intake by Small & Large Suppressers

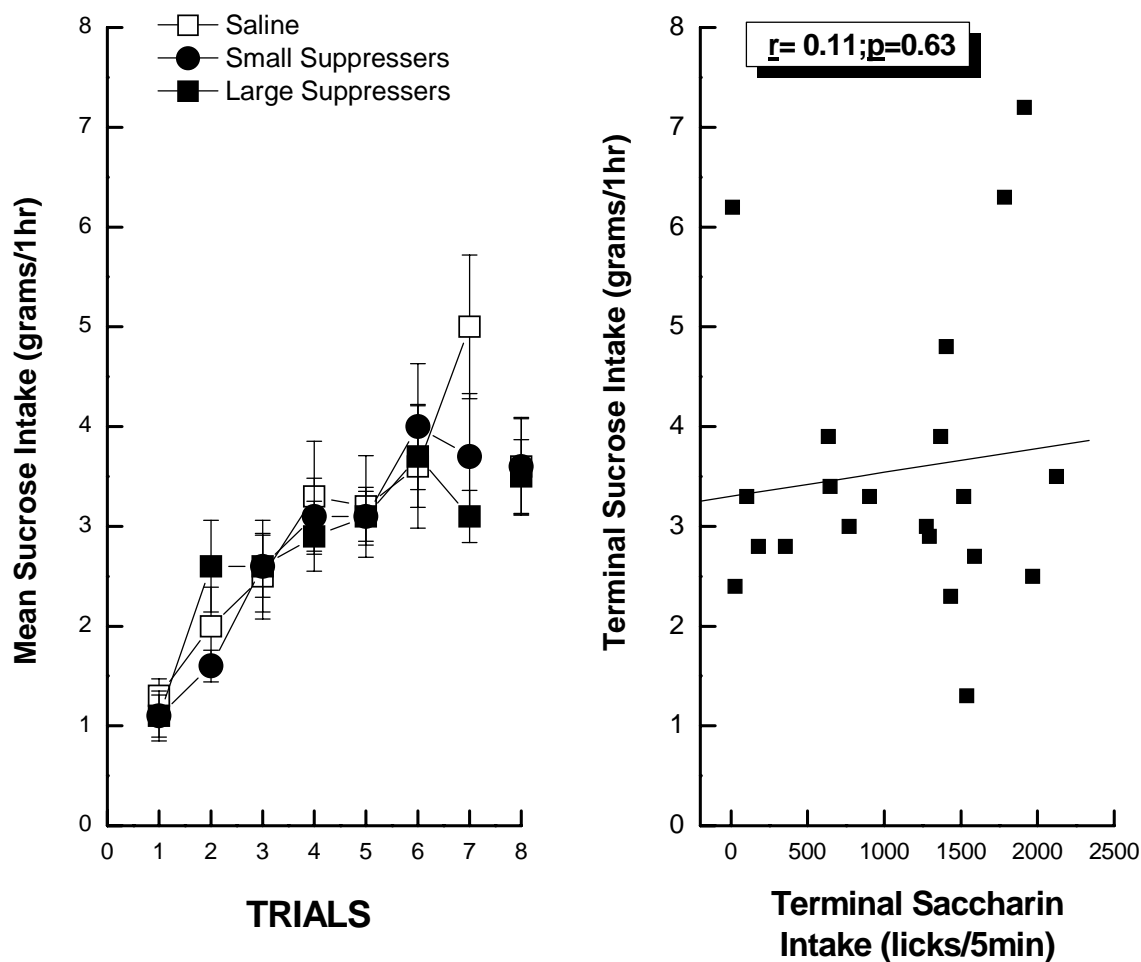


Figure 4.5: The left panel shows mean (\pm SEM) sucrose intake/h for each group across 8 consecutive days. The right panel shows terminal sucrose intake graphed as a function of terminal saccharin intake and the results of a correlation analysis showing that there was no relationship between sucrose intake and terminal saccharin intake.

Sucrose Intake and Cocaine Self-Administration. Although there was no relationship between sucrose intake and the conditioned suppression of saccharin intake, the data were analyzed to test whether there would be a relationship between sucrose intake and cocaine SA. To this end, the cocaine rats were divided into two groups, the High Sucrose Feeders (HSF) and the Low Sucrose Feeders (LSF), using the median split of sucrose intake on Day 8 (stress day), see Figure 4.6, left panel. Results of a 2 x 8 mixed factorial ANOVA varying group (HSF and LSF) and day (1-8) showed that sucrose intake was, as expected, significantly greater for the HSF group than for the LSF group. This conclusion was supported by a significant main effect of Group, $F(1,19)=15.99$, $p<0.001$. The right panel of Figure 4.6 shows the mean number of infusions across trials for saline rats, HSF rats, and LSF rats. Newman Keuls post hoc comparisons of a significant Group (Saline, HSF, LSF) x Trials (1-13) interaction, $F(24,336)=2.43$, $p<0.001$, showed that the mean number of infusions made by the HSF group was significantly higher than that made by the saline group on trials 9-13, $p_s <0.05$. The mean number of cocaine infusions made by the LSF group was not significantly different from the number of infusions made by the rats in the saline group, $p>0.05$.

Cocaine SA by High & Low Sucrose Feeding Groups (Day 8 median split)

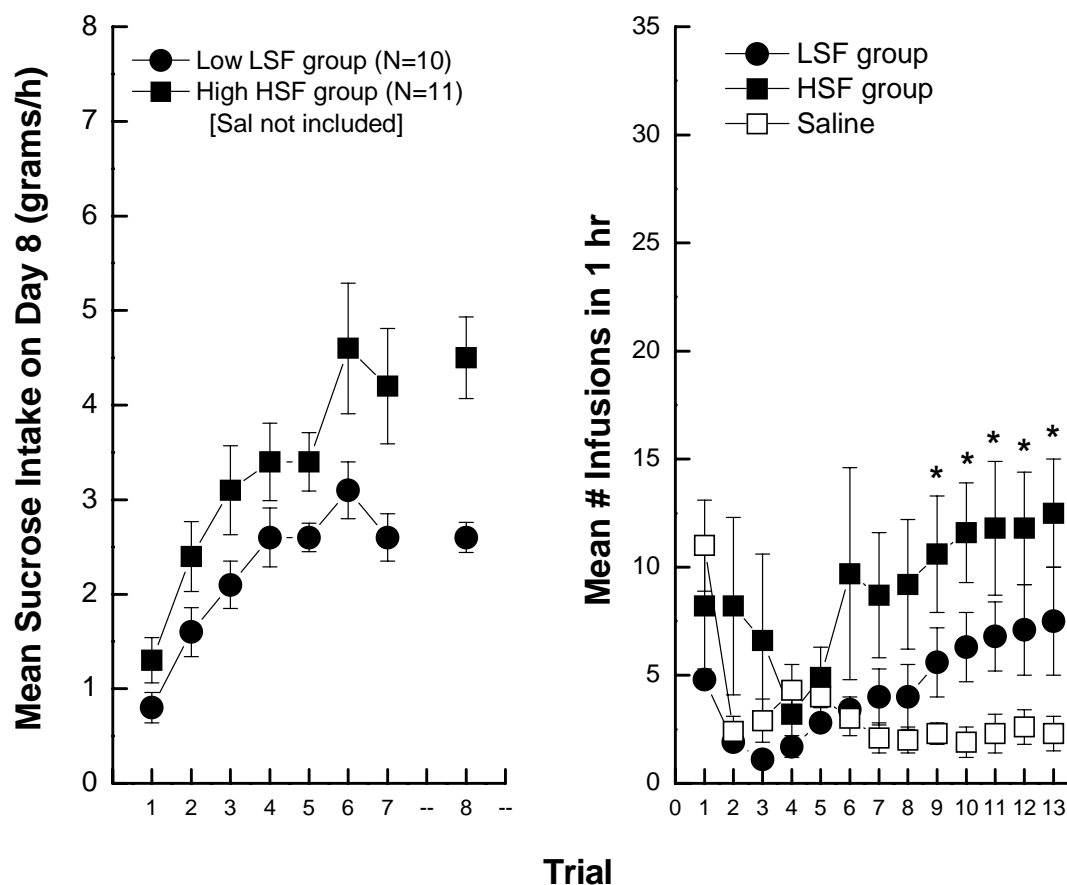


Figure 4.6: The left panel shows mean (\pm SEM) sucrose intake for cocaine rats divided into High (HSF) and Low (LSF) sucrose feeding groups using the median split on Day 8 (stress day) sucrose intake. The right panel shows mean (\pm SEM) number of infusions for the saline, HSF, and LSF groups. *Significantly different from saline.

Next, cocaine SA was graphed for high and low sucrose feeders on Day 7 to test if the rats that consumed the most sucrose, in the absence of stress, also would self-administer the most cocaine. To this end, sucrose intake, saccharin intake, and cocaine SA data were re-graphed for HSF and LSF groups that were determined using the median split of Day 7 sucrose intake. These graphs are presented in Figure 4.7. The sucrose intake data, shown in the left panel, were analyzed using a 2 x 8 mixed factorial ANOVA varying sucrose group (HSF and LSF) and day (1-8). Newman Keuls post hoc comparisons of a significant Group x day interaction, $F(7,133)=2.57$, $p<0.05$, showed that the HSF Group consumed more sucrose than the LSF Group on Days 6-8, $p_s <0.05$. The infusion data (shown in Figure 4.7, right panel) were analyzed using a 3 x 13 mixed factorial ANOVA varying group (HSF, LSF, saline) and trial (1-13). Newman Keuls post hoc comparisons of a significant Group x Trial interaction, $F(24,336)= 2.76$, $p<0.001$, showed that HSF group made more infusions than the saline group on trials 10, 12, and 13, $p_s<0.05$. The HSF group did not differ from the LSF group on any trials and the LSF group did not differ from the saline group on any trials, $p_s>0.05$.

Observation of the right panels of Figures 4.6 and 4.7 suggests there is a relationship between day 7 and 8 sucrose intake and cocaine SA. Correlation analyses were conducted to test whether sucrose intake on either Day 7 or Day 8 would be significantly correlated with terminal cocaine SA. The results from these analyses are shown in Figure 4.8. Sucrose intake was not correlated with cocaine SA on either Day 7 (left panel) or Day 8 (right panel), $p_s>0.05$. Although

high sucrose feeders take more cocaine than low sucrose feeders as a group, high intake of granulated sucrose is not a reliable predictor for subsequent cocaine SA.

Cocaine SA by High & Low Sucrose Feeding Groups (Day 7 median split)

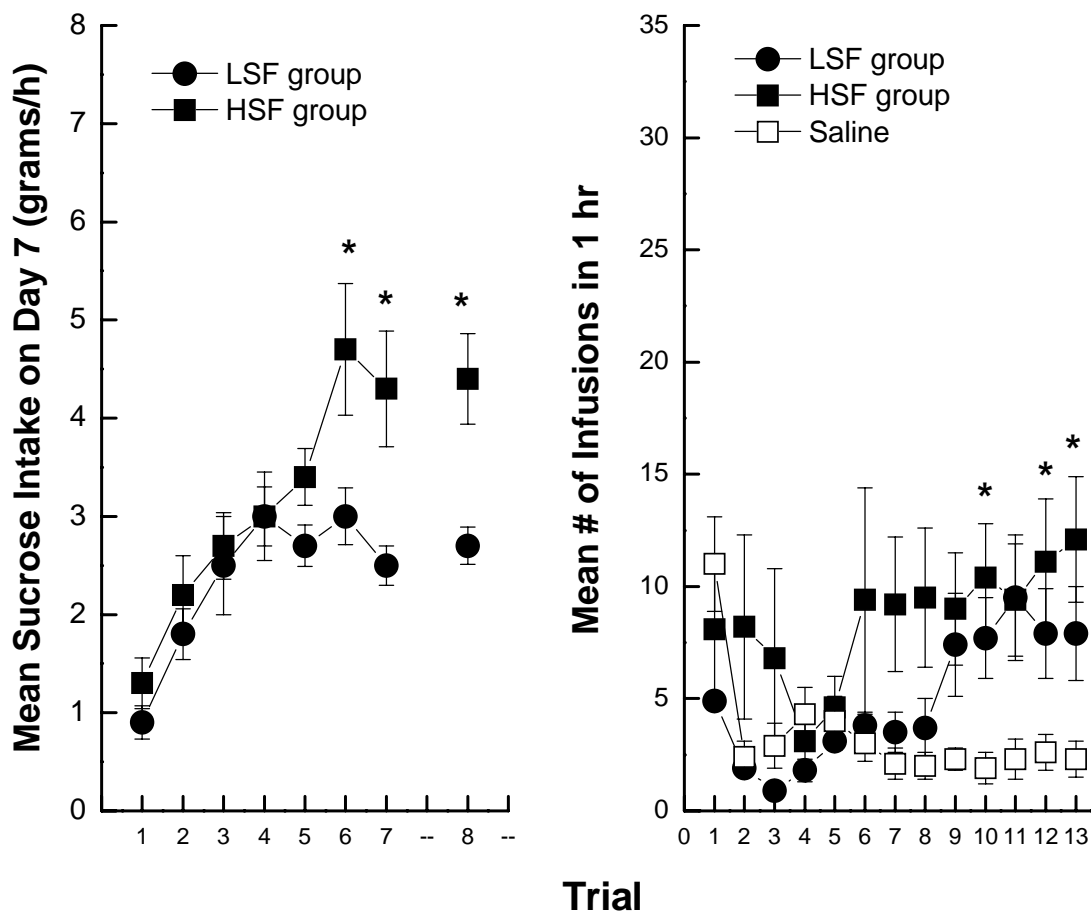


Figure 4.7: The left panel shows mean (\pm SEM) sucrose intake for cocaine rats divided into high (HSF) and low (LSF) sucrose feeding groups using the median split of Day 7 (no stress) sucrose intake. The right panel shows mean (\pm SEM) number of infusions by the saline, HSF, and LSF groups. *Significantly different from saline.

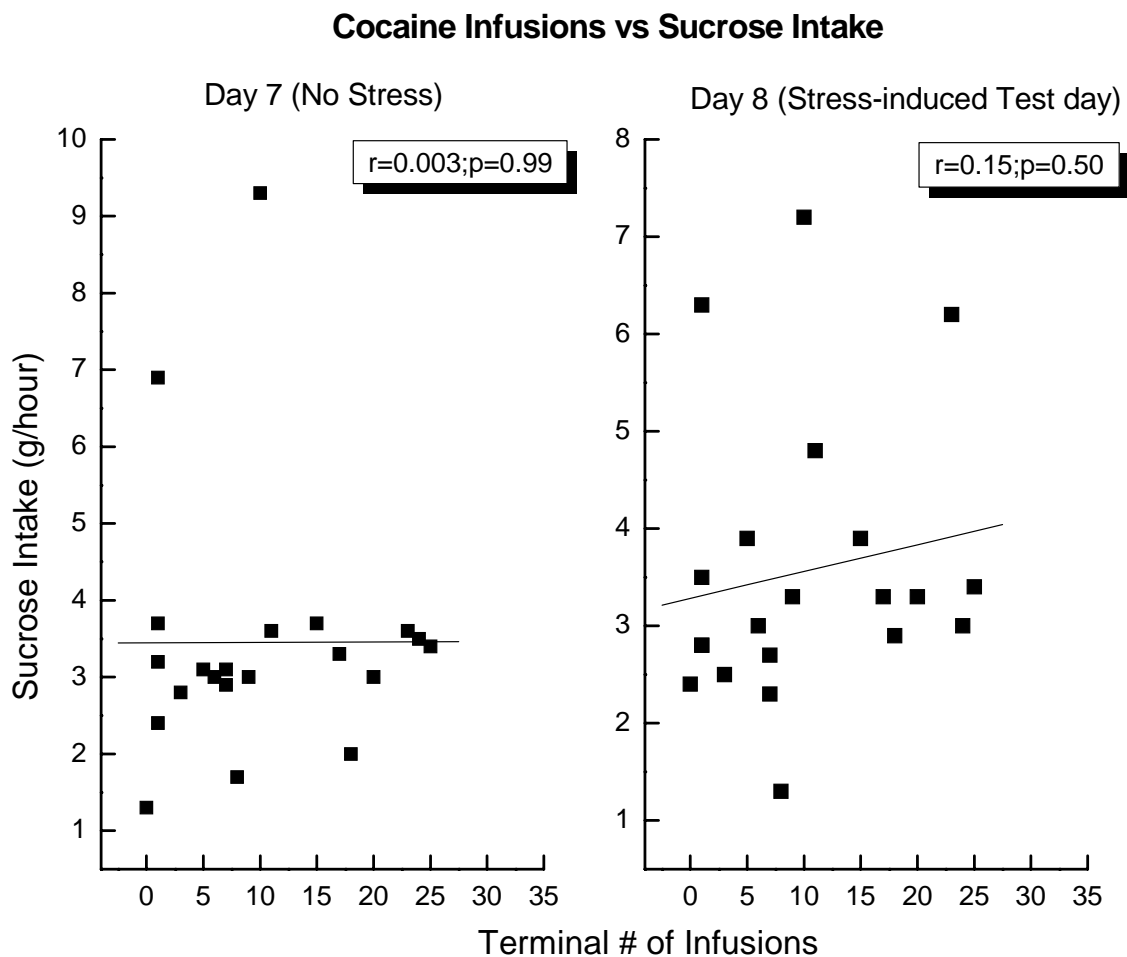


Figure 4.8: Both panels show sucrose intake for cocaine rats as a function of the terminal number of cocaine infusions. The left panel shows individual sucrose intake on Day 7 when no stress was administered to the rat and the right panel shows individual sucrose intake on Day 8 when a mild stressor was presented prior to accessing the sucrose. Results of correlation analyses showed that there was no relationship between sucrose feeding and cocaine SA on either day.

Sucrose Intake and Rate of Acquiring Cocaine SA. Although sucrose intake was not correlated with terminal cocaine SA, it remains possible that sucrose intake was related to how quickly cocaine SA behavior was acquired. Indeed, Gosnell et al. (2000) showed that sucrose intake was related to the rate of acquisition of cocaine SA, but not the level of SA once the behavior was acquired. The next set of correlation analyses tests the possibility that sucrose intake was correlated with the rate of acquisition. The results of these analyses are shown in Figure 4.9. The number of trials it took to reach either 10 (top panel) or 15 (bottom panel) cocaine infusions was used to measure the rate of acquisition. The number 14 was assigned to animals that never reached the criteria since there were a total of 13 trials. The number of trials to reach criteria was plotted as a function of both Day 7 intake (left panel) and Day 8 intake (right panel). Although none of the four analyses revealed any significant relationships, there was a trend toward a relationship between the rate of acquisition and stress-induced sucrose intake. This observation is consistent with previous findings (Gosnell et al., 2000) that show how stress precipitates individual differences in drug SA behavior.

Sucrose Intake & Rate of Acquiring Cocaine SA

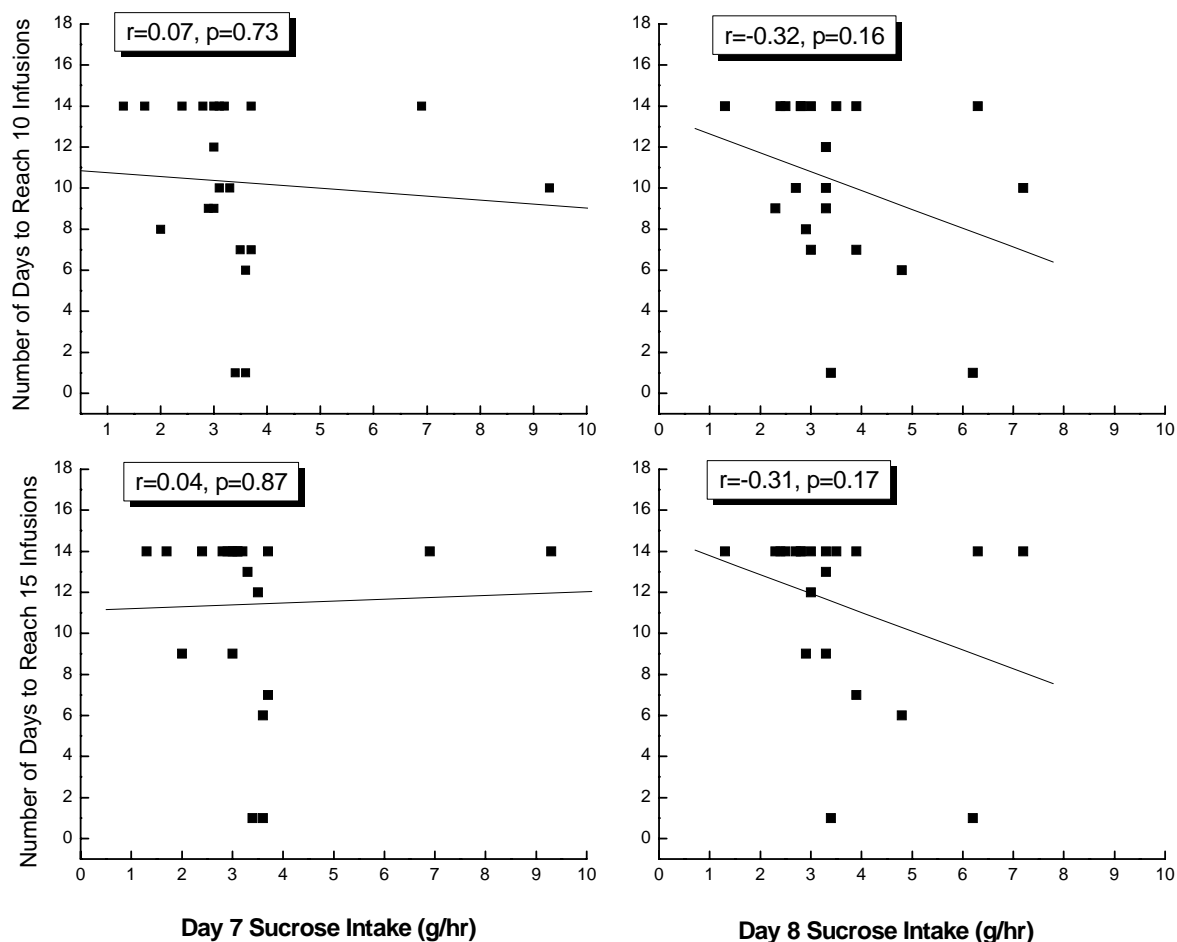


Figure 4.9: The number of trials taken to reach either 10 (top panels) or 15 (bottom panels) cocaine infusions was used to measure the rate of acquisition and plotted as a function of sucrose intake on Day 7 (no stress, see left panels) and Day (stress, see right panels). The number 14 was assigned to rats that never reached the criteria.

Sucrose Intake and CORT. The final set of correlation analyses tested whether sucrose intake would be associated with saccharin cue-induced elevations in CORT levels at test. The results are shown in Figure 4.10 with CORT graphed as a function of Day 7 sucrose intake in the left panel and Day 8 sucrose intake in the right panel. Sucrose intake was not correlated with post-conditioning CORT levels in this study, p s > 0.05.

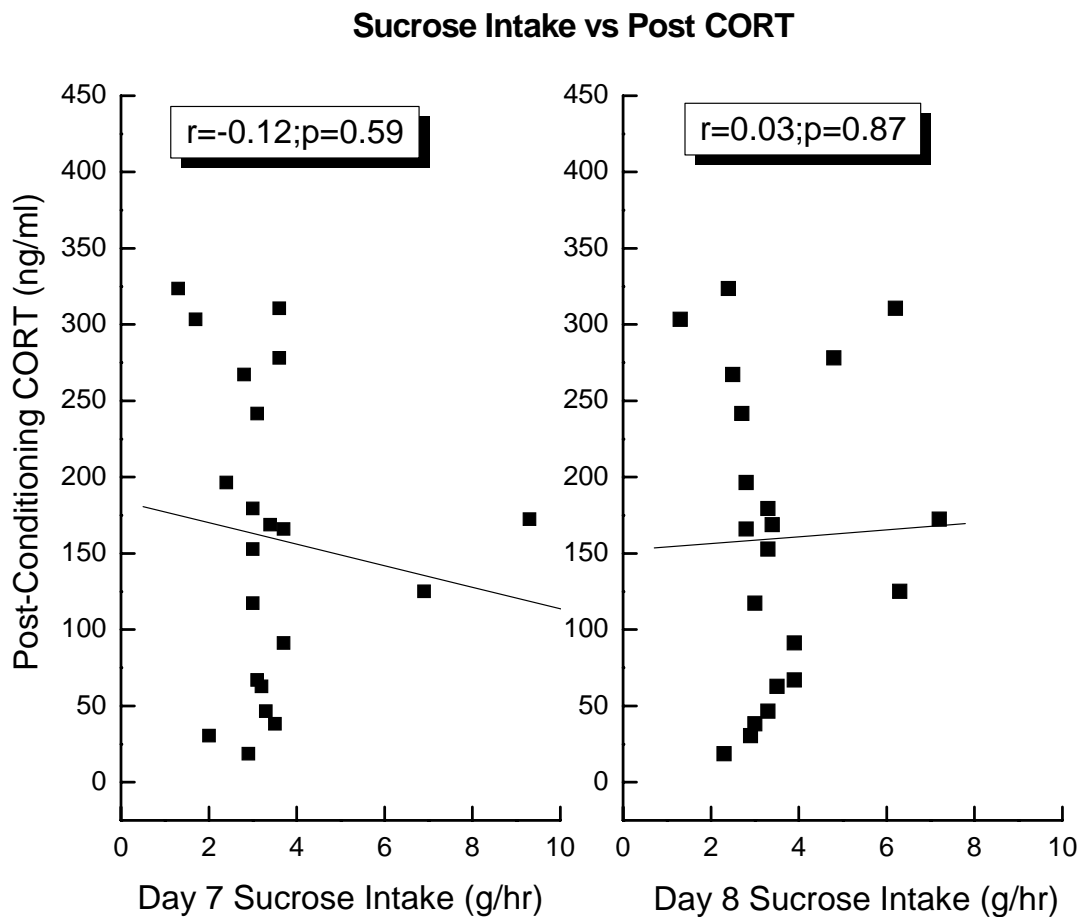


Figure 4.10: Post-conditioning CORT levels (ng/ml) as a function of intake (g/h) of granulated sugar on Day 7 (left panel) and on Day 8 (right panel). The results of correlation analyses are also shown.

Discussion

In the present study, we examined the relationship between sucrose intake and cocaine SA behavior in a reward comparison paradigm. Greater sucrose intake following the presentation of a mild stressor has been shown to predict faster and greater amphetamine and cocaine SA (DeSousa et al., 2000; Gosnell, 2000). Consistent with these findings, the present study shows that stress-induced intake of sucrose tended to be associated with faster and greater cocaine self-administration. There were definite group differences whereby High Sucrose Feeders (HSF), as a group, self-administered more cocaine than did the Low Sucrose Feeders (LSF). This is consistent with the pattern of behavior reported by Gosnell (2000). It was predicted that if this were the case, as it was, then greater intake of sucrose also would predict greater cocaine-induced devaluation of the saccharin CS. This, however, was not the case. Similarly, *group* differences in sucrose intake failed to be associated with group differences in saccharin intake. Interestingly, the HSF rats that self-administered the most cocaine were not necessarily large suppressers, and vice versa. Although there was no relationship between sucrose and saccharin intake, exposure to the sweet granulated sucrose may have attenuated the suppressive effects of cocaine.

One possible explanation for why the suppressive effects of cocaine were attenuated in the present study is that generalization occurred between the two sweet stimuli. From a learning perspective, when exposure to a non-reinforced stimulus (i.e., granulated sucrose) retards the subsequent development of a CS-

US association, it is referred to as latent inhibition (Lubow and Moore 1959; Lubow 1973; Lubow 1989). In the present study, latent inhibition likely occurred as a result of the sweet taste of the granulated sucrose being generalized to the liquid saccharin cue. As a result, the rats did not learn the association between the saccharin CS and the cocaine US as well or as quickly as they might have if they had not been exposed to the sucrose. This would explain why the magnitude of suppression in CS intake in the present experiment was less than that of other experiments. In Chapter 3 and in the Grigson & Twining (2000) study, where the standard taste-drug design was employed without the sucrose component, the large suppressers reduced intake of the saccharin CS by trial 2 or 3. In the present study, the large suppressers did not reduce intake until trial 11. This delayed avoidance of the saccharin cue most likely occurred as a result of latent inhibition.

An alternative explanation is that experience with the naturally rewarding sucrose 'protected' the rats from immediate devaluation of the natural saccharin reward. Specifically, the experience with one sweet tastant set the stage for a stronger response by the brain's pleasure system to another sweet tasting reward, in this case, saccharin. Consequently, the rats may have just as quickly learned the CS-US association but were slow to demonstrate learning as measured by suppression of CS intake. Regardless, there is still the question of whether any 'protective' effects of sucrose would have occurred separate from latent inhibition or through overlapping mechanisms. While these two possibilities may be more distinct than similar, exposure to the granulated sucrose may have

protected the rats from devaluing the natural saccharin reward at least, in part, through generalization.

Nevertheless, exposure to sucrose did not significantly affect responding for the less similar drug US. In the present study, cocaine rats made more infusions than saline rats by trial 9 and in the Grigson and Twining (2000) study, by trial 6. This may have been due to a protective effect of the sucrose on cocaine SA, but this conclusion is difficult to draw without data from a control group that was not exposed to sucrose. In any case, exposure to granulated sucrose seems to have affected subsequent saccharin intake behavior more than it did cocaine SA behavior.

Plasma CORT levels were assessed 15 min following the presentation of the saccharin CS before and after conditioning. The results showed that CORT levels, in general (pre and post), were lower than those observed previously (e.g., Chapter 3). In Chapter 3, CORT levels were unusually high across the board. In the present study, precautionary measures, such as extra handling and longer habituation periods, were implemented to prevent unusually high CORT levels. These measures were successful in solving the problem. That is, pre-conditioning CORT levels were lower and there were no differences among groups (e.g., saline, small, and large). Consistent with previous findings, post-conditioning CORT levels were significantly elevated for both small and large suppresser rats. The finding that the large suppresser rats did not demonstrate greater post-conditioning CORT levels than the small suppressers, however, is not consistent with previous findings.

There are several possible reasons for why greater cocaine-induced avoidance of saccharin intake (e.g., by the large suppressers) was not correlated with greater elevations in CORT at test. First, prior exposure to the granulated sucrose may have had a protective effect on both intake of the saccharin CS and HPA-axis activity. In support, several studies have shown that sucrose ingestion can affect regulation of the HPA axis (Laugero 2001; Laugero, Bell et al. 2001; Bell, Bhargava et al. 2002; Laugero, Gomez et al. 2002; Dallman, Akana et al. 2003). Specifically, ingestion of sucrose lowers stress-induced secretion of CORT (Bell, Bhargava et al. 2002), and normalizes central expression of CRF messenger RNA and energy balance in adrenalectomized rats (Laugero, Bell et al. 2001).

By the same token, the extra handling should not be discounted as a reason for lower post-conditioning CORT levels in the large suppressers. Studies have shown that environmental factors such as postnatal rearing conditions and experimenter handling can attenuate stress-induced activation of the HPA-axis (Nunez, Ferre et al. 1996; Vallee, Mayo et al. 1997; Garipey, Rodriguez et al. 2002; Brake, Zhang et al. 2004). Furthermore, early handling in rats promotes a more rapid post-stress recovery in CORT levels and significant attenuation of amphetamine-induced conditioned place preference (Campbell and Spear 1999). Taken together, these findings suggest that extra handling and experience with naturally rewarding stimuli can prevent drug-induced devaluation of other naturally rewarding stimuli (i.e., the saccharin CS) and protect normal HPA-axis functioning.

Latent inhibition may have attenuated not only suppression of CS intake but also cue-induced CORT elevations at test. If this were the case, cocaine-induced elevations in CORT may have occurred, but this unconditioned effect may not have transferred completely to become the conditioned effect at test. Thus, delayed learning may have been expressed through both the consummatory response and the hormonal response.

In conclusion, the results of this experiment do not support the hypothesis that sucrose preference and reward comparison behavior are mediated by similar underlying neurobiological mechanisms. Indeed, the data demonstrate that they are not. This conclusion is supported by the finding that the rats that made up the HSF group were not the same rats that made up the large suppresser group. The results of this study do, however, support the conclusion that mechanisms underlying sucrose preference in rats overlap with those underlying sensitivity to the rewarding properties of drugs of abuse. Indeed, there was a tendency for HSF rats to self-administer more drug than LSF rats and to acquire self-administration more quickly. Taken together, when a higher preference for sweets is found to predict greater drug SA, it does not necessarily mean that the individual also will be more likely to devalue other naturally rewarding stimuli. A potentially valuable finding is that individual responsiveness to drugs of abuse, stress, and subsequent devaluation of natural rewards, can all be influenced by increased postnatal handling and exposure to naturally rewarding stimuli.

PART 2

INDIVIDUAL DIFFERENCES IN REWARD COMPARISON AND GENETICS

While greater sensitivity to drugs of abuse can be influenced by various environmental factors, individual vulnerability also can be determined by a genetic predisposition for a greater sensitivity to the rewarding properties of a drug of abuse. The experiments presented in Chapters 5-7 were primarily designed to investigate the extent to which individual differences in reward comparison behavior are driven by genetics. Individual differences in the reward comparison paradigm have consistently emerged whereby approximately half of the Sprague-Dawley rats avoid intake of the saccharin cue following taste-drug pairings, while the other half do not. This fifty percent split in behavior provides a strong premise on which to hypothesize that there is a powerful genetic component to cocaine-induced suppression of saccharin intake. If cocaine-induced avoidance of saccharin intake is mediated by a simple genetic system driven by as few as two or three genes, then behavioral traits should be transferred to the offspring, possibly in a single generation and would not diverge further in subsequent generations. If, on the other hand, the difference between the high and low lines increases steadily over several generations, this would strongly suggest that many genes, rather than just one or two, contribute to variation in behavior (Plomin, DeFries et al. 2001). A selective breeding experiment was designed to test this hypothesis by mating extreme large and

small suppressers to evaluate the genetic influence, if any, that there would be on the first generation.

Before breeding male and female rats for this trait, it was important to determine whether female rats also would demonstrate large individual differences in the reward comparison paradigm. This was the experimental objective for Chapter 5. Testing female rats would not only provide female parents for the selective breeding study, but also help characterize how gender influences individual differences in our paradigm.

Chapter 5

REWARD COMPARISON IN FEMALE RATS

Male and female rats, like humans, respond differently to drugs of abuse. In humans, females are more likely than males to report greater sensitivity to cocaine and increased craving following exposure to cocaine-associated cues (Kosten, Kosten et al. 1996; Robbins, Ehrman et al. 1999). The finding that women begin using cocaine and enter treatment at earlier ages than men suggests that women are more sensitive to cocaine's rewarding properties (Griffin, Weiss et al. 1989; Mendelson, Weiss et al. 1991). In support, female rats demonstrate greater behavioral sensitization than male rats in response to both acute and chronic administration of cocaine (Glick and Hinds 1984; van Haaren and Meyer 1991; Bowman and Kuhn 1996; Walker, Cabassa et al. 2001). Female rats also acquire cocaine SA more rapidly than male rats (Lynch and Carroll 1999) and work harder for an infusion of cocaine on a progressive ratio schedule (Roberts, Bennett et al. 1989). Furthermore, during reinstatement of extinguished cocaine SA, females respond more than males to priming injections of cocaine (Lynch and Carroll 2000). Taken together, these findings suggest that female rats, like female humans, are more likely than male rats to not only acquire cocaine SA but also reinstate cocaine-seeking behavior following periods of abstinence.

Although there are many possible reasons for differences between male and female rats in sensitivity to cocaine, several findings attribute these differences to ovarian hormones. The fluctuation in estrogen and progesterone levels in female rats influences both cocaine-stimulated locomotor behavior (Walker, Cabassa et al. 2001) and cocaine SA behavior (Roberts, Bennett et al. 1989). For example, Walker et al. (2001) showed that cocaine-induced horizontal movement is attenuated in ovariectomized females placed in an open field while castrated males demonstrate increased locomotor activity. Others have shown that female rats will not only exhibit greater cocaine SA but also work harder for cocaine during estrous than in other stages of the estrous cycle (Roberts, Bennett et al. 1989; Lynch, Arizzi et al. 2000). In humans, women in the follicular phase of their menstrual cycle demonstrate higher peak plasma cocaine levels following intranasal administration than women in the luteal phase (Lukas, Sholar et al. 1996).

Ovarian hormones may contribute to these gender differences primarily through their influences on two physiological systems. First, greater responsiveness to cocaine during proestrus and estrous may be related to increased activity in the brain dopamine system. In support of this, dopamine release and re-uptake in the mesolimbic dopamine system fluctuates over the estrous cycle (Shimizu and Bray 1993; Thompson and Moss 1997). In addition, the administration of estradiol increases dopamine receptor density in the striatum (Hruska 1986). The hypothalamic-pituitary-adrenal axis is the second system prone to gender differences and is influenced by the fluctuation of ovarian

hormone levels during the estrous cycle. Female rats demonstrate an exaggerated activation of the HPA axis in response to cocaine when compared with male rats (Kuhn and Francis 1997). This is not surprising because, compared with male rats, female rats demonstrate enhanced diurnal peaks of corticosterone (Critchlow, Liebelt et al. 1963) and greater activation of the HPA axis in response to other physical and social stressors (Le Mevel, Abitbol et al. 1978; Le Mevel, Abitbol et al. 1979; Ehlers, Kaneko et al. 1993; Aloisi, Steenbergen et al. 1994). Ovarian hormones also influence stress hormone activation. Haas and George (1988) have shown that CRH synthesis is decreased by ovariectomy and restored by the administration of estradiol. When cocaine is administered, female rats exhibit up to three times as much cocaine-induced ACTH secretion as males and this increase is most evident during proestrus (Walker, Francis et al. 2001), when estradiol levels are the highest. In sum, greater sensitivity to cocaine in female rats compared with male rats can be attributed to a more active HPA axis. This is further compounded by the fluctuation of ovarian hormone levels and their stimulatory actions on the mesolimbic dopamine reward system.

The following experiment tests whether differences between male and female rats will occur in the reward comparison paradigm. Gender differences in cocaine-induced avoidance of saccharin intake have been studied before but the subjects were Wistar, not Sprague Dawley, rats (van Haaren and Hughes 1990). Female Wistar rats were shown to be more sensitive than males to the

suppressive effects of cocaine. It is predicted that this also will be true for female Sprague Dawley rats.

The first objective of this study determines whether female Sprague-Dawley rats will be more sensitive than males to the suppressive effects of cocaine. The second objective determines whether there will be large individual differences in cocaine-induced suppression of CS intake among female rats. The third objective determines whether female rats, like males, will experience cue-induced elevations in CORT following taste-drug pairings. Given that female rats will demonstrate individual differences in both CS intake and CORT, the fourth objective tests whether greater avoidance of the saccharin cue will be associated with greater elevations in CORT. The results from this study will provide valuable data regarding female behavior to a literature based largely on male subjects.

Methods

Subjects. This experiment was conducted in two replications. There were a total of 48 naïve, female, Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing between 202 and 240 g at the beginning of each experiment (N=24/replication). Furthermore, a total of 24 naïve, male, Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing between 230 and 320 g were included in the second but not in the first replication. All rats were individually housed in stainless steel hanging cages in a temperature and humidity controlled animal care facility with a 12 hr light-dark cycle (lights on at 7 am). Food and water were available ad libitum, except where noted otherwise.

Apparatus, tastant and drug. The saccharin solution and drug were prepared and presented as described in Chapter 2. The experiment took place in the home cage from 9:30 to 11:30 a.m..

Procedure. All rats were handled and weighed once a day throughout the experiment. Deprivation state. Following several days of handling, all rats were placed on a water deprivation regimen in which they were given 5 min access to dH₂O on the front of the cage each morning and 1 h access each afternoon to maintain proper hydration. All rats were kept on this daily regimen until morning intake stabilized. Saccharin pre-exposure and pre-conditioning CORT. Twenty-four h following the last 5 min access period to water, and 48 hours prior to the first taste-drug pairing, all rats were pre-exposed to the 0.15% saccharin CS for 5 min. Blood samples (approximately 0.4 ml) were collected by means of a tailcut 15 min after saccharin pre-exposure and stored for later evaluation. This was

designed to control for any possible unconditioned effect of saccharin on baseline CORT. The 15 min time point replicates the procedure used previously (Gomez, Leo et al. 2000) and is approximately how long it takes to detect a rise in circulating levels of CORT (Fluttert, Dalm et al. 2000). Conditioning. The rats were matched on the basis of 5 min water intake on the last day of water training and saccharin intake on the pre-exposure day and divided into 2 drug conditions: saline (n=24; 16 females, 8 males) or cocaine (n=48; 32 females, 16 males). More subjects were placed in the cocaine group to allow for an assessment of individual differences. During conditioning, the rats were given 5 min access to 0.15% saccharin and, after a 5 min ISI, injected with saline or 10 mg/kg, s.c. cocaine. There was one such CS – US pairing every other day for a total of 7 pairings, followed by one CS only test. All rats were given 5 min access to water each morning on the days between conditioning trials and 1 h every afternoon to rehydrate. Post-conditioning corticosterone. A second blood sample (approximately 0.4 ml) was collected from the tail 15 min after access to the saccharin CS on the final CS-only test trial. No injections were given on this day.

Tracking Estrous with Vaginal Lavages. In the second experiment only, females were lavaged to track estrous. The estrous cycle was tracked daily following afternoon waters. The cotton swab method (as opposed to the dropper method) was adopted for this study. First, the cotton tip of a swab was moistened with isotonic saline and then inserted into the vagina. This was then smeared onto a clean microscope slide. A new swab was used for each female and four smears were fit onto a single slide. The slides were air dried and then

immersed into absolute methyl alcohol for 5 min. Next, the slides were placed in Giemsa stain (undiluted) for 2 min, rinsed with water, and air dried.

CORT Radioimmunoassay. Blood samples were analyzed for CORT as described above for Exp 1.

Results

All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc comparisons were conducted, when appropriate, using the Newman Keuls test, with alpha set at 0.05.

Saccharin (CS) Intake. For females, the main effect of replication was not significant, $F < 1$, following a 2 x 2 x 8 repeated measures ANOVA varying replication (2), drug group (saline and cocaine), and trial (1-8). Therefore, the data from both studies were combined, re-analyzed, and presented accordingly.

Saccharin intake data for both males and females are shown in Figure 5.1. The data were analyzed using a 2 x 2 x 8 mixed factorial ANOVA varying gender (male and female), group (saline and cocaine), and trial (1-8). As expected, a significant main effect of Drug, $F(1,68)=63.72$; $p < 0.001$, indicated that overall consumption of the saccharin CS was significantly lower for cocaine-treated rats than for the saline-treated rats. Newman Keuls post hoc comparisons of a significant Gender x Drug x Trials interaction, $F(7,476)=3.52$; $p < 0.001$, showed that cocaine-treated female rats (left panel) significantly reduced intake of the saccharin CS compared with saline-treated rats on Trials 2-8, $p_s < 0.05$. For

cocaine-treated male rats (right panel), consumption of the saccharin CS was significantly lower than that of saline-treated males on Trials 3-8, $p < 0.05$.

Although there was no significant main effect of Gender, $p > 0.05$, male cocaine-treated rats did consume more of the saccharin CS than females on the first trial and consumed significantly less of the saccharin CS on Trial 4 and then again on Trials 7 and 8, $p < 0.05$.

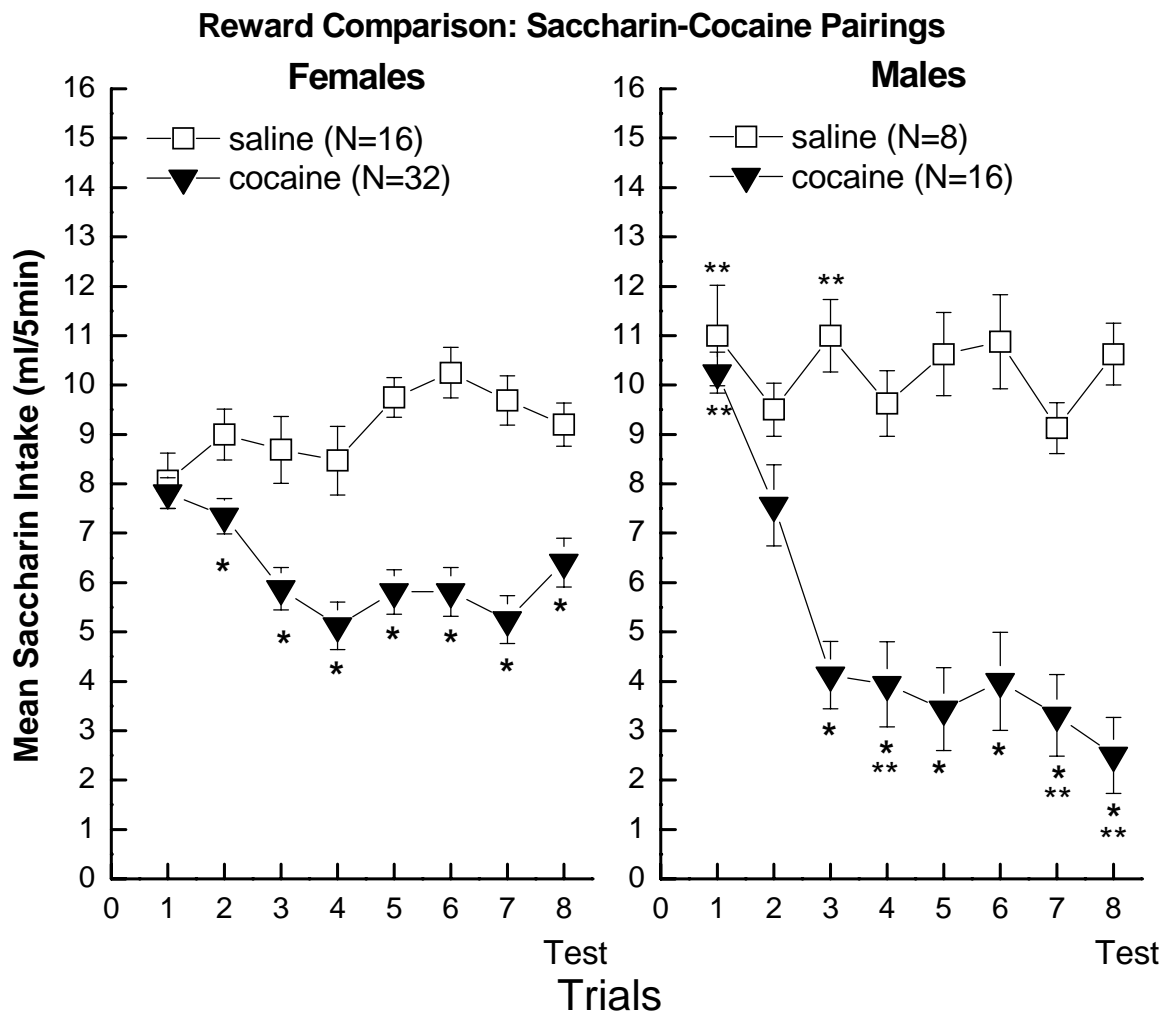


Figure 5.1: Mean (+/- SEM) intake of 0.15% saccharin (ml/5 min) across seven pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the females are shown in the left panel and data for males are shown in the right panel. * Different from saline, ** different from females, $p < 0.05$.

Large individual differences in CS intake were present among both male and female cocaine-treated rats, see Figure 5.2. As a result, the cocaine-treated rats were divided into two separate groups (large suppressers and small suppressers) based on a median split of terminal (Trial 8) saccharin intake. The data were then regraphed and analyzed using a mixed factorial 3 x 2 x 8 ANOVA varying group (saline, large suppresser, and small suppresser), gender (male and female), and trial (1-8). The results of this analysis revealed a significant Group x Gender x Trials interaction, $F(14,462)=2.64$, $p<0.001$. Newman Keuls post hoc tests of this interaction showed that female large suppresser rats (left panel, Figure 5.2) consumed significantly less of the saccharin CS than both the saline-treated rats and the small suppresser rats on Trials 3-8, $p_s<0.05$. The small suppresser rats exhibited a smaller reduction in intake that attained significance only on Trials 5-7, $p_s<0.05$. Male large suppresser rats (right panel) consumed significantly less of the saccharin CS than saline-treated rats on Trials 2-8, $p_s<0.05$, and less than the small suppresser rats on Trials 4-8, $p_s<0.05$. The male small suppresser rats still demonstrated a strong reduction in intake compared to the saline group that was significant on Trials 3-8, $p_s<0.05$. Although overall suppression in saccharin intake appeared to be more pronounced in males than in females, this effect was heavily carried by the large suppressers. Indeed, additional post hoc comparisons of the same 3-way ANOVA showed that male large suppressers demonstrated greater avoidance of the saccharin cue than female large suppressers on Trials 4-6 and then again on Trial 8 while intake by the male small suppressers was significantly reduced

compared to female small suppressers only on Trial 8, $p_s < 0.05$. Saccharin intake also differed between males and females on the first trial, $p_s < 0.05$, but this difference likely reflects body weight differences with males being larger than females.

To determine the magnitude to which body weight differences accounted for differences in intake, intake to weight ratios were calculated and those values were graphed (see Figure 5.2b) and analyzed with the same 3 x 2 x 8 ANOVA described above. The results of this analysis, unlike the results above, did not reveal a significant 3 way interaction, $F(14,462)=1.5$, $p > 0.05$. There was, however, a significant main effect of Gender, $F(1,66)=66.2$, $p < 0.001$, showing that females, overall, consumed more of the saccharin CS than males, $p < 0.05$. Thus, taking body weight into account eliminated gender differences in saccharin intake.

These data show that female Sprague-Dawley rats, like males, are sensitive to the suppressive effects of cocaine. Furthermore, small and large suppressers emerged among female rats. These individual differences among female rats, however, were not as great as those demonstrated by males in the present and in past studies (Gomez, Leo et al. 2000). Females seemed to be more resistant to the suppressive effects of cocaine than males. Few, if any, females demonstrated complete avoidance of saccharin intake. Although reasons for these gender differences in suppression are not clear, gender differences in CTA learning have been reported (Weinberg, Gunnar et al. 1982). In this study, gender differences in CTA learning (using milk and LiCl) occurred

only if rats were food and water deprived. While both males and females suppressed intake of the CS, females subsequently recovered to pretoxicosis intake levels faster than males. This finding is consistent with the present results that suggest that females are more resistant than males in suppressing intake of a naturally rewarding stimulus such as saccharin or milk. As mentioned above, gender differences also have been reported by van Haaren (1990) in a study using a paradigm similar to the present experimental design. In this study, a 0.1% saccharin solution was paired with the administration of 5, 10 or 20 mg/kg cocaine for different groups of fluid deprived male and female Wistar rats. The results of this study showed that females were more sensitive than males to the suppressive effects of a 20 mg/kg dose of cocaine and this was the only dose in which a consistent decrease in CS consumption was observed. This finding is not consistent with the present results which show that males are more sensitive than females to the suppressive effects of a 10 mg/kg dose of cocaine. Possible reasons for this discrepancy between studies include the use of different rat strains and also the use of different concentrations of saccharin (0.1% vs 0.15%).

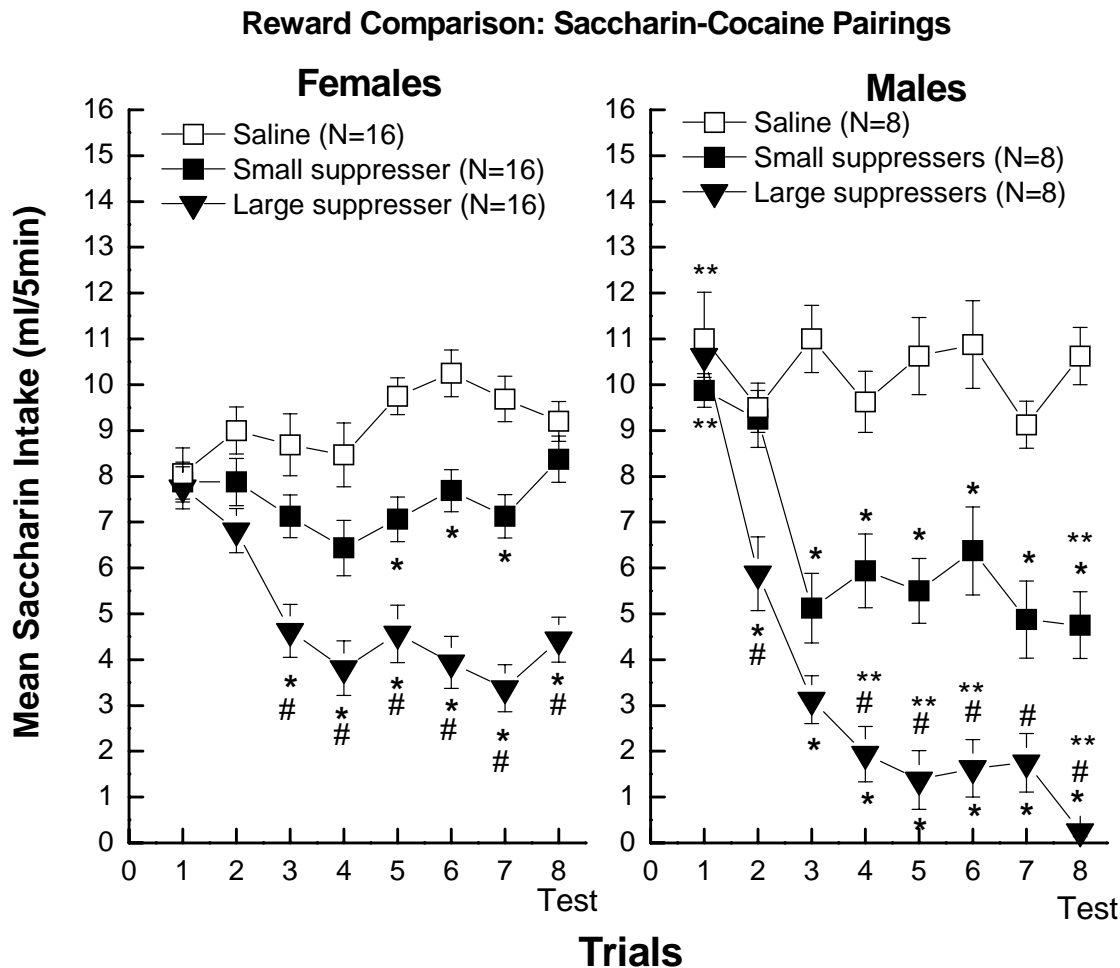


Figure 5.2 a: Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) for saline controls, small suppressors, and large suppressors across 7 pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for female rats are shown in the left panel and data for male rats are shown in the right panel. *Significantly different from saline-injected controls, # different from small suppressors, ** different from females, $p < 0.05$.

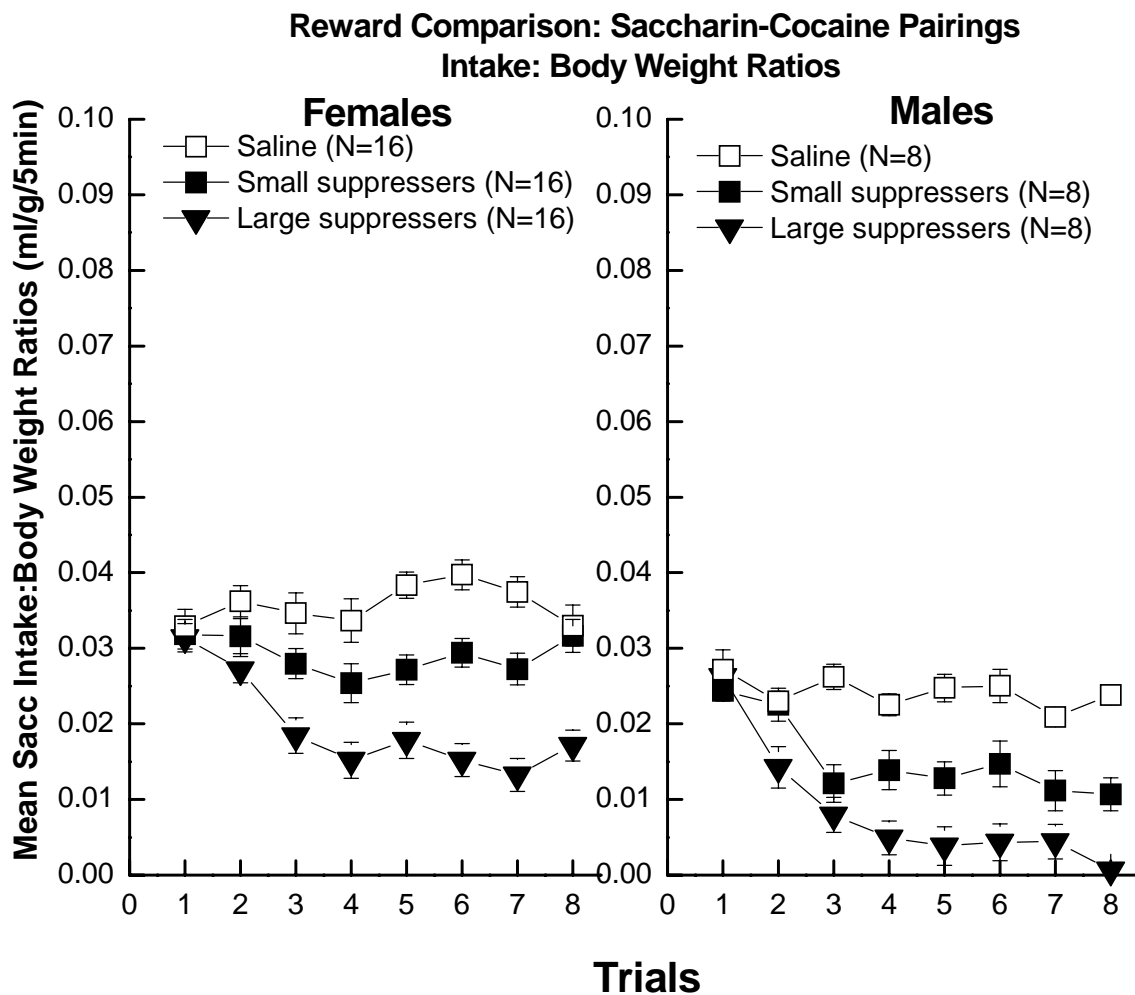


Figure 5.2 b: Mean (\pm SEM) intake per gram of body weight of 0.15% saccharin (ml/g/5 min) for saline controls, small suppressors, and large suppressors across 7 pairings with either saline or cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for female rats are shown in the left panel and data for male rats are shown in the right panel.

Corticosterone. The CORT data (shown in Figure 5.3) were analyzed using a 3 x 2 x 2 repeated measures ANOVA varying group (saline, small suppressers, and large suppressers), gender (male and female), and trials (pre- and post-conditioning). Although the 3-way Group x Gender x Trials interaction was not significant, post hoc comparisons of a significant Group x Trials interaction, $F(2,65)=7.99$, $p<0.001$, showed that post-conditioning CORT levels were significantly elevated from pre-conditioning to test for both small and large suppressers, $p_s<0.05$ but not for the saline controls, $p_s>0.05$. This pattern also was reflected by the significant main effects of Drug, $F(2,65)=5.92$, $p<0.01$, and Trial, $F(1,65)=15.9$, $p<0.001$, indicating, respectively, that cocaine-treated rats demonstrated higher CORT levels than saline treated controls overall, and post-conditioning CORT levels were higher than pre-conditioning CORT levels. These Group patterns in CORT were statistically the same for males and females as indicated by the lack of a significant main effect of Gender or any significant interactions thereof, $p_s>0.05$.

Although results from the above analysis of the CORT data did not indicate any differences between males and females, the behavioral data (saccharin intake) indicated that males and females were, in fact, different. As a result, the CORT data were re-analyzed separately for males and females using a 3 x 2 repeated measures ANOVA varying group (saline, small suppressers, and large suppressers) and trials (pre- and post-conditioning). For females (see Figure 5.3, left panel), post hoc comparisons of a significant Group x Trials interaction, $F(2,44)=5.24$, $p<0.01$, showed that female large suppressers were

the only group that exhibited a significant elevation in CORT from pre- to post-conditioning, $p < 0.05$. Both large and small suppressers, however, demonstrated greater levels of circulating CORT at test than did the saline group, $p_s < 0.05$. For males (see Figure 5.3, right panel), post hoc comparisons of a significant Group x Trials interaction, $F(2,21) = 4.95$, $p < 0.05$, showed that both large and small suppressers exhibited a significant elevation in CORT from pre- to post-conditioning, $p_s < 0.05$. Both large and small suppressers also demonstrated greater post-conditioning CORT levels compared with that of the saline group, $p_s < 0.05$.

Correlation analyses were conducted to determine whether there was a relationship between terminal saccharin intake and post-conditioning CORT (see Figure 5.4). The results revealed that, for females, there was no relationship between saccharin intake and CORT at test (left panel) and, for males, there was a slight negative, but non-significant, relationship in which greater avoidance of saccharin intake was associated with greater post-conditioning CORT levels (right panel). The finding that males did not demonstrate a significant relationship between terminal CS intake and CORT is surprising. One possible reason for this discrepancy is that there were fewer subjects included in this analysis compared with previous studies ($N=16$ vs. $N=32$).

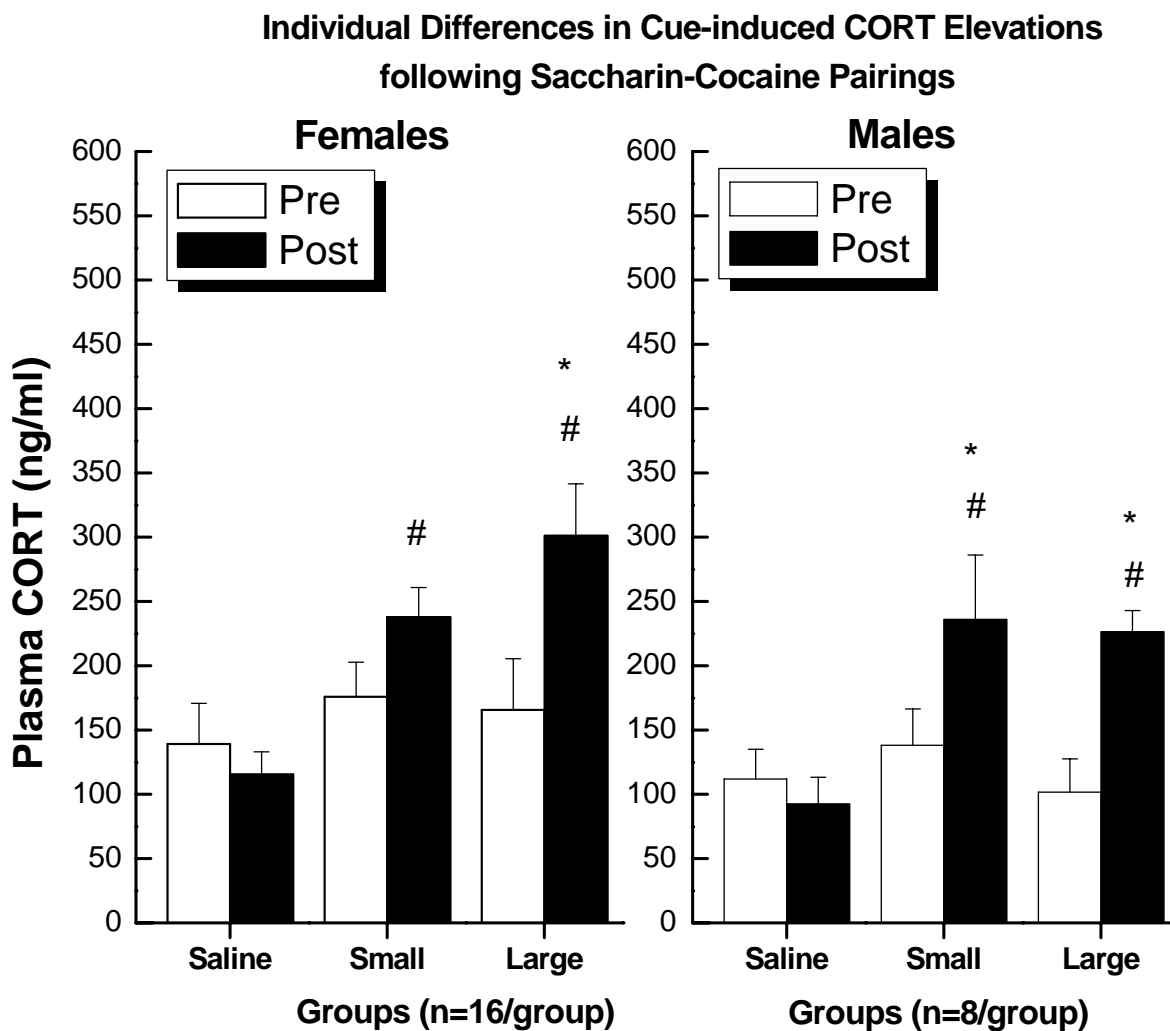


Figure 5.3: Mean (+/- SEM) pre- and post-conditioning CORT levels (ng/ml) across saline controls, small suppressers, and large suppressers for females (left panel) and males (right panel). #Significantly different from saline group post-conditioning CORT levels, * significantly different from pre-conditioning CORT levels within the same Group, $p < 0.05$.

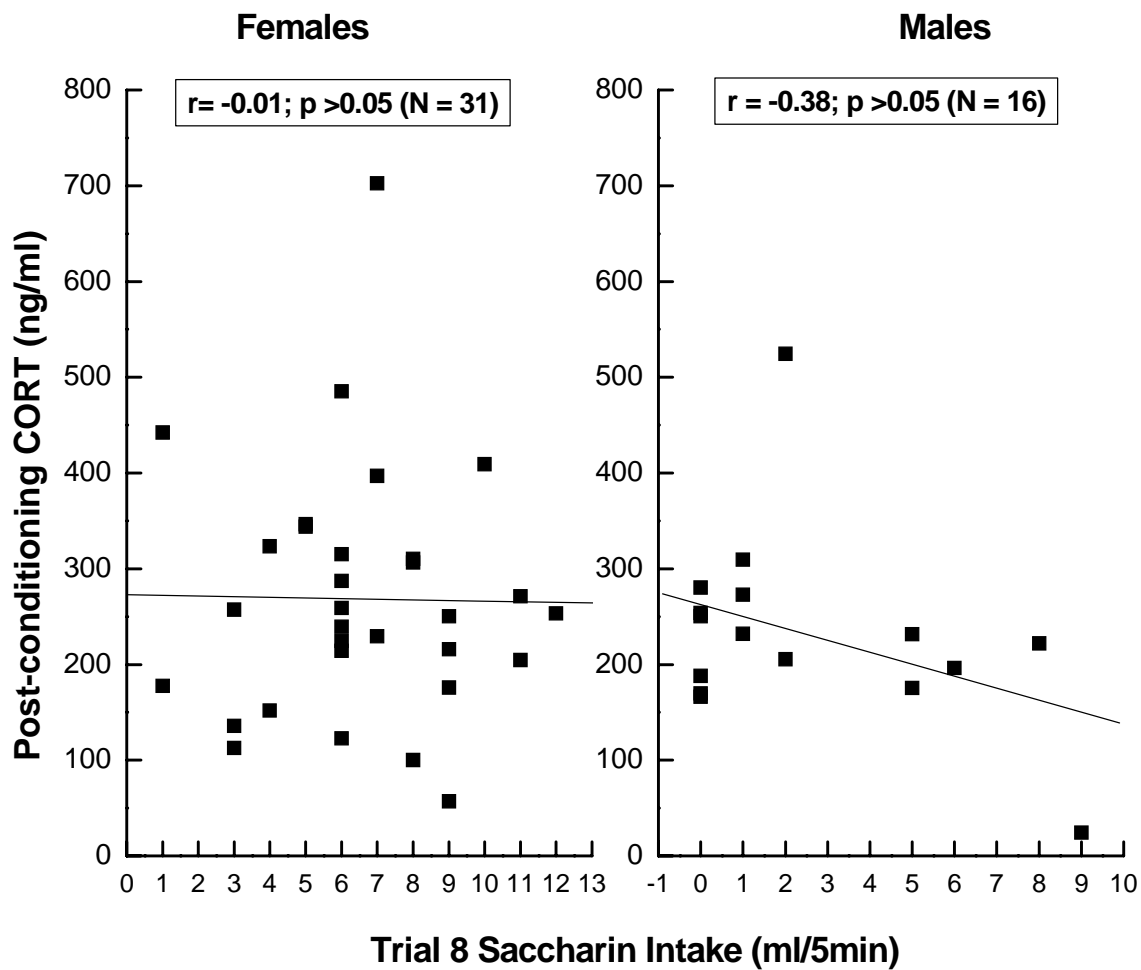


Figure 5.4: Intake of the saccharin conditioned stimulus as a function of post-conditioning corticosterone levels at test for cocaine-treated females (left panel) and males (right panel).

Estrous. Daily lavage revealed that female rats did not demonstrate synchronous estrous cycling. There also were no apparent patterns in saccharin intake and estrous cycling (data not shown). On Trial 1, only six of the sixteen cocaine-treated females were observed to be in estrous. Of those six, only two females went on to become large suppressers. Three females were observed to be in proestrous and only one of those three went on to become a large suppresser. By Trial 2, five of the sixteen females were in estrous and three of those five went on to become large suppressers. By the end of the experiment, there were three females that stopped showing any sign of regular cycling and instead, exhibited smaller, abnormal vaginal cells. Two of three females with seemingly disrupted estrous cycling demonstrated the greatest avoidance of the saccharin cue.

Discussion

The present study showed that female Sprague-Dawley rats suppress intake of a saccharin CS when it is paired with the administration of cocaine. Moreover, suppression of saccharin intake by females was highly variable with two distinct populations emerging such that half of the rats greatly avoided the saccharin CS (the large suppressers) following taste-drug pairings and the other half (the small suppressers) did not. Female rats treated with cocaine also demonstrated elevations in CORT following presentation of the saccharin cue at Test. Indeed, CORT was elevated for male and female rats in both, the large and small suppresser, groups. There was, however, no significant relationship between suppression of saccharin intake and test day CORT levels. Although there was a tendency for greater avoidance of the saccharin cue to be associated with greater cue-induced CORT elevations in males, there was no relationship between the two variables in females.

While a few studies report that female rats are sensitive to the suppressive effects of cocaine (Goudie, Dickins et al. 1978; van Haaren and Hughes 1990; Ferrari, O'Connor et al. 1991), the present study provides the first report of large individual differences being present in females following taste-drug pairings. Ferrari et al. (1991) found that female Long Evans rats will suppress intake of saccharin when paired with cocaine but the authors did not report any individual differences in intake, nor did they assess CORT levels in the female rats. One possible reason for the lack of large individual variability among cocaine-treated

females in the study conducted by Ferrari et al. (1991) is that they used a higher dose of cocaine (18 vs 10 mg/kg), respectively. In the Van Haaren and Hughes (1990) study, suppression in intake was evident only when using a 20 mg/kg dose and not when using a 10 mg/kg dose. They also tested a different strain (Wistar) and used a lower concentration of saccharin (0.1%). Thus, interpretation of the present findings is limited to the Sprague-Dawley strain when pairing a 0.15% concentration of saccharin with the sc administration of 10 mg/kg cocaine to fluid deprived subjects.

Although CORT was elevated at test for both cocaine treated groups, reasons for the absence of a significant relationship between CS intake and CORT are not clear. For males, this finding is unusual but there was at least a tendency for greater avoidance of the saccharin cue to be associated with greater elevations in CORT. This relationship may have attained significance if more male subjects were tested (e.g., 48 males instead of 24). For females, this finding may or may not be unusual since this is the only report of this particular finding. Weinberg (1982) measured CORT levels before and after the start of CTA conditioning (with milk and LiCl serving as the CS and the US) and found that females had higher pre-session plasma CORT levels than males. This finding is consistent with the results of the present experiment which also showed higher pre-conditioning CORT levels for female Sprague Dawley rats. Weinberg (1982) did a second assessment of CORT levels on the first reexposure day to the milk CS and sex differences emerged again. Specifically, females demonstrated suppression in CORT relative to pre-session levels while males demonstrated an

increase in CORT. This finding suggests that males and females experience different patterns in HPA axis activity within taste drug pairing designs. This is not surprising in light of the many studies that have found differences in HPA axis activity between males and females (Critchlow, Liebelt et al. 1963; Le Mevel, Abitbol et al. 1979; Ehlers, Kaneko et al. 1993; Aloisi, Steenbergen et al. 1994; Kuhn and Francis 1997). As mentioned earlier, estrous is a factor that influences CORT independent of conditioning or withdrawal and, therefore, may substantially contribute to the difficulty in observing a direct relationship between intake and CORT as it is observed in males.

The estrous cycle was not associated with suppression of CS intake on any trial. One possible reason for this is that the estrous cycle for these females was not synchronized. That is, each individual female was in a different phase of her cycle when exposed to the saccharin CS and the cocaine US for the first time. For this reason, the perceived hedonic value of both stimuli may have been different depending on which phase of the estrous cycle the female was in. As mentioned earlier, female rats will SA more, and work harder for, cocaine during estrous than in the other phases of her cycle (Roberts, Bennett et al. 1989; Lynch, Arizzi et al. 2000). Thus, the phase of estrous probably contributed to the variation in cocaine-induced suppression of saccharin intake. Vaginal lavages performed to monitor estrous also influence the behavioral response to acute cocaine (Walker, Nelson et al. 2002). This study showed that repeated lavage decreases the enhanced cocaine-induced locomotor activity during proestrous and estrous. A separate experiment in the same study showed that a conditioned

place preference is acquired for the compartment where vaginal lavages took place. The apparent reinforcing effects of vaginal lavages observed in this study were suggested to be related to a sexual representation of the mating process. Regardless, females in the first set of the present experiment were not lavaged and still did not demonstrate a relationship between CORT and suppression in saccharin intake at test.

There are at least four possible reasons why female rats are more resistant to the suppressive effects of cocaine than male rats. First, the drug may be less rewarding for the females. This is not likely, however, because female rats not only acquire cocaine SA more rapidly (Lynch and Carroll 1999) but also work harder for a cocaine infusion than male rats, on a progressive ratio schedule (Roberts, Bennett et al. 1989). Second, female rats “protect” their intake of food, when deprived, more than males. In support of this idea, Weinberg et al. (1982) showed that gender differences in CTA performance occur only when rats are deprived. When deprived, females protect intake of a novel milk CS that is paired with the administration of LiCl. Because all the rats were deprived in the present study, females may have been protecting their intake of the sweet saccharin CS. Third, sweet cues may be more rewarding for females than for males. This also is not likely, however, as gender differences in sweet preferences have not been reported (Konkle, Baker et al. 2003; Ackroff and Sclafani 2004). Fourth, gender differences could be attributed to body weight differences. Although weight differences did not contribute to differences in overall intake of the saccharin CS (i.e., intake to weight ratios revealed that

females actually drank more saccharin per gram of body weight than males), the amount of drug available to act in the brain may have been greater in males than in females. The present study employed a mg/kg dose regimen that resulted in more drug being administered to males than females.

If weight differences contribute to gender differences in cocaine-induced suppression of saccharin intake, it is important to consider why. First, because the weight difference between males and females is largely in adipose tissue, perhaps more drug is absorbed into this tissue for males leaving less drug available to act in the brain. If this were the case, then greater suppression by the males would be associated with less drug being available to act in the brain relative to females. This possibility is not likely, however, because suppression should become greater as the drug concentration becomes higher. One other possibility is that, despite the extra weight in males, more cocaine ultimately reaches the brain of a heavier male. This possibility could be tested by measuring the amount of cocaine in the brain and blood in a dose response study testing both males and females.

Gender differences in drug pharmacokinetics have been reported before. Becker et al (1982) found that the systemic administration of amphetamine (1.0 – 10.0 mg/kg) results in significantly higher drug levels in the female brain than in the male's. This finding is not consistent, however, with that of another showing that there are no gender differences in the metabolism of cocaine in plasma and in brain tissue following a 15 mg/kg, ip, injection of cocaine (Bowman, Vaughan et al. 1999). It remains unknown whether brain concentrations of cocaine still

would be the same if the rats were given subcutaneous injections. Regardless, brain levels of drug did not vary with the estrous cycle or with gonadectomy in either study. This finding suggests that, regardless of drug concentration, gender differences are more heavily influenced by the effects of gonadal hormones on mesostriatal dopamine activity.

In sum, female rats, like males, are sensitive to the suppressive effects of cocaine but there are gender differences in both behavioral and hormonal responses that deserve further investigation. A more thorough evaluation of how females behave in the reward comparison paradigm would provide additional clues for understanding why rats suppress intake of saccharin when it is paired with a drug of abuse. Moreover, it is important to continue evaluating gender differences in response to drugs of abuse to find treatment better suited for men vs women who suffer with addiction.

Chapter 6

GENETIC BASIS OF INDIVIDUAL DIFFERENCES: A SELECTIVE BREEDING STUDY

Individual differences in responsiveness to drugs of abuse and, ultimately, the propensity to become addicted are influenced by both environmental and genetic factors. The experiments in Chapters 2 through 4 were designed to investigate individual differences in behavioral and CORT responses within the realm of environmental factors. A significant by-product of these studies, however, is the emergence of large individual differences in cocaine-induced avoidance of saccharin intake. As a result, the cocaine-treated rats were divided into two separate groups, half were large suppressers and the other half were small suppressers. This bimodal distribution in the drug-treated population may reflect a simple genetic system (i.e., 2 or 3 genes) that influences drug-induced suppression of saccharin intake (for review see Plomin, DeFries et al. 2001). The finding above that female rats, like males, also exhibit individual differences in cocaine-induced suppression of saccharin intake facilitated the selective breeding of small and large suppressers.

Selective breeding is a powerful tool for identifying characteristics that correlate with drug sensitivity and vulnerability to drug abuse. As mentioned earlier, rodents have been selectively bred for many drug and taste related traits. Examples include high and low intakes of a sweet saccharin solution (Carroll,

Morgan et al. 2002), and various responses to ethanol (Eriksson 1969; Crabbe, Kosobud et al. 1987; Crabbe, Young et al. 1987), opiates (Belknap, Danielson et al. 1987), nicotine (Smolen and Marks 1991; Smolen, Marks et al. 1994) and cocaine (Smolen and Marks 1991; Marley, Arros et al. 1998). One characteristic that is correlated with the consumption of sweets and drugs of abuse is emotional reactivity. For example, the Maudsley Reactive (MR) and Maudsley Nonreactive (MNRA) rats, selectively bred for high and low open-field defecation, respectively, differ in consummatory contrast paradigms that involve reward comparison (Rowan and Flaherty 1991). Specifically, MNRA rats are more sensitive to changes in saccharin or sucrose concentrations that occur during successive negative, simultaneous, and anticipatory contrast. The Syracuse high (SHA) and low avoidance (SLA) rats provide another example that differences in emotional reactivity (measured by avoidance in a two way shuttle box) are linked to differences in consummatory contrast behavior (Flaherty and Rowan 1989). By the same token, rats have been selected specifically for differences in successive negative contrast (i.e., using a shift from 32% to 4% sucrose) and subsequently tested for emotionality and drug responsiveness (Flaherty, Krauss et al. 1994). Interestingly, these two lines (i.e., large and small contrast) did not differ in response to several seemingly related phenotypes such as the response to the absolute rewarding value of sucrose or cocaine, open-field defecation, and anticipatory contrast, suggesting that sensitivity to reward reduction is mediated by its own separate neurophysiological circuit. As many selective breeding endeavors have proven to be useful, it is expected that selective breeding for

large and small cocaine-induced suppression of saccharin intake not only will become equally useful but will also fill a unique niche that addresses both taste and drug sensitivity simultaneously in our reward comparison paradigm.

Results from the selective breeding study are presented here in Chapter 6. These experiments tested the hypothesis that there is a strong genetic component to cocaine-induced suppression of saccharin intake. As was discussed earlier, if cocaine-induced avoidance of saccharin intake is mediated by a simple genetic system, then behavioral traits should be transferred to selectively bred offspring, possibly, in a single generation. The selective breeding experiment tested this hypothesis by mating small and large suppressors to evaluate the genetic influence, if any, that there would be on the first and subsequent generations. Selective breeding was carried out for three generations. Each generation was tested first in the passive reward comparison design using cocaine (Experiment 1) and then later tested in a lithium chloride conditioned taste aversion (CTA) design (Experiment 2), (see Table 1 for summary).

<u>Generation</u>	<u>Experiments</u>
<p>S1: N=59</p> <p>Small (13 M, 16 F) Large (12 M, 18 F)</p> <p>* 2 litters make up small offspring & 3 make up large.</p>	<p>1. Passive Cocaine</p> <p>2. LiCl CTA</p>
<p>S2: N=73</p> <p>Small (12 M, 10 F) Large (20 M, 31 F)</p> <p>* 2 litters make up small offspring & 5 litters make up large.</p>	<p>1. Passive Cocaine</p> <p>2. LiCl CTA</p>
<p>S3: N=25</p> <p>Small (6 M, 4 F) Large (8 M, 7 F)</p> <p>* 1 litter makes up the large offspring and 2 litters make up the small.</p>	<p>1. Passive Cocaine (No CORT)</p> <p>2. LiCl CTA</p>

Table 1. This table summarizes how many rats were bred for each generation and the experiments that were conducted and presented in this chapter.

Experiment 1

Cocaine Conditioned Taste Avoidance

All three generations were first tested in the passive reward comparison design. Briefly, the standard protocol was employed where a 0.15% saccharin solution served as the CS and was paired with the passive administration of cocaine (10 mg/kg/s.c.), which served as the US. CORT responses to the presentation of the saccharin cue also were assessed before and after conditioning (e.g., taste-drug pairings).

Methods

Subjects. A total of 3 generations were produced and tested in the passive reward comparison design (see Table 1). The first generation (S1) was produced using individuals chosen from the population that served as subjects in Chapter 5 above (the 2nd replication). Thus, the original (P1) population consisted of 48 non-sibling (N=24 of each gender) Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC). The second (S2) and third (S3) generations were produced using individuals chosen from the S1 and S2 populations, respectively. All rats were housed and maintained as described for Chapter 2.

Apparatus, tastant, and drug. The apparatus was the same as that described in Chapter 2. The tastant and drug were prepared and presented as described in Chapter 2.

Procedure. The procedure was the same as that described in Chapter 5.

CORT Radioimmunoassay. The RIS was conducted as described in Chapter 2.

Experiment 1 Results: First Selectively Bred Generation (S1)

Of the 48 rats (24 males and 24 females) in the initial parent population, the most extreme small (N = 2 males and 2 females) and large (N = 3 males and 3 females) suppressers were successfully mated to bring forth 5 litters (N = 59), also see Table 6.1. All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc tests were conducted, when appropriate, using Newman Keuls tests, with alpha set at 0.05.

Saccharin (CS) Intake. The saccharin intake data (shown in Figure 6.1) indicated that in the first generation (S1), the large suppresser line demonstrated greater avoidance of the saccharin cue over trials than the small suppresser line. This conclusion was supported by a significant Lineage x Trial interaction, $F(7,385)=4.89$, $p<0.05$, following a 2 x 2 x 8, repeated measures, ANOVA varying Lineage (small and large), gender (male and female), and trial (1-8). The results of this analysis did not reveal a significant main effect of Gender or 3-way interaction, $p_s >0.05$. The behavioral data, however, indicated that, like their parents, males tended to suppress intake of the saccharin CS more greatly than did females. Consequently, the intake data were analyzed separately for males and females using a 2 x 8 ANOVA varying lineage (small and large) and trial (1-8) and is presented below.

Males (see Figure 6.1, left panel). Although the main effect of Lineage was not significant, $p>0.05$, the results of the same analysis showed that, over trials, the large suppresser line demonstrated greater suppression of saccharin intake than the small suppresser line. Support for this conclusion came from a

significant Group x Trial interaction, $F(7,161)=2.88$, $p<0.01$. Newman Keuls post hoc comparisons of this significant 2-way interaction revealed that while the male small suppresser line demonstrated a slight, but non-significant, decrease in intake across trials, the large suppresser line exhibited a robust drop in saccharin intake that became significant by Trial 3, $p<0.05$. The two lines also differed on the first trial because the large suppresser line consumed more of the saccharin CS than did the small suppresser line, $p <0.05$. There also was a difference in the same direction on the CS pre-exposure day but the trend was not significant. (unpaired t-test), $p>0.05$. Females (see Figure 6.1, right panel). The results of a similar 2 x 8 ANOVA revealed a significant Line x Trial interaction, $F(7,224)=2.12$, $p<0.05$, indicating that females also exhibited different intake patterns as a function of lineage. Post hoc comparisons of this interaction showed that, while the female large suppresser line demonstrated a significant decrease in intake by Trial 3, $p<0.05$, intake by the small suppresser line was not different from the first trial until Trial 8, $p<0.05$. The two lines also were different on Trial 7 where the large suppresser line significantly reduced their intake of the saccharin CS compared with the small, $p<0.05$. The female small suppresser line, in general, demonstrated greater consumption of the saccharin CS compared with the large suppresser line and this conclusion was supported by a significant main effect of Lineage (large vs. small) collapsed across trials, $F(1,32)=5.01$, $p<0.05$. Thus, while both small and large suppresser females initially consumed the same amount of the saccharin CS on Trial 1, large suppresser females experienced a greater reduction in intake over trials than did the females born to small

suppressing parents. Results from an unpaired t-test comparing intake on the saccharin CS pre-exposure day, however, showed that the mean intakes were significantly different, $p < 0.05$, with the large suppresser line consuming more of the novel saccharin solution than the small.

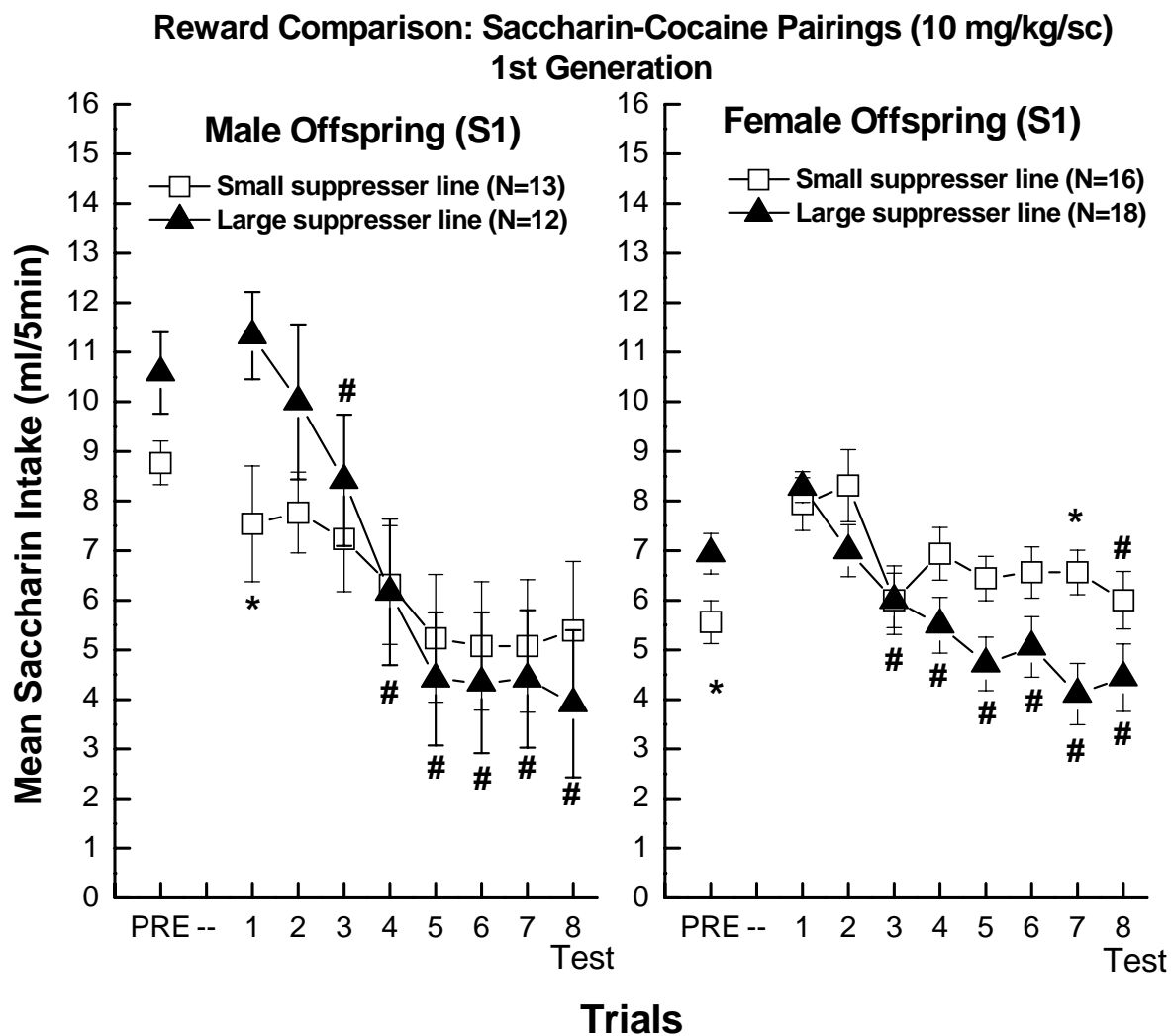


Figure 6.1. Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) on the CS pre-exposure day and across seven pairings with cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the first generation of selectively bred male small and large suppressers are shown in the left panel and data for females are shown in the right panel. * Different from large, #different from Trial 1, $p < 0.05$.

Corticosterone. The CORT data (shown in Figure 6.2) were first analyzed using a 2 x 2 x 2, mixed factorial, ANOVA varying lineage (large and small), gender (male and female), and trial (pre and post-conditioning). The results of this analysis revealed that, in general, CORT became significantly elevated from pre- to post-conditioning, as indicated by a significant main effect of Trial, $F(1,55)=4.18$, $p<0.05$. The results also revealed a significant main effect of Gender, $F(1,55)=11.3$, $p<0.05$, indicating that overall CORT was higher for females than for males but this effect was carried by high post-conditioning CORT levels in females. Post hoc comparisons of a significant Gender x Trial interaction, $F(1,55)=4.2$, $p<0.05$, revealed that although both males and females (collapsed across small and large groups) experienced an increase in CORT from pre- to post-conditioning, $p_s<0.05$, females demonstrated higher levels of circulating CORT at test than did males. The main effect of Lineage was not significant, $p>0.05$, indicating that, when collapsed across gender and trial, mean CORT levels were not different between the small and large suppresser lines. Although the present analysis did not result in a significant Lineage x Gender or 3-way interaction, $p_s>0.05$, the behavioral data indicated that males and females were different. As that reason, the CORT data for males and females were analyzed separately using a 2 x 2 ANOVA varying lineage (large and small) and trial (pre- and post-conditioning).

Males (see Figure 6.2, left panel). The male small and large suppresser lines demonstrated similar pre- to post-conditioning CORT patterns to those observed previously. This conclusion was supported by a significant main effect

of Trial, $F(1,23)=14.4$, $p<0.05$, indicating that post-conditioning CORT levels were higher than pre-conditioning levels. The main effect of Lineage and Lineage x Trial interaction, however, were not significant, $F_s<1$, $p_s>0.05$. Females (see Figure 6.2, right panel). Unlike males, female pre- to post-conditioning CORT patterns appeared different for the large and small suppresser lines. Specifically, the large suppresser line demonstrated greater elevations in pre- to post-conditioning CORT than did the small suppresser line. Although, this trend was not significant, as indicated by a non-significant main effect of Lineage, $p>0.05$, and Lineage x Trial interaction, $F(1,32)=3.4$, $p>0.05$.

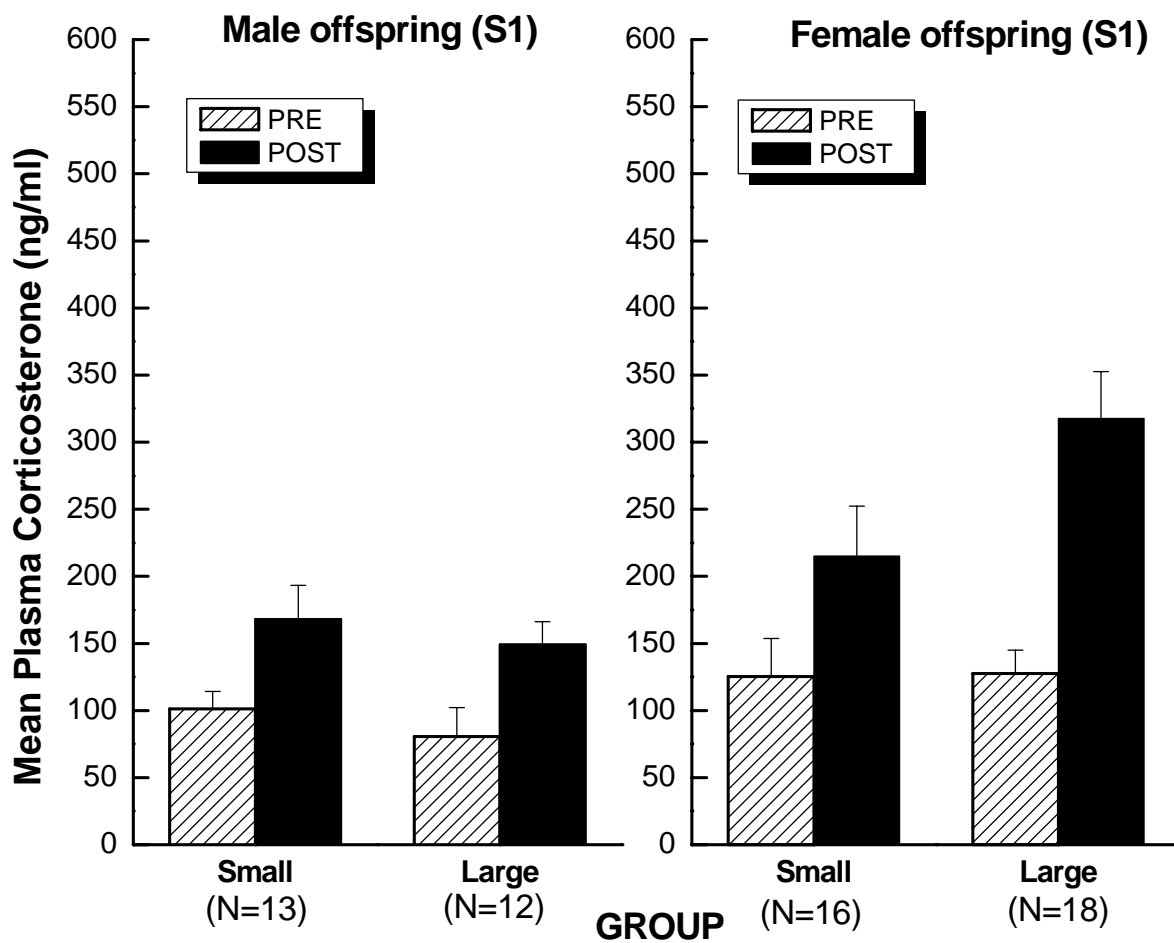
CORT: Pre- & Post-Conditioning

Figure 6.2. Mean (\pm SEM) pre- and post-conditioning CORT levels (ng/ml) for the first generation of selectively bred small and large suppresser males (left panel) and females (right panel).

Intake and CORT Correlations. A series of correlation analyses were conducted to assess the relationship between individual saccharin intake and CORT on test day. For both, males (Figure 6.3, left panel) and females (right panel), a negative linear relationship between individual intake and CORT emerged whereby greater avoidance of the saccharin cue was significantly correlated with greater elevations in CORT at test. This relationship is consistent with what has been observed previously.

Plotting small and large suppresser lines separately, on the other hand, revealed patterns within the groups that were not consistent with patterns observed previously. While greater avoidance of the saccharin cue tended to be associated with greater elevations in CORT at test for male offspring, regardless of line (see Figure 6.4), this was not the case for female offspring. Given the tendency for the female large suppresser line (see Figure 6.5) to have the highest CORT levels at test, the finding that there was no relationship between saccharin intake and CORT at test for this was surprising (Figure 6.5, right panel). A marked negative correlation, however, was obtained in the small suppressing line.

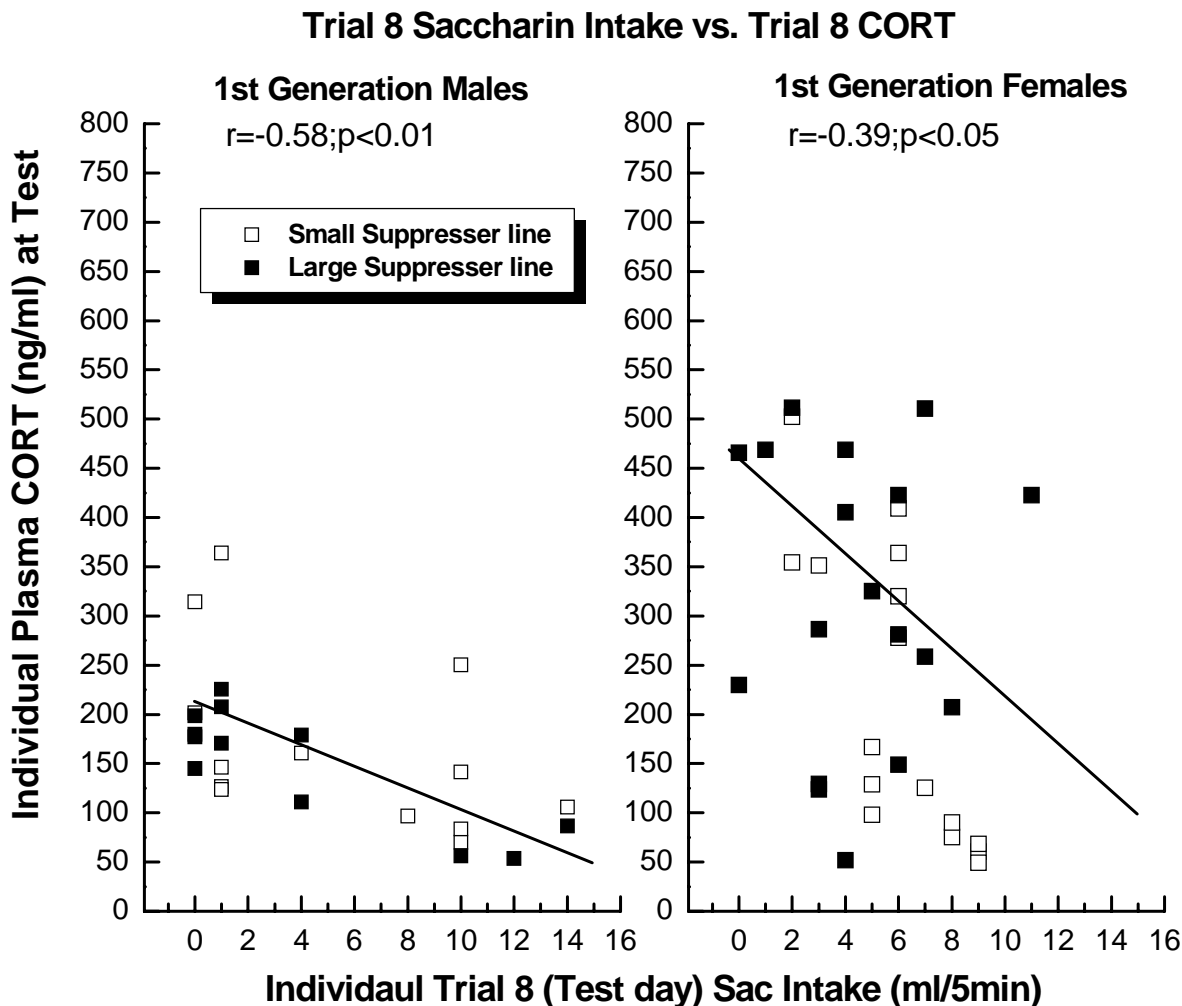


Figure 6.3: Correlation analyses were conducted separately for males and females to assess the relationship between terminal saccharin intake and post-conditioning CORT levels. Intake of the saccharin CS was graphed as a function of post-conditioning CORT levels at test for first generation cocaine-treated male (left panel), and female (right panel), small and large suppressers.

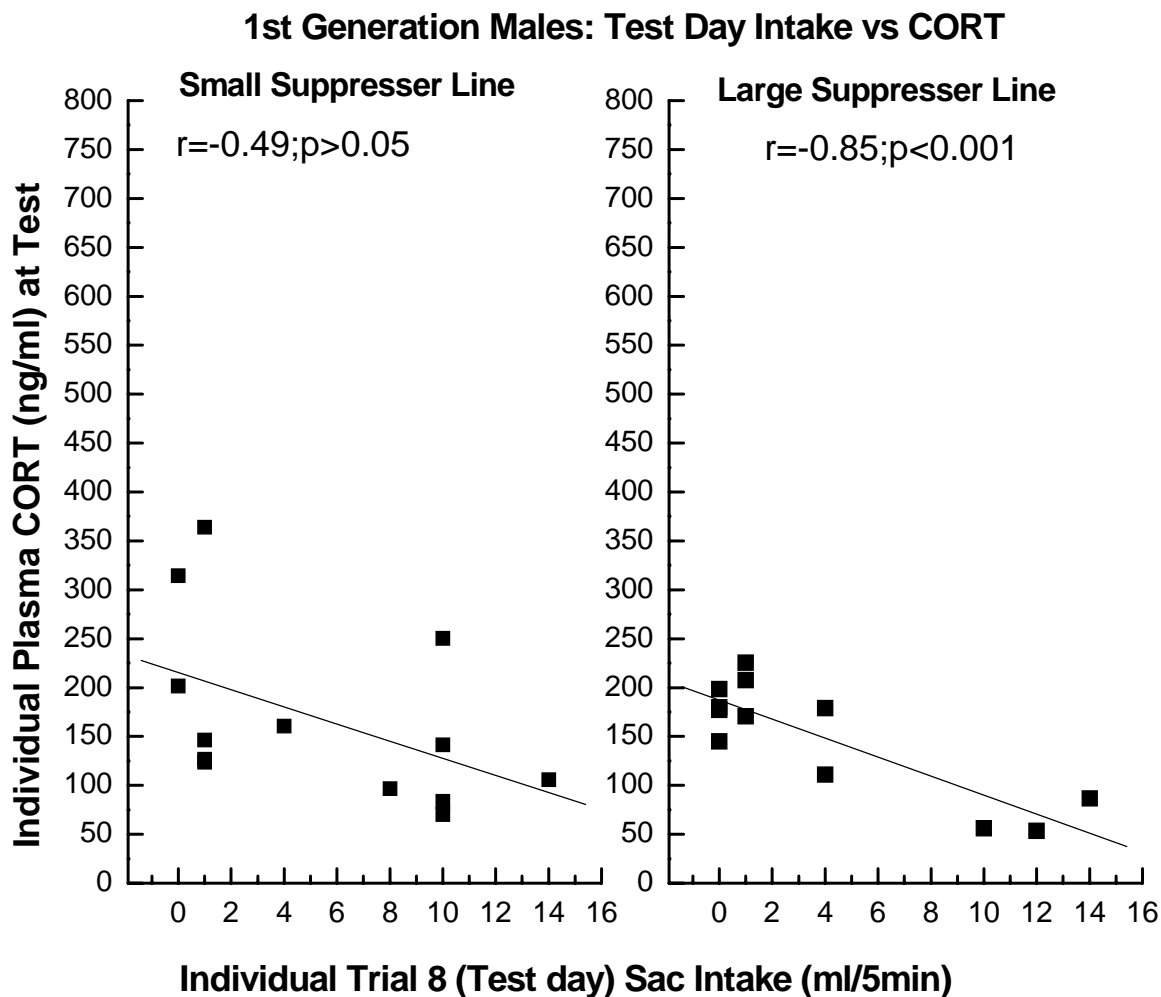


Figure 6.4: Correlation analyses were conducted separately for first generation male small and large suppressers. Intake of the saccharin CS was graphed as a function of post-conditioning CORT levels for male small (left panel), and large (right panel), suppressers.

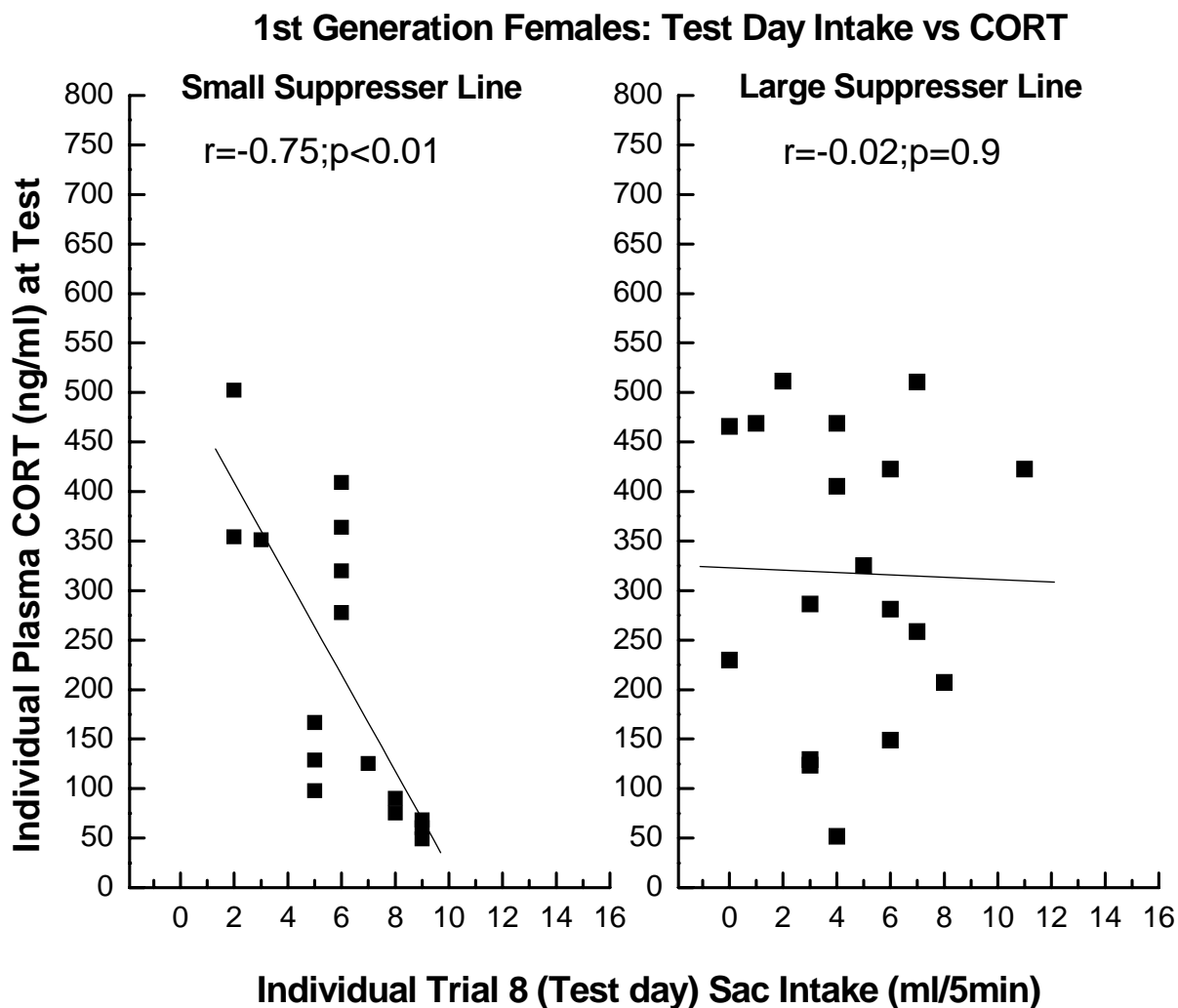


Figure 6.5: Correlation analyses were conducted separately for first generation female small and large suppressers. Intake of the saccharin CS at test was graphed as a function of post-conditioning corticosterone levels for females from the small (left panel), and the large (right panel), suppressing line.

Experiment 1 Results: Second Selectively Bred Generation (S2)

Of the 59 rats in the S1 population, the most extreme small (N = 2 males and 2 females) and large (N = 5 males and 5 females) suppressers were successfully mated to bring forth 7 litters (N = 73), also see Table 6.1. All data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc tests were conducted, when appropriate, using Newman Keuls tests, with alpha set at 0.05.

Saccharin (CS) Intake. The behavioral data (shown in Figure 6.6) indicated that second generation (S2) offspring behaved much like their first generation parents (S1). That is, the large suppresser line demonstrated greater avoidance of the saccharin cue than the small suppresser line. The overall separation as a function of lineage by Trial 8 was much greater in the second generation than in the first. These conclusions were supported by the results of a 2 x 2 x 8 ANOVA varying lineage (small and large), gender (male and female), and trial (1-8). A significant main effect of Lineage, $F(1,69)=43.8$, $p<0.05$, main effect of Trial, $F(7,483)=14.5$, $p<0.05$, and Group x Trials interaction, $F(7,483)=20.8$, $p<0.05$, indicated that the large suppresser line consumed less of the saccharin CS than the small suppresser line across trials (figure not shown for data collapsed across males and females). It should be noted that the Line x Trial interaction did not attain significance for the first generation as it did here. Furthermore, males from the large suppressing line demonstrated greater avoidance of the saccharin cue than did females from the large suppressing line as indicated by a significant Lineage x Gender interaction, $F(1,69)=8.6$, $p<0.05$. The main effect of Gender and the 3-way interaction, however, were not

significant, $p < 0.05$. Next, the data were analyzed separately for males and females using a 2 x 8 ANOVA varying lineage (large and small) and trial (1-8) and are presented below.

Males (see Figure 6.6, left panel). As in the first generation, the male large suppresser line demonstrated greater avoidance of the saccharin cue than the small suppresser line. This conclusion was supported by a significant main effect of Lineage, $F(1,28)=28.7$, $p < 0.05$, and Lineage x Trial interaction, $F(7,196)=12.9$, $p < 0.05$. The main effect of Lineage did not attain significance, however, in the first generation, indicating a greater separation occurred in the second generation. Post hoc comparisons of the 2-way interaction in the present analysis indicated that the large suppresser line experienced a quick and significant drop in saccharin intake by Trial 2, $p < 0.05$, while the small suppresser line continued to consume the same amount of saccharin through Trial 8. Furthermore, intake as a function of lineage became significantly different by Trial 2 and remained different through Trial 8, $p < 0.05$. As in the first generation, initial consumption of the novel saccharin solution tended to be greater for the large suppresser line than for the small suppresser line, but this effect did not attain significance, $p > 0.05$. Females (see Figure 6.6, right panel). As observed for males, the female large suppresser line demonstrated greater suppression of saccharin intake than the small suppresser line. This conclusion was supported by a significant main effect of Lineage, $F(1,41)=10.8$, $p < 0.05$, and a significant Lineage x Trial interaction, $F(7,287)=7.4$, $p < 0.05$. Post hoc comparisons of this 2-way interaction revealed that saccharin intake was significantly reduced for the

large suppresser line by Trial 3, $p < 0.05$, and intake was not suppressed by the small suppresser line. Separation between the two lines in this generation also occurred more quickly than for first generation with intake significantly reduced for the large suppresser line by Trial 3 and remaining different through Trial 8, $p < 0.05$. As observed in the first generation, mean consumption of the novel saccharin CS was significantly greater for the large suppresser line than for the small suppresser line, $p < 0.05$.

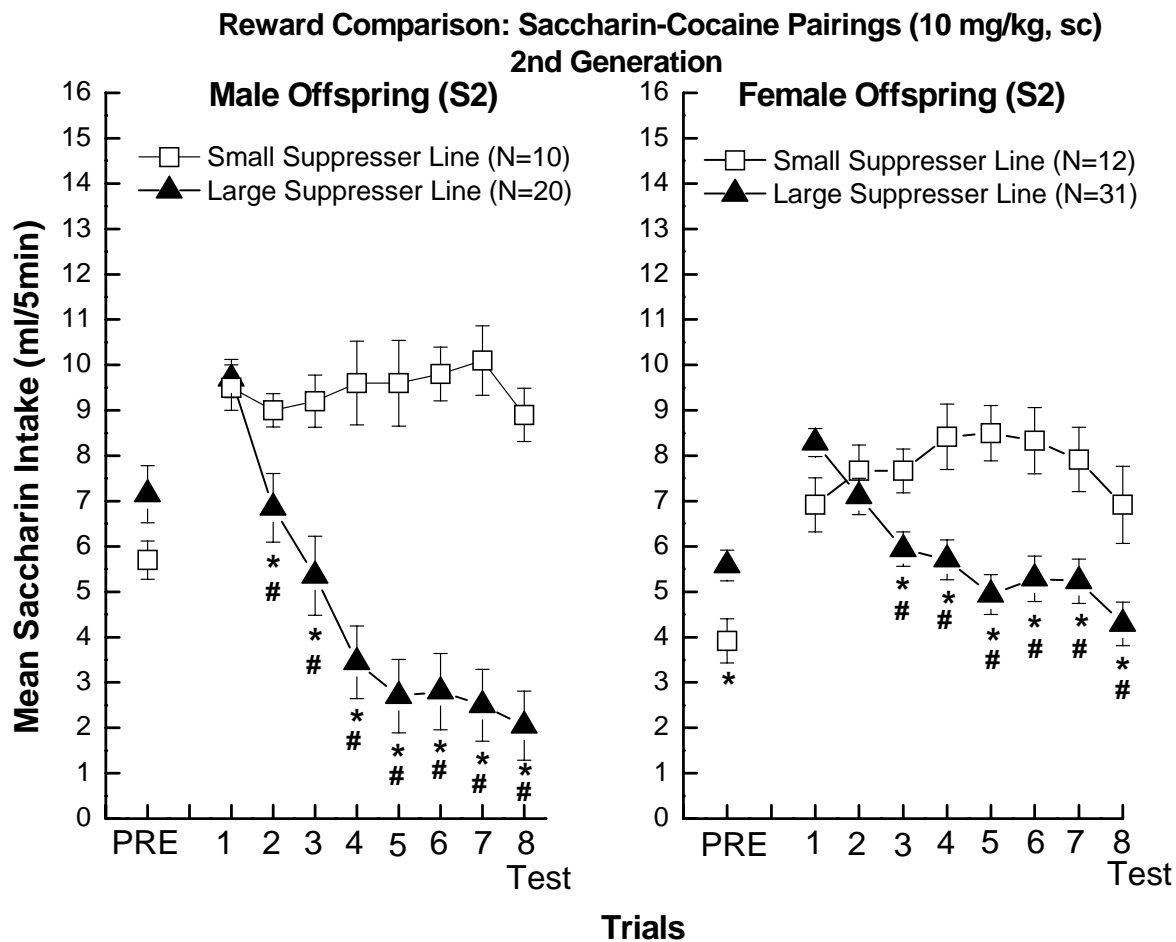


Figure 6.6. Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) on the CS pre-exposure day and across seven pairings with cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the second generation of selectively bred male small and large suppressors are shown in the left panel and data for females are shown in the right panel. * Different from small, #different from Trial 1, $p < 0.05$.

Corticosterone. There was one female large suppresser that did not give blood and was therefore dropped from the analysis. The CORT data (shown in Figure 6.7) were first analyzed using a 2 x 2 x 2 mixed factorial ANOVA varying gender (male and female), lineage (large and small) and trial (pre and post-conditioning). Unlike that observed for the first generation, the main effect of Trial was not significant, $F < 1$, $p > 0.05$, indicating that overall CORT for the second generation did not increase from pre- to post-conditioning. The main effect of Lineage also was not significant, $p > 0.05$. Post hoc comparisons of a significant Lineage x Trial interaction, $F(1,68) = 20.1$, $p < 0.05$, however, revealed that the large suppresser line (males and females combined, figure not shown) did demonstrate an increase from pre- to post-conditioning CORT levels. The small suppresser line, however, demonstrated the reverse effect; CORT levels decreased from pre- to post-conditioning, $p_s < 0.05$. The decrease in CORT from pre- to post-conditioning observed for the small suppresser line appeared more pronounced in females but reasons for the observed decrease are unclear.

The main effect of Gender, significant for the first generation, was not significant for the S2 population, $F < 1$, $p > 0.05$, and there were no significant interactions, $p_s > 0.05$. The behavioral data indicated, as it did for the S1 population, that males and females were different. Thus, the CORT data were analyzed separately for males and females using a 2 x 2, repeated measures ANOVA varying lineage (large and small) and trial (pre- and post-conditioning) and the results are presented below.

Males (see Figure 6.7, left panel). Although there was not a significant main effect of Lineage, $p > 0.05$, a significant main effect of Trial, $F(1,28) = 7.1$, $p < 0.05$, indicated that, overall, CORT levels became significantly elevated from pre- to post-conditioning. This effect was primarily carried by the large suppresser line. A significant elevation in pre- to post-conditioning CORT was not observed for the small suppresser line because pre-conditioning CORT levels were significantly elevated compared with the large suppresser line. These findings were revealed following post hoc comparisons of a significant Lineage x Trial interaction, $F(1,28) = 11.1$, $p < 0.05$. Females (see Figure 6.7, right panel). The results of a similar 2 x 2 ANOVA for female CORT data showed that neither the main effect of Lineage nor the main effect of Trial were significant, $p_s > 0.05$. Post hoc comparisons of a significant Lineage x Trial interaction, $F(1,40) = 13.6$, $p < 0.05$, however, showed that CORT was elevated at test in the large suppresser line, $p < 0.05$, but not in the small suppresser line, $p > 0.05$. This finding is consistent with those observed for males. Although there was a tendency for pre-conditioning CORT levels to be higher for the small suppresser line, this effect was not significant, $p > 0.05$. Post-conditioning CORT levels, on the other hand, were significantly elevated in the large suppresser line compared with the small, $p < 0.05$.

CORT: Pre- & Post-Conditioning

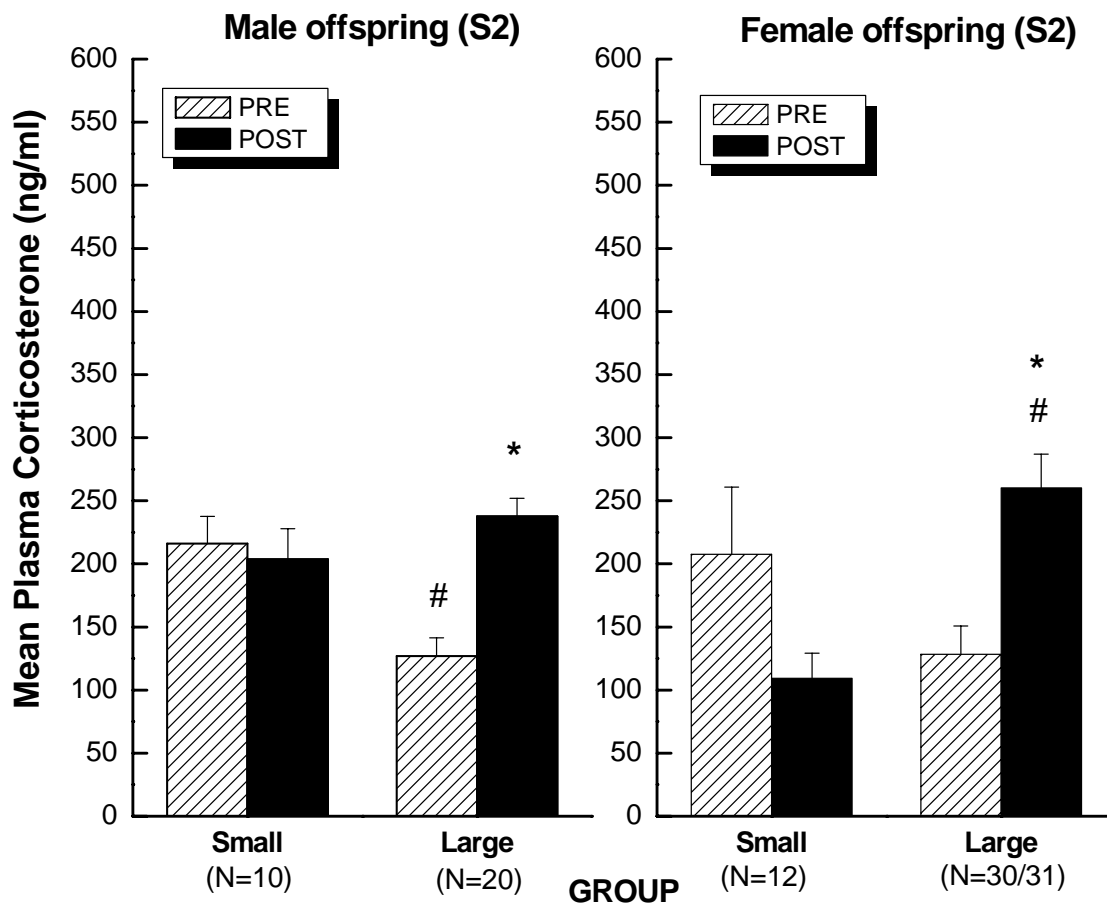


Figure 6.7. Mean (\pm SEM) pre- and post-conditioning CORT levels (ng/ml) for the second generation of selectively bred small and large suppresser males (left panel) and females (right panel). * Different from pre, # different from small.

Intake and CORT Correlations. A series of correlation analyses were conducted to assess the relationship between individual saccharin intake and CORT at test. As in the first generation, both males (Figure 6.8, left panel) and females (right panel) of the second generation exhibited individual differences such that greater avoidance of the saccharin cue was significantly correlated with greater elevations in CORT at test. When the small and large suppresser lines were plotted separately, however, there were no longer any significant relationships between intake and CORT (see Figures 6.9 and 6.10). Dissipation of a significant relationship between intake and CORT might reflect further separation of the two lines. There was less variability in both intake and CORT within both the small and large suppresser lines in the second generation. Compared with the first generation, the individual CORT data tend to hover over its respective segment of the x-axis (i.e, large suppresser offspring over the lower part of the x-axis and small suppresser offspring over the higher end of the x-axis). As a result, it became more difficult to examine large individual differences in the second generation because the populations became more homogenous.

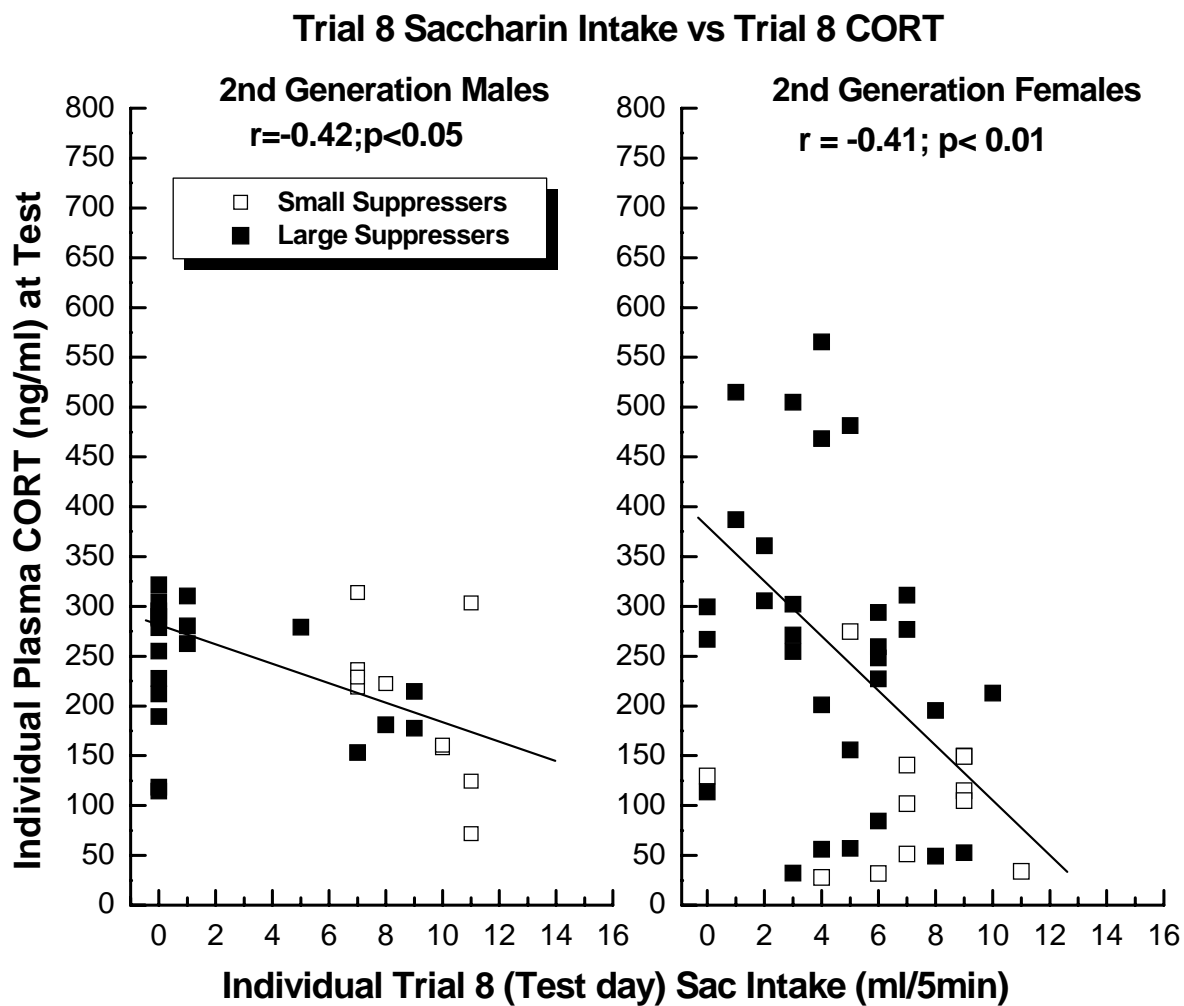


Figure 6.8: Intake of the saccharin CS graphed as a function of post-conditioning CORT levels at test for second generation cocaine-treated male (left panel), and female (right panel), small and large suppressors.

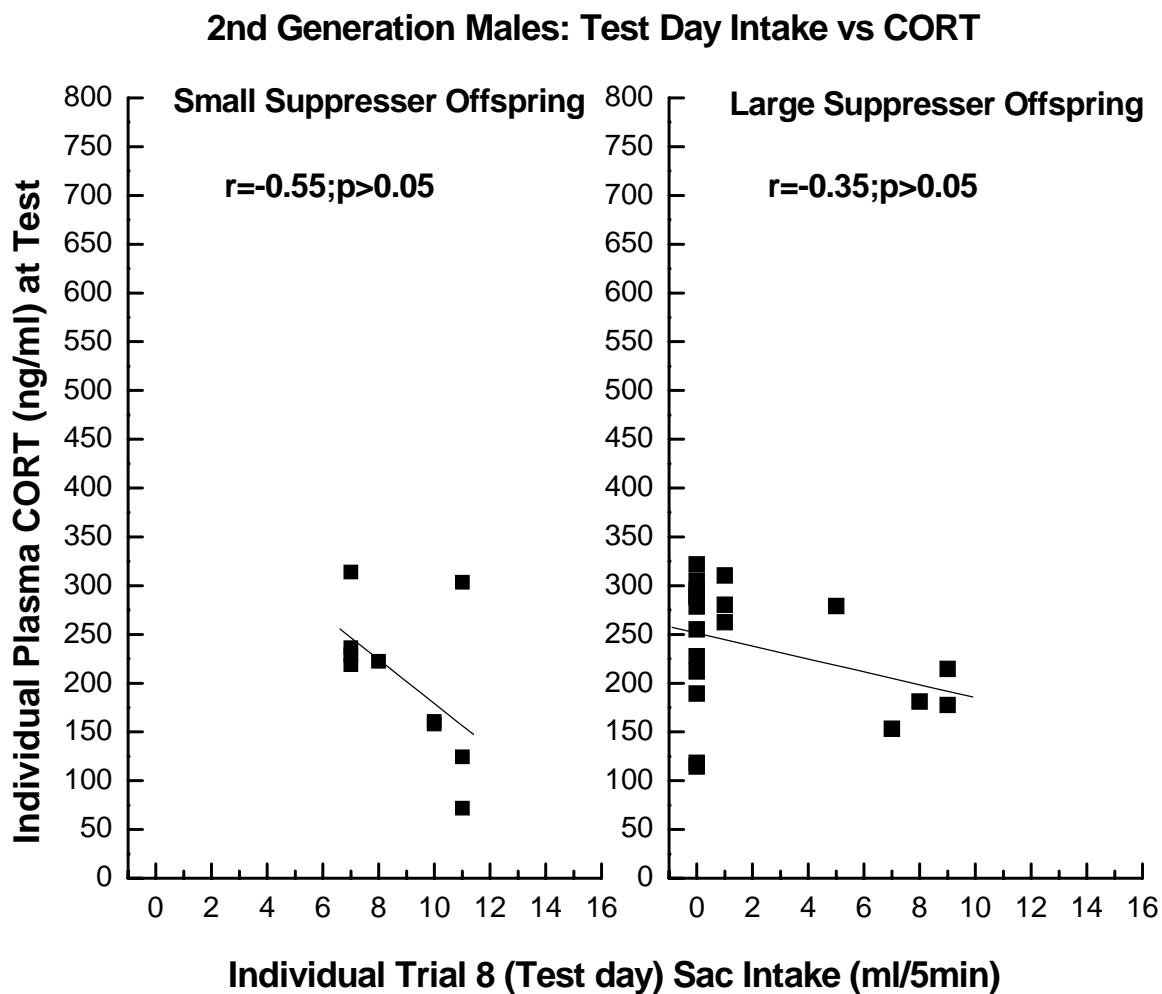


Figure 6.9: Intake of the saccharin CS graphed as a function of post-conditioning corticosterone levels for male small (left panel) and large (right panel), suppressors.

2nd Generation Females: Test Day Intake vs CORT

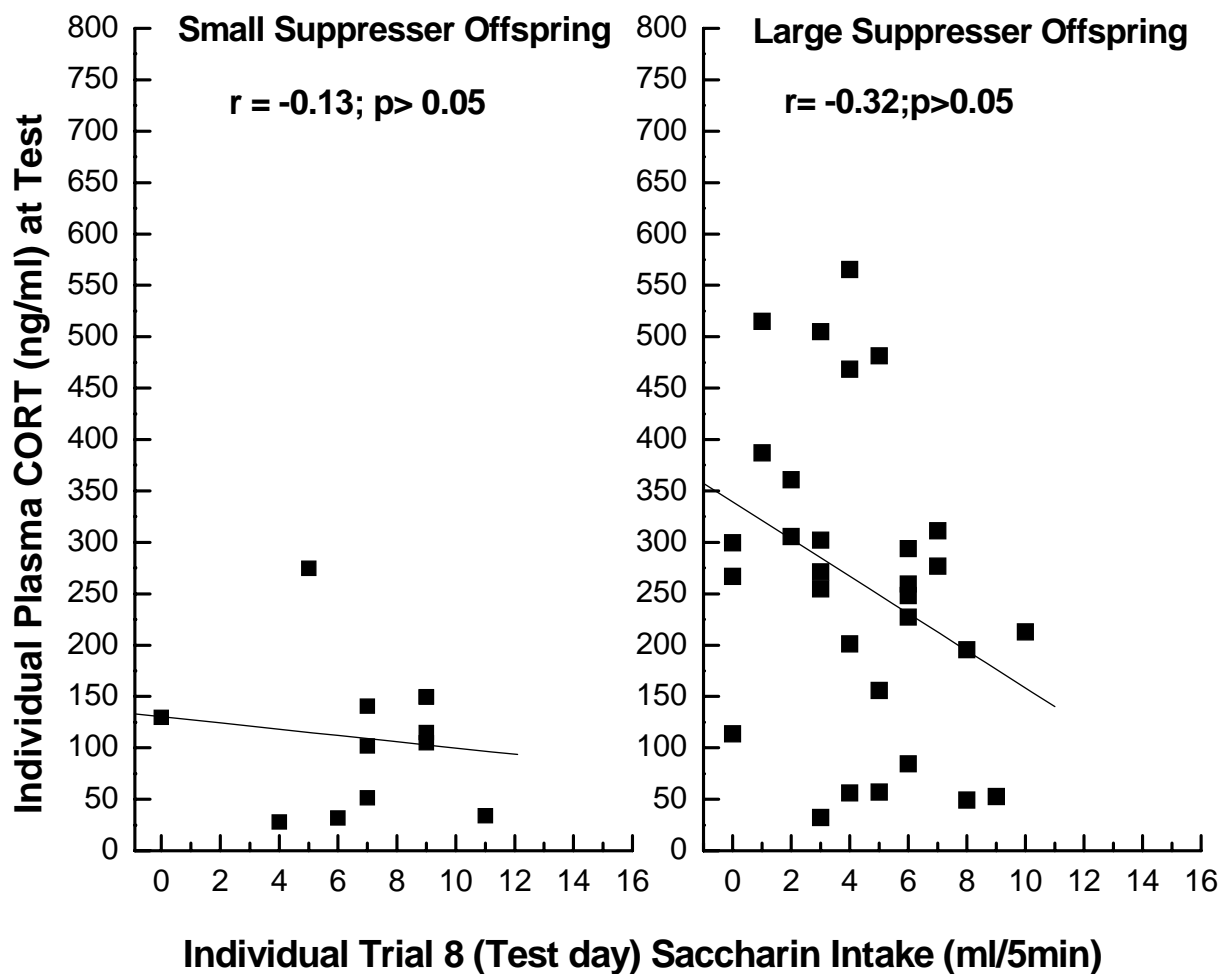


Figure 6.10: Intake of the saccharin conditioned stimulus graphed as a function of post-conditioning corticosterone levels for female small (left panel) and large (right panel), suppressers.

Results: Third Selectively Bred Generation (S3)

Of the 73 rats in the S2 population, the most extreme small (N = 2 males and 2 females) and large (N = 1 male and 1 female) suppressers were successfully mated to bring forth 3 litters (N= 25), also see Table 6.1. All the data were analyzed with Statistica using mixed factorial ANOVAs. Post hoc tests were conducted, when appropriate, using Newman Keuls tests, with alpha set at 0.05.

Saccharin (CS) Intake. The saccharin Intake data (shown in Figure 6.11) were first analyzed using a 2 x 2 x 8 ANOVA varying lineage (small and large), gender (male and female), and trial (1-8). As observed for the first two generations, the large suppresser line demonstrated greater reduction of saccharin intake over trials than the small suppresser line. Although the 3-way interaction was not significant, $p > 0.05$, this conclusion was supported by a significant main effect of Lineage, $F(1,21)=11.5$, $p < 0.05$, main effect of Trial, $F(7,147)=17.6$, $p < 0.05$, and Lineage x Trial interaction, $F(7,147)=4.4$, $p < 0.05$ (figure not shown for collapsed male and female data). These effects also were significant for the S2 population but not for the S1 population. Neither the main effect of Gender nor the Lineage x Gender interaction were significant, $p_s > 0.05$. The Gender x Trial interaction, however, was significant, $F(7,147)=4.1$, $p < 0.05$, indicating that, overall, males significantly reduced their intake of saccharin over trials compared with females. Next, the data were analyzed separately for males and females using a 2 x 8 ANOVA varying lineage (small and large) and trial (1-8) and the results are presented below.

Males (see Figure 6.11, left panel). The male large suppresser line demonstrated greater avoidance of the saccharin cue than the small suppresser line. This conclusion was supported by a significant main effect of Lineage, $F(1,12)=11.8$, $p<0.05$, and Lineage x Trial interaction, $F(7,84)=4.1$, $p<0.05$. These results parallel those for the 2nd generation. Post hoc comparisons of the 2-way interaction in the present analysis indicated that the large suppresser line experienced an immediate drop in saccharin intake that became significant by Trial 3, $p<0.05$. The small suppresser line, on the other hand, consumed the same amount of the saccharin CS through Trial 8, $p>0.05$. The difference in intake between the two lines became significant by Trial 2 and remained different through Trial 8, $p<0.05$. Unlike for the S1 and S2 populations, intake of the novel saccharin CS on the pre-exposure day did not differ between the small and large suppresser lines for the S3 population, $p>0.05$. Females (see Figure 6.11, right panel). Although the female large suppresser line suppressed their intake of the saccharin CS more than the small suppresser line, the results of a similar 2 x 8 ANOVA showed that neither the main effect of Lineage nor the Lineage x Trial interaction were significant, $p>0.05$. This was likely due to a relatively small sample size. The main effect Trial was significant, $F(7,63)=2.7$, $p<0.05$, however, indicating that overall intake of saccharin decreased over trials. As observed for the first and second generations, the mean consumption of the novel saccharin CS tended to be greater for the large suppresser line, but this tendency did not attain significance in the S3 females, $p>0.05$. In sum, the S3 population behaved similarly to the S1 and S2 populations.

CORT has yet to be determined for S3 offspring.

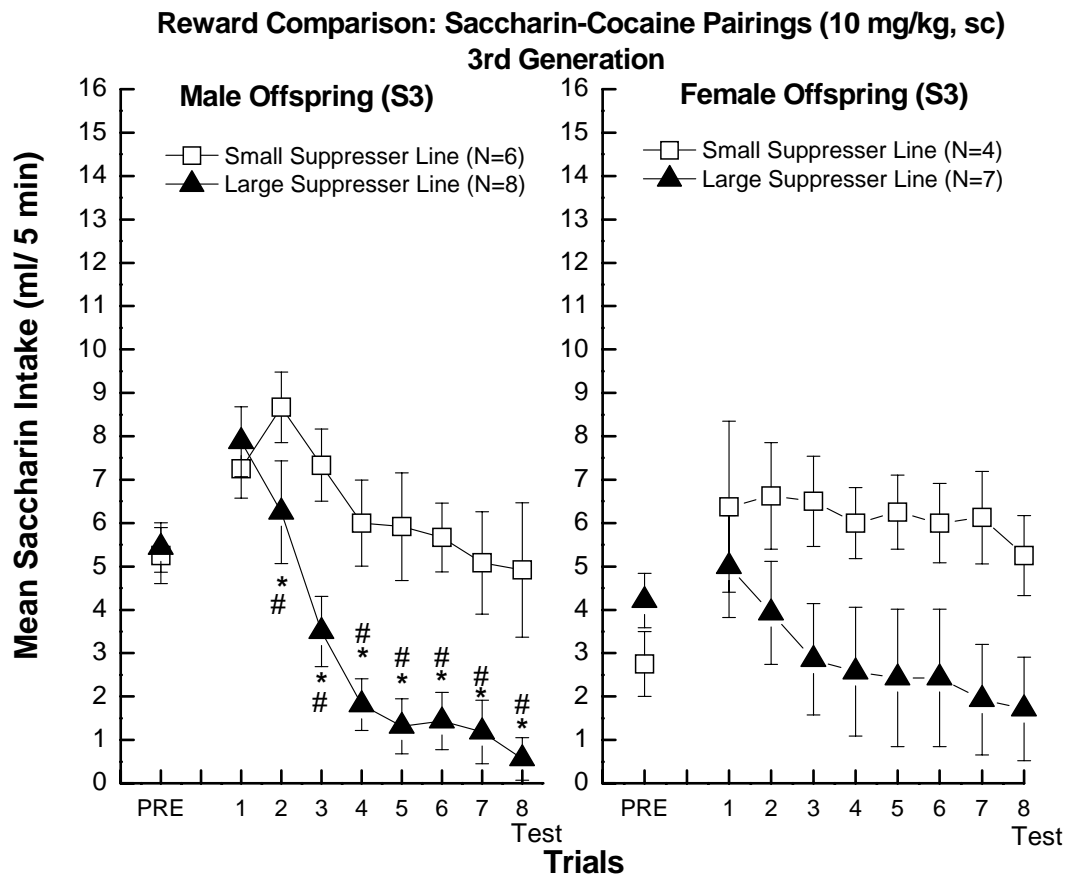


Figure 6.11. Mean (\pm SEM) intake of 0.15% saccharin (ml/5 min) on the CS pre-exposure day and across seven pairings with cocaine (10 mg/kg, sc) followed by a saccharin CS only test trial. Data for the third generation of selectively bred male small and large suppressors are shown in the left panel and data for females are shown in the right panel. * Different from small, #different from Trial 1, $p < 0.05$.

Comparing All Generations. Figure 6.12 shows mean terminal (Trial 8) saccharin intake for each generation. Population means are shown in the left panel and breeder means are shown in the right panel with males (top panel) and females (bottom panel) also presented separately. These graphs depict separation of the selectively bred small and large suppresser lines over generations. Indeed, the two lines diverged significantly as a function of terminal saccharin intake for each population. This conclusion was supported by results of a 4 x 2 x 1 ANOVA varying generation (P1, S1, S2, and S3) and lineage (small or large) with Trial 8 saccharin intake as the dependent variable (combined data for males and females not shown). ANOVAs were conducted separately for males and females of each of the 2 population data sets. Male population. The small and large suppresser lines for the male population diverged significantly by the second generation. Support for this conclusion comes from post hoc comparisons of a significant Generation x Lineage interaction, $F(3, 77)=4.2$, $p<0.05$. The main effect of the Lineage also was significant, $F(1,77)=15.6$, $p<0.05$, indicating that the large suppresser line had significantly lower Trial 8 saccharin intake than the small suppresser line over generations. Female population. The results of a similar analysis for the female population confirmed that, consistent with the male data, the small and large suppresser lines were significantly different over generations. This conclusion was supported by a significant main effect of the Lineage, $F(1,96)=10.9$, $p<0.05$. The Generation x Lineage interaction, however, did not attain significance, $F(3,96)=1.4$, $p>0.05$. Male and female breeders. Similar patterns in behavior were observed for the

male and female breeders. Because the sample size was only 1 rat for the S2 breeders of the small suppresser line, these data were not analyzed but still presented for the purpose of comparison.

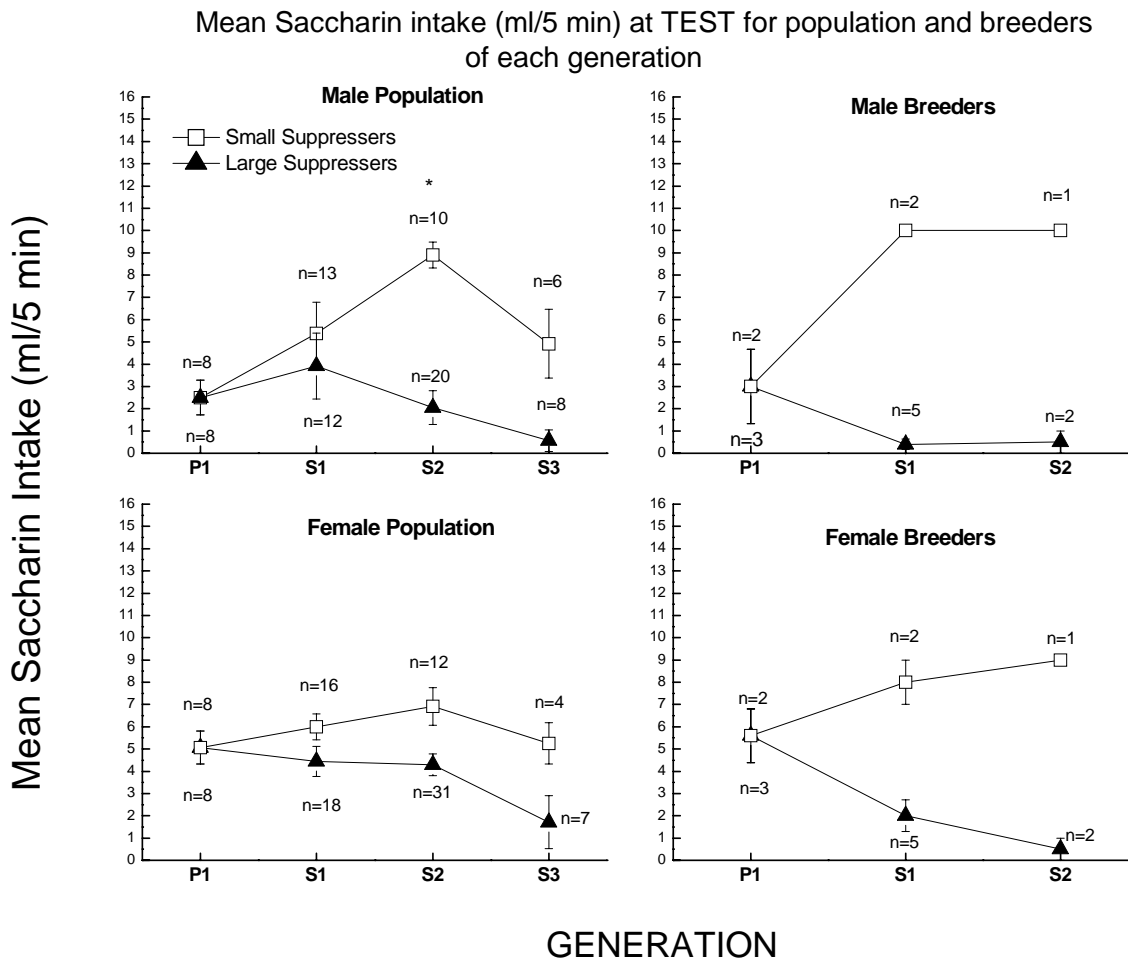


Figure 6.12. Mean terminal CS intake (i.e., intake at test) is shown for each population (P1, S1, S2, and S3) in the left panel and for the breeders of each generation in the right panel. Data are graphed separately for males (top panel) and for females (bottom panel).

Experiment 2

Lithium Chloride (LiCl) Conditioned Taste Aversion

In the next set of experiments, rats were tested using a LiCl CTA design to determine whether strain differences in drug-induced avoidance of a taste cue reflect changes in sensitivity to reward or in associative learning, separate from reward. LiCl- and drug-induced suppression of saccharin intake is thought to be mediated by separately in the brain. Changing the nature of the CS, the caloric value of the CS, or the deprivation state of the animal affects drug- and sucrose-induced suppression of CS intake, but does not affect a LiCl CTA (Flaherty, Grigson et al. 1991; Flaherty, Turovsky et al. 1994; Grigson 1997; Gomez and Grigson 1999; Grigson, Lyuboslavsky et al. 1999)). Lesions of the gustatory thalamus attenuate the suppressive effects of sucrose and of drugs of abuse, but not of LiCl. This finding indicates a certain divergence in the neuronal pathways underlying taste avoidance that is mediated by aversive and rewarding properties (Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005). Finally, reward-preferring Lewis rats are more sensitive than less-preferring Fischer 344 rats to the suppressive effects of cocaine and sucrose but are equally sensitive to a LiCl-induced CTA (Glowa et al., 1994; Grigson and Freet, 2000; but see Kosten et al., 1994).

This experiment tested the hypothesis that these selectively bred large suppressors are similar to the reward-preferring Lewis rats while the small

suppressers are similar to the less-preferring Fischer 344 rats. If this hypothesis is true, then both, the small and the large suppresser, lines will demonstrate equal LiCl-induced CTAs.

Experiment 2: Methods

Subjects. Three sets of rats were run in the LiCl CTA design; 15 from the S1 generation (4 M Lg; 4 M Sm; 3 F Lg; 4 F Sm, all breeders); 26 from the S2 generation (7 M Lg; 3 M Sm; 9 F Lg; 7 F Sm, half breeders); 27 from the S3 generation (8 M Lg; 9 M Sm; 7 F Lg; 3 F Sm). The S1 rats were run about 3 months before the S2 and S3 rats that were run together in the same study. The experiment was conducted in the home cages in the morning hours. All rats were housed and maintained as described in Chapter 2.

Apparatus, tastant and drug. The apparatus was the same as that described in Chapter 2. The solutions were prepared in advance and presented as described in Chapter 2. Polycose (0.03 M) served as the tastant for the S1 generation, and sodium chloride (0.1 M), served as the taste CS for the S2 and S3 generations. The 0.009 M LiCl (Sigma, St. Louis, MO) solution was prepared with NaCl to adjust molarity when needed, to either 0.018 or 0.15 M.

Procedure. All rats were handled and weighed once a day throughout the experiment. Deprivation state. Following several days of handling, all rats were placed on a water deprivation regimen in which they were given 5 min access to dH₂O on the front of the cage each morning and 1 h access each afternoon to maintain proper hydration. All rats were kept on this daily regimen until morning intake stabilized. Conditioning. During conditioning, the rats were given 5 min access to either 0.03 M Polycose (for S1) or 0.1 M NaCl (for S2 and S3), and after a 5 min ISI, injected intraperitoneally (ip) with either 0.009 M (for S1) or 0.018 M LiCl (for S2 and S3). The LiCl concentration was increased from 0.009

to 0.018 in the 2nd and 3rd generations to reduce intake further for the females and the male small suppressers. During tests of the S1 generation using 0.009M LiCl, all the females and the male small suppressers failed to reduce CS intake significantly. Thus, we needed a higher concentration that was sufficient to induce suppression in the groups that did not respond to the lower 0.009 M concentration. There was one such CS – US pairing every other day for a total of 6 pairings. There were two additional pairings for S1 and one additional pairing for S2 and S3 using 0.15 M LiCl, followed by a final CS only test. All the rats were given 5 min access to water each morning on the days between conditioning trials and 1 h every afternoon to rehydrate.

Experiment 2 Results: First Selectively Bred Generation (S1)

Polydose (CS) Intake. Figure 6.13 shows Polydose intake following pairings with LiCl for males (left panel) and for females (right panel). The male large suppresser line demonstrated the quickest and strongest CTA over trials compared with the male small suppresser line and with females. Post hoc comparisons of a significant Gender x Lineage x Trial interaction, $F(8,88)=2.92$, $p<0.05$, confirmed that Polydose intake for the male large suppresser line was significantly reduced relative to that of the small suppresser line on Trials 6 and 7 and that of the female large suppresser line on Trial 7, $p_s<0.05$. This difference diminished, however, when the higher LiCl dose was administered, reducing intake for all the groups to under one ml. Females were more resistant to the suppressive effects of the 0.009 M dose of LiCl, regardless of which line they came from. Although there was a tendency for the female small suppresser line to exhibit more suppression than the large suppresser line, this effect was not significant on any of the trials, $p_s>0.05$.

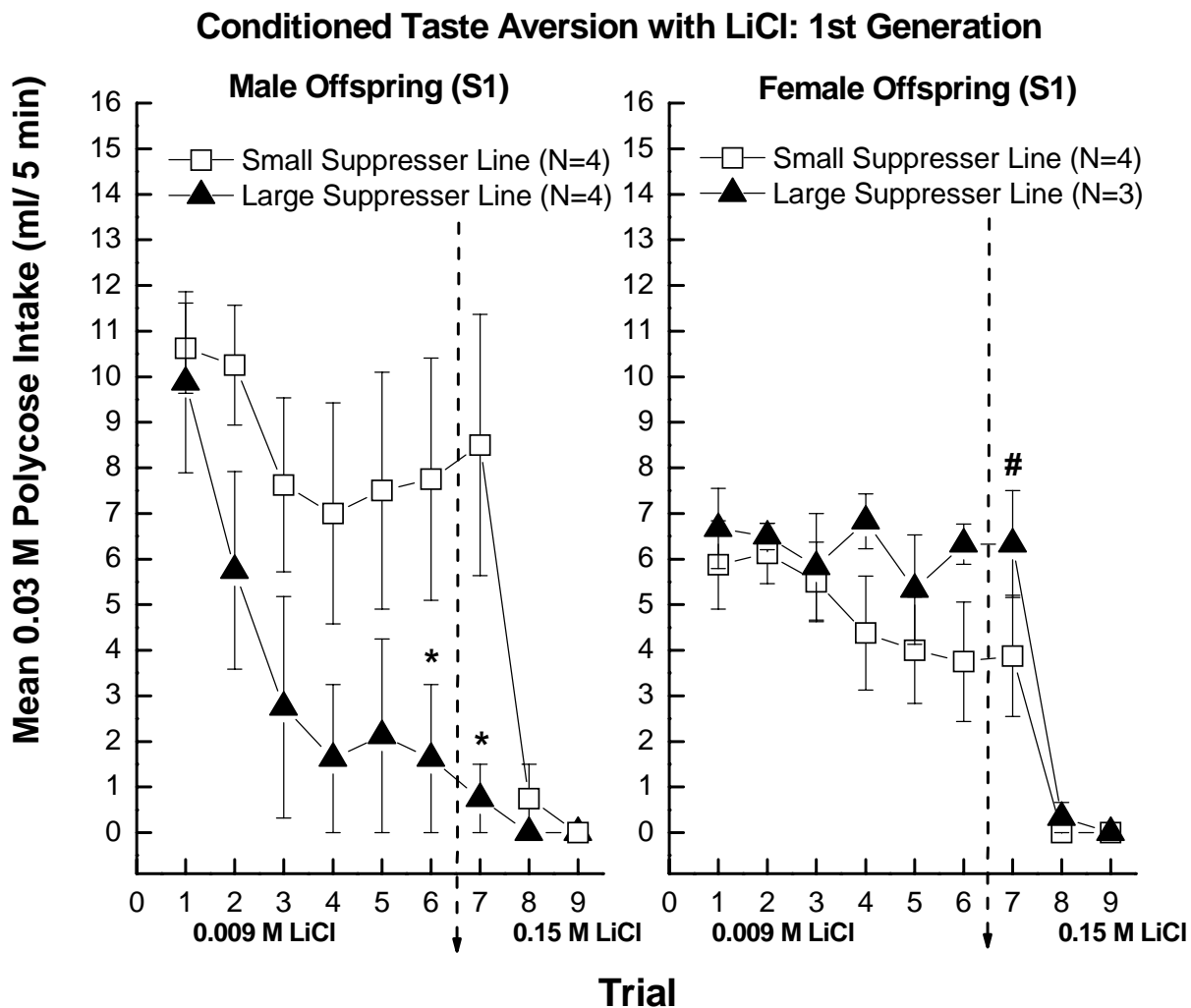


Figure 6.13. Mean (\pm SEM) intake of 0.03 M Polycose (ml/5 min) when paired with 0.009 M LiCl (trials 1-6) and 0.15 M LiCl (trials 7 and 8) followed by one CS only test trial. Data for the first generation of selectively bred male small and large suppressors are shown in the left panel and data for females are shown in the right panel. * Different from small, #different from male large suppressors, $p < 0.05$.

Results: Second Selectively Bred Generation (S2)

NaCl (CS) Intake. Figure 6.14 shows NaCl intake following pairings with LiCl for males (left panel) and for females (right panel). A salt solution was used in this experiment so that the CS would not generalize to the saccharin CS. In the first generation, the large suppresser line was more sensitive to the suppressive effects of LiCl and this may have been due to generalization. Despite this manipulation, the male large suppresser line still was more sensitive than all the other groups to the suppressive effects of LiCl. The intake data were analyzed using a 2 x 2 x 8 repeated measures ANOVA varying gender (male or female), lineage (small or large) and trial (1-8). Although the 3-way interaction was not significant, $F(7,3.3)=1.5$, $p>0.05$, the main effect of Lineage, $F(1,22)=14.4$, $p<0.05$, Trial, $F(7,154)=70.7$, $p<0.05$, and the Lineage x Trial interaction, $F(7,154)=4.8$, $p<0.05$, were significant. That is, both males and females in the large suppresser line were more sensitive than those in the small suppresser line to the suppressive effects of LiCl. Although the main effect of Gender was not significant, $F<1$, the Gender x Lineage and the Gender x Trial interactions were ($F(1,22)=6.0$, $p<0.05$; $F(7,154)=4.8$, $p<0.05$, respectively). In other words, males (especially the large suppresser males) were more sensitive to the suppressive effects of LiCl than were females. Next, intake data were analyzed separately for males and females using a 2 x 8 repeated measures ANOVA varying lineage (large and small) and trial (1-8). Male NaCl intake. The S2 large suppresser line, as in the S1 population, demonstrated greater CTA over trials than the small suppresser line. This conclusion was supported by a significant main effect of

Lineage, $F(1,8)=27.4$, $p<0.05$, Trial, $F(7,56)=40.6$, $p<0.05$, and Lineage x Trial interaction, $F(7,56)=4.3$, $p<0.05$. Indeed, post hoc comparisons of this 2-way interaction revealed that NaCl intake was significantly different for the two groups by Trial 2 and remained different through Trial 6, $p_s<0.05$. This difference was eliminated by use of the higher LiCl dose (0.15 M), reducing intake for both groups to less than one ml. Female NaCl intake. Females of the S2 generation, like females of the S1 generation, were less sensitive than males to the effects of LiCl as indicated by little difference between the small and the large suppressing lines. Indeed, the results of a similar 2 x 8 ANOVA confirmed that female large and small suppressers behaved alike as neither the main effect of Lineage, $F(1,14)=1.0$, $p>0.05$, nor the Lineage x Trial interaction, $F(7,98)=1.3$, $p>0.05$, were significant.

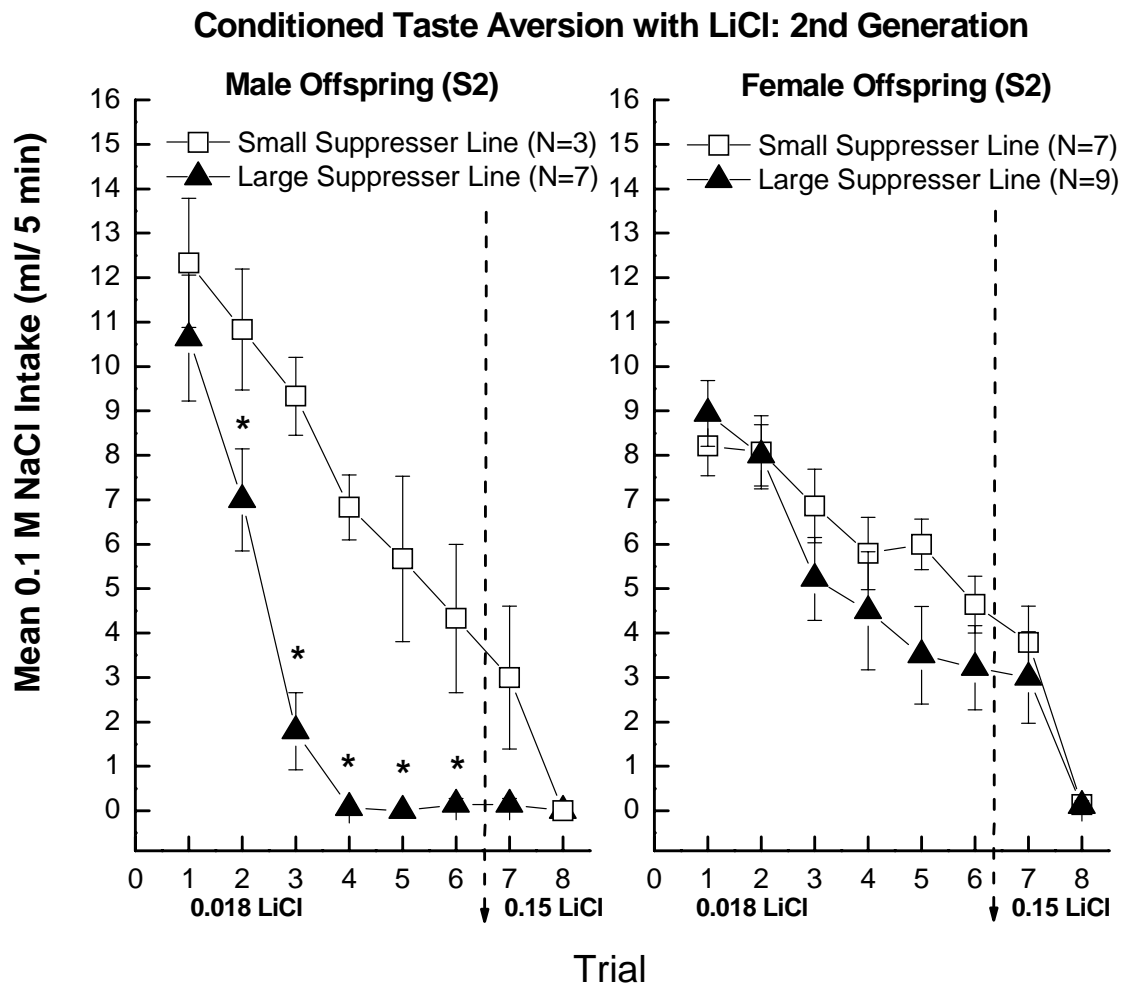


Figure 6.14. Mean (\pm SEM) intake of 0.1 M NaCl (ml/5 min) when paired with 0.018 M LiCl (trials 1-6) and 0.15 M LiCl (trial 7) followed by one CS only test trial. Data for the second generation of selectively bred male small and large suppressors are shown in the left panel and data for females are shown in the right panel. * Different from small, $p < 0.05$.

Results: Third Selectively Bred Generation (S3)

NaCl (CS) Intake. Figure 6.15 shows NaCl intake following pairings with LiCl for males (left panel) and for females (right panel). The males of the S3 generation, like the males of the previous generations, again appeared to be more sensitive to the effects of LiCl than females. Support for this conclusion was provided by a significant Gender x Trial interaction, $F(7,161)=8.6$, $p<0.05$, following a 2 x 2 x 8 repeated measures ANOVA varying gender (male or female), lineage (small or large) and trial (1-8). Neither the main effect of Gender, $p>0.05$, nor the Gender x Lineage interaction, $F<1$, however, were significant. A significant main effect of Lineage, $F(1,23)=5.8$, $p<0.05$, indicated that overall intake for the large suppressing line was lower than that for the small suppressing line. Neither the Lineage x Trial nor the 3-way interactions were significant, $ps>0.05$.

Male NaCl intake. Results of a 2 x 8 repeated measures ANOVA confirmed that the male large suppresser line, collapsed over trials, was more sensitive to the effects of LiCl than the small suppresser line. This conclusion was supported by a significant main effect of Lineage, $F(1,15)=5.4$, $p<0.05$. The 2-way interaction, however, did not attain significance, $F(7,105)=1.2$, $p>0.05$.

Female NaCl intake. The results of a similar 2 x 8 ANOVA showed that the female small and large suppresser lines, unlike the male lines, did not exhibit differences in NaCl consumption, as indicated by a nonsignificant main effect of Lineage, $F(1,8)=1.6$, $p>0.05$, and Lineage x Trial interaction, $F<1$.

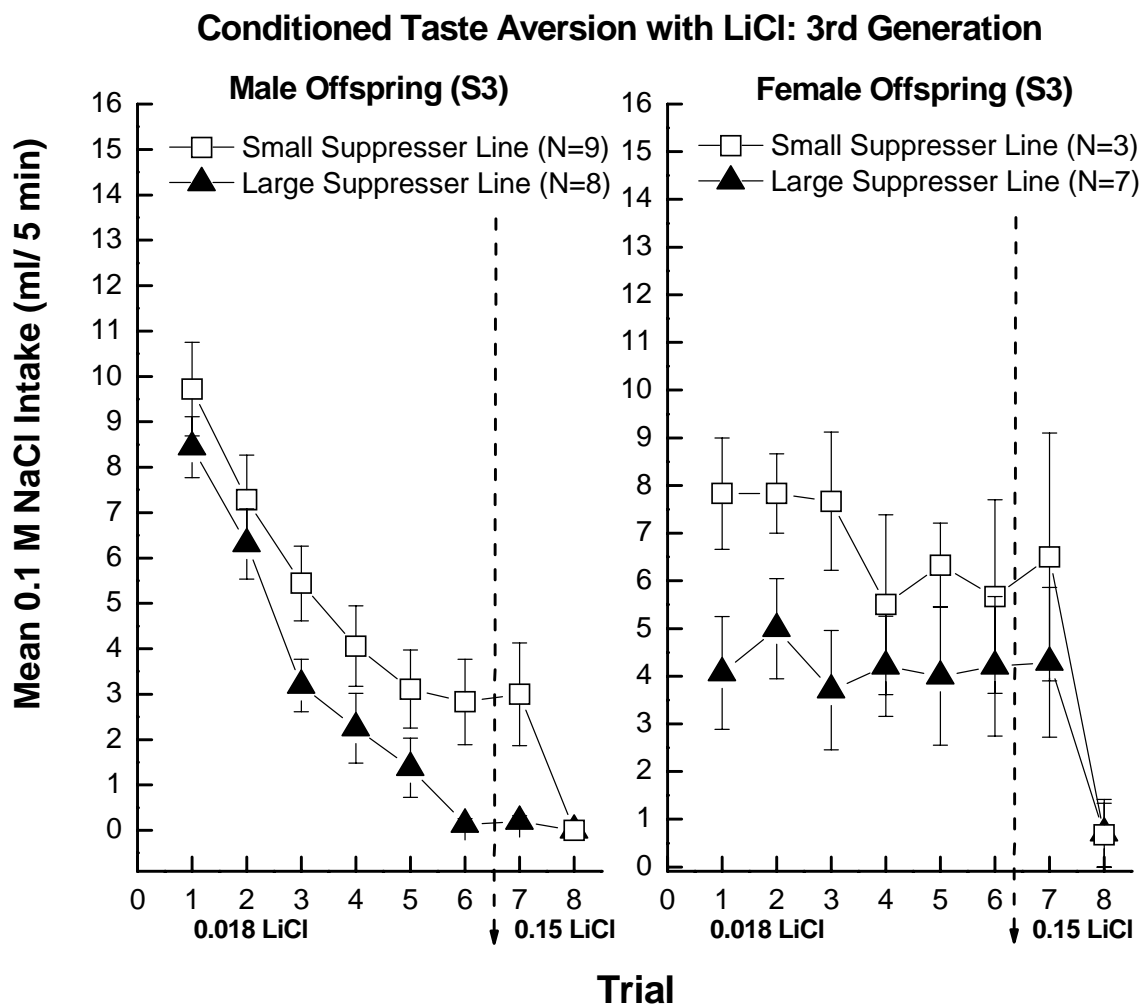


Figure 6.15. Mean (\pm SEM) intake of 0.1 M NaCl (ml/5 min) when paired with 0.018 M LiCl (trials 1-6) and 0.15 M LiCl (trial 7) followed by one CS only test trial. Data for the third generation of selectively bred male small and large suppressors are shown in the left panel and data for females are shown in the right panel.

Comparing All Generations. Figure 6.16 shows mean terminal CS intake for each generation. Only population means were graphed since the breeders for the S1 generation were no longer alive at testing. Furthermore, the S1 and S2 rats that were kept alive for CTA testing were, for the most part, the same rats used to breed the S2 and S3 populations, respectively. Data for males and females are presented separately. These graphs depict the overall trend in CTA learning for the small and large suppresser lines over generations. Data for males and females were analyzed separately using a mixed factorial ANOVA varying generation (S1, S2, and S3) and lineage (small and large) with Trial 7 CS intake as the dependent variable. Male populations. Collapsed across trials, the small suppresser line was less sensitive than the large to the suppressive effects of LiCl across all 3 generations. This conclusion was supported by a significant main effect of Lineage, $F(1,29)=6.4$, $p<0.05$. By the second and third generations, however, the difference in intake between the two lines became smaller as the small suppresser line became more sensitive to the effects of LiCl. A significant main effect of Generation, $F(2,29)=3.7$, $p<0.05$, indicated that overall Trial 7 CS intake went down significantly over generations and this drop was carried largely by the small suppresser line. Indeed, post hoc comparisons of a significant Lineage x Generation interaction, $F(2,29)=12.26$, $p<0.05$, revealed that the two lines were significantly different from each other only in the first generation, $p<0.05$. Female populations. The female small and large suppressing lines, on the other hand, did not show any differences in CTA learning over generations. This conclusion was supported by a non-significant

main effect of Lineage, $F < 1$, Generation, $F(2,27) = 1.3$, $p > 0.05$, and Lineage x Generation interaction, $F(2,27) = 1.05$, $p > 0.05$.

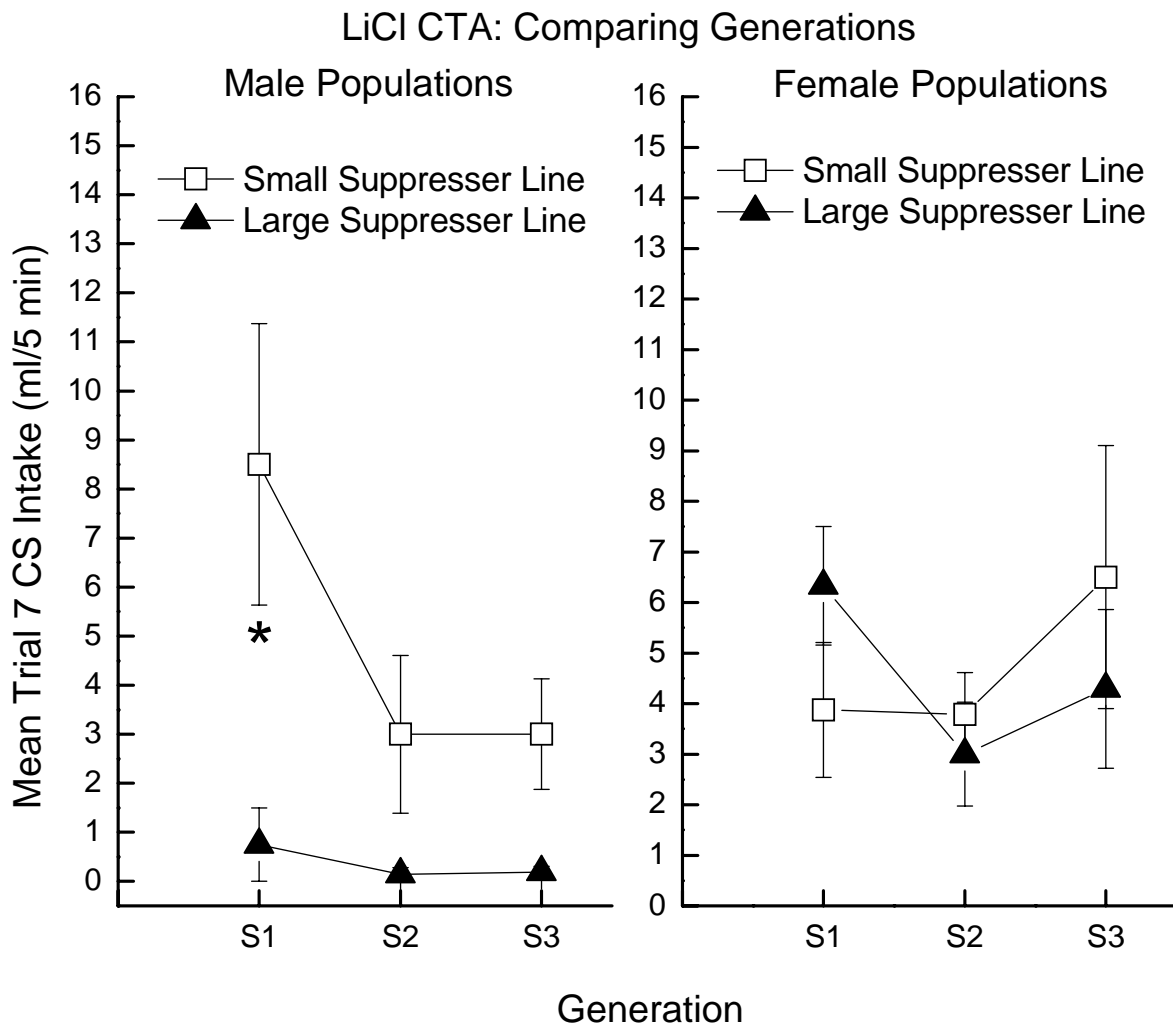


Figure 6.16. Mean Trial 7 CS intake is shown for each male population (S1, S2, and S3) in the left panel and for each female population in the right panel.

Discussion

The primary objective in selectively breeding small and large suppressers in the passive reward comparison design was to determine the extent to which the trait, cocaine-induced avoidance of saccharin intake, is influenced by genetics. The results from this study suggest that there is, indeed, a major hereditary component affecting the behavior at hand. This conclusion was primarily supported by a significant divergence in the small and large suppresser lines by the second generation. Other selective breeding attempts have taken up to 30 or even 50 generations for significant separation to occur. These data suggest that the individual vulnerability to the suppressive effects of cocaine can be determined by a genetic predisposition for a greater sensitivity to the rewarding properties of a drug of abuse.

The results from this study also shed light on the nature of the genetic system influencing the behavior. Clues for determining whether the underlying genetic system is simple (i.e., 1 or 2 genes), or complex (i.e., many genes), come from the pattern of separation between the small and large suppresser lines (Plomin, DeFries et al. 2001). If the small and large suppresser lines had separated steadily over many generations, then many genes were probably involved with variation in the behavior. If, however, only one or two genes were responsible for the behavior, the two lines would separate after only a few generations and they would not diverge any further in later generations. In the present study, the latter pattern of separation was observed suggesting that

individual variability in cocaine-induced suppression of saccharin intake is mediated by a relatively simple genetic system.

In addition to evaluating saccharin intake responses following pairings with cocaine, the pre- and post-conditioning CORT responses to the presentation of the saccharin CS also was assessed. CORT was evaluated to determine whether patterns in saccharin intake still would be associated with patterns in CORT for selectively bred small and large suppressers. For the male, but not the female, population, greater avoidance of the saccharin cue was associated with greater CORT levels at test. Selectively bred females did not demonstrate a clear relationship between saccharin intake and CORT at test. This finding was not unexpected, however, because greater avoidance of saccharin intake had not yet been found to be associated with greater elevations in post-conditioning CORT even for outbred female Sprague-Dawley rats.

While patterns in post-conditioning CORT were consistent with past observations, patterns in pre-conditioning CORT appeared quite different by the second generation. Specifically, both male and female small suppresser offspring exhibited relatively high pre-conditioning CORT levels compared with the first generation. In past experiments (see Chapter 2), pre-conditioning CORT levels were not only lower than post-conditioning CORT levels, but also did not differ across groups (i.e, small, large, and saline). For both generations, the large suppresser line demonstrated the expected increase in plasma CORT from pre- to post-conditioning, but the small suppresser line did not. In fact, for the female

small suppresser line, there was actually a decrease in CORT from pre- to post-conditioning.

It is not clear why the small suppresser line demonstrated higher pre-conditioning CORT levels than the large suppresser line in the second generation. One possible explanation is the degree of neophobia to the novel saccharin CS experienced by each group. For example, the small suppresser line consistently consumed less of the novel taste cue than the large suppresser line. Neophobia probably does not explain sensitivity in cocaine-induced suppression of saccharin intake because significant differences between small and large suppressers on the saccharin pre-exposure day have not been observed in past experiments. Furthermore, it is difficult to tease apart whether increased neophobia was being 'bred' into the small suppressers and, in turn, causing the elevation in pre-conditioning CORT levels or, alternatively, increased sensitivity in the HPA axis was being 'bred' into the small suppressers and, in turn, causing increased neophobia. In any case, neither increased neophobia, nor increased pre-conditioning CORT levels, are likely the primary cause for greater avoidance of the saccharin cue.

One important issue in interpreting these data is determining what, specifically, was being 'bred' into or out of the two lines. While the goal was to breed differential sensitivity to the suppressive effects of cocaine into two distinct populations (i.e., the small and large suppressers), it remains unknown why rats suppress intake of saccharin when paired with the administration of cocaine. Determining the answer to this question is an essential component in assessing

what was being bred into each line. As discussed earlier, a great deal of evidence supports that it is the rewarding, rather than aversive, properties of cocaine that lead to devaluation (i.e., suppression of saccharin intake) of the naturally rewarding saccharin solution. There also is the possibility, however, that the propensity for better associative learning (e.g., conditioning) are driving differences in suppression of saccharin intake. Thus, three possible accounts of the data are differences in sensitivity to rewarding properties of the drug, aversive properties of the drug, and learning.

The results from the LiCl CTA experiments argue against a learning deficit or CTA account of the data. If learning differences or differential sensitivity to the aversive properties of cocaine were the underlying causes of small or large suppression in the present study, then one would expect the large suppresser line to demonstrate a larger LiCl CTA than the small suppresser line. Although this was the case in the first generation, this difference decreased in the second and third generations. Meanwhile, differences in CS intake increased over generations for the cocaine experiments. This trend may reflect certain traits being selected out as others are being selected in.

There are several possible reasons for why LiCl CTA differences between the two lines diminished over generations. First, the large suppresser line may have, initially, been more sensitive to the aversive effects of LiCl than the small suppresser line. If this were the case, it then becomes possible that the large suppresser line also were more sensitive to the aversive, rather than rewarding, properties of cocaine. This is probably not the case, however, because significant

differences between the two lines diminished when the higher dose (0.018 M) was implemented. A second reason is that the small suppresser line may have an associative learning deficit. If this were the case, however, it would only be true for males because female small and large suppressers exhibited no differences in their rate and magnitude of avoidance in any of the generations. Furthermore, the difference for males became smaller with each generation. The gradual coming together of the two lines also could be attributed to the higher 0.018 M dose being implemented in the second and third generations. This higher dose may have reduced intake further for the small suppresser line. Regardless, the possibility remains that a learning difference existed in the first generation and was being selectively bred out in the second and third generations. If so, then the diminished difference between the two lines was not caused by the higher dose of LiCl. In support of this idea, there still was a slight difference in CTA learning between the small and large suppresser lines in the second generation when the higher dose (0.018M) of LiCl was implemented.

One observation that held true for all three generations was that females tended to be less sensitive to the suppressive effects of LiCl than males. Females also were less sensitive to the suppressive effects of cocaine. Reasons for a similar pattern of resistance to the suppressive effects of both cocaine and LiCl are not clear but these findings are consistent with those from Chapter 5. As suggested in Chapter 5, female rats may protect their intake of food and fluids more than males. These findings also suggest a common mechanism for cocaine- and LiCl- induced avoidance of CS intake.

Although early separation of the small and large suppresser lines suggests a strong genetic component, other possible reasons for early separation should be addressed. For example, the environmental rearing style or maternal behavior also can account for differences in behavior. Because maternal behavior was not recorded in the present study, it is possible that large suppressing mothers attend to their litters in a different manner than small suppressing mothers. There is substantial evidence to support that differences in maternal behavior can affect the phenotype of developing and adult offspring (Gomez-Serrano, Tonelli et al. 2001; Walker, Deschamps et al. 2004; Zhang, Parent et al. 2004; Levine 2005). It is important to determine what the effects of cross-fostering would be on the outcome of small and large suppresser offspring. If the results were the same after cross-fostering, then the data would argue in favor of a strong genetic predisposition toward becoming a small or a large suppresser. On the other hand, if separation did not occur as quickly as observed in the present study, then the data would support that individual differences in cocaine-induced suppression of saccharin intake are caused, primarily, by post natal environmental factors.

There are several other issues that should be addressed in a separate study to bring forth a better interpretation of these data. For example, the genetic diversity and independence of breeders in the parent stock is largely unknown. Current practice in selectively breeding phenotypes is to use defined stock that would maximize genetic heterogeneity in the foundation stock (i.e., the N/Nih heterogeneous stock from an 8-way outcross). Animals also can be obtained

from several different breeding colonies to increase genetic diversity of the founding population. A second issue is the need for two independent replicate selection lines to allow for the strongest possible conclusions about potential genetic correlations. Replicate lines are important because they help control for random genetic drift and false associations (Henderson 1997). A third issue is sample size. There were, initially, 12 breeding pairs and by the second generation, there were only 3 or 4. Some problems that occurred as a result of low pregnancy rates were small populations, inbreeding, and reduced viability. An insufficient number of families also make it difficult to avoid confounding selection with litter/founder effects. All of these issues will need to be addressed.

Despite several flaws, the results of the present study provide a solid premise from which to attempt a second breeding study. As with any complex drug-related behavior, it is important to understand both the behavioral and the genetic determinants of that behavior. These data suggest a strong genetic component to cocaine-induced avoidance of saccharin intake. This finding opens doors to future studies designed to identify behavioral, endocrine, neurochemical, and genomic differences that may predict, early in life, which individuals would be more sensitive to the reinforcing properties of drugs of abuse. These future studies would facilitate a greater understanding of the complex interactions between genetic background and environmental changes during development, which most directly influence addictive behavior later in life.

Chapter 7

GENETIC BASIS OF INDIVIDUAL DIFFERENCES: A SURVEY AMONG INBRED MOUSE STRAINS

Chapter 6 showed that a greater propensity to avoid intake of a naturally rewarding saccharin solution following pairings with cocaine can be selectively bred into out-bred Sprague-Dawley rat populations. The present chapter adopts the opposite approach in exploring the genetic basis of reward comparison behavior. The experiments in Chapter 7 tests whether a greater predisposition to become a large or a small suppresser already exists among certain inbred mouse strains with known genetic backgrounds. One major advantage to comparing inbred, as opposed to outbred, strains is that the task of narrowing down which genes mediate reward comparison behavior can be accomplished more easily using genetically homogeneous populations of rodents. Four inbred strains of mice were tested in the passive taste-drug pairing design, making this the first strain survey of a behavior not yet characterized among mice. The data show that the C57BL/6Jbg (B6), DBA/2Jbg (D2), A/JJbg, and Sv129Jbg mouse lines exhibit substantial differences in avoidance of saccharin intake following pairings with cocaine.

Although this is the first survey of reward comparison behavior characterized in mice, it should be mentioned that two inbred rat strains, Lewis and Fischer 344, have previously been tested in our laboratory. In these experiments (Grigson and Freet 2000), we found that reward-preferring Lewis

rats are more sensitive than the less-preferring Fischer rats to the suppressive effects of cocaine. Ideally, these two strains could serve as promising candidates for genetic experiments designed to identify the number and chromosomal location of the genes which encode reward comparison behavior. As a model for genetic analysis, the mouse is better established than the rat and offers a better chance at identifying the genetic determinants of susceptibility to drug-induced devaluation of natural rewards.

There were several reasons for choosing B6 and D2 mice as two of the four strains to test in the reward comparison paradigm. Mice from the B6 and D2 strains have been known to differ greatly on many drug-related phenotypes. For example, while cocaine acts as an effective reinforcer in both strains, C57BL/6J mice acquire intravenous cocaine self-administration faster than DBA/2J mice (Grahame and Cunningham 1995). C57BL/6J mice also have been shown to SA greater amounts of cocaine not only when self-administering intravenously (Kuzmin and Johansson 2000), but also when cocaine is consumed orally (Morse, Erwin et al. 1993). Moreover, when compared with D2 mice, B6 mice consume greater amounts of other drugs of abuse, such as ethanol and morphine (McClearn and Rodgers 1959; Belknap, Crabbe et al. 1993; Belknap, Crabbe et al. 1993). Finally, Seale and Carney (1991) found that the DBA strain is markedly hyporesponsive to the rewarding actions of cocaine and the authors speculated that the DBA strain has an inherent generalized appetitive defect.

Although these strains have been tested across several drug-related designs, this is the first time that both strains were simultaneously characterized

in the reward comparison design. There is only one other report of how mice behave when tested in the taste-drug pairing design. In this study, Risinger and Boyce (2002) found that, in DBA/2J mice, conditioned taste avoidance induced by morphine or cocaine occurs in a similar pattern with that observed for rats. Furthermore, these data supported the reward comparison hypothesis by showing that greater avoidance occurs with a more preferred saccharin cue than with a neutral salt cue. The DBA/2J strain, however, was the only strain tested by Risinger and Boyce. The main reason for testing two additional strains in the present study, Sv129lbg and A/Jlbg, was for comparison since drug responses for these strains are not as well characterized as they are for D2 and B6 mice. A second experiment examined the effect of strain on intake of the saccharin solution across a range of concentrations in D2, B6, and Sv129 mice. Mice from the A/J strain were not tested in Experiment 2.

Methods: Experiment 1

Subjects and Apparatus (also see Table 2). This experiment was conducted in two replications. There were a total of four inbred mouse strains tested in this study: C57BL/6lbg, DBA/2lbg, A/Jlbg, and Sv129lbg. Collectively, there were 89 naïve, male, mice (provided by the Institute for Behavioral Genetics, Boulder, CO) that were between two and three months of age at the beginning of the experiment. They weighed between 15 and 25 g throughout the experiment. After four days of being group housed, all mice were individually housed in stainless steel bread-pan cages lined with woodchip bedding in a temperature and humidity controlled animal care facility with a 12:12 hr light dark cycle (lights on at 7 am). The experiment was conducted in home cages from 9:00 – 11:00 am. Bedding was replaced as needed. Solutions were presented in inverted graduated cylinders with stainless steel spouts protruding into the home cage. Intake was measured to the nearest one tenth ml.

		C57	DBA	A/J	Sv 129
Rep 1	Saline	2	1	2	n/a
	10 mg	4	4	4	n/a
	30 mg	4	4	4	n/a
		10	9	10	
Rep 2	Saline	3	3	3	3
	10 mg	4	3	3	3
	20 mg	8	7	7	4
	30 mg	4	1	1	3
		19	14	14	13
TOTAL combined	Saline	5	4	5	3
	10 mg	8	7	7	3
	20 mg	8	7	7	4
	30 mg	8	5	5	3
		29	23	24	13

Table 2. This table summarizes the number and strain of mice used for each drug group in Experiment 1. The group numbers are listed separately for Replication 1 and Replication 2 with combined totals shown at the bottom.

Procedure. Handling & weights: Mice were not handled prior to the beginning of the experiment. Handling occurred when weights were taken every other afternoon before an injection day. Weights for all of the mice remained stable throughout the course of the experiment. Water deprivation and training. All mice were first allowed to acclimate to the new environment continuously (24 hr/ day) over 1 – 10 days. All mice were then placed on a fluid deprivation schedule for 6 days in which they were given 1 hour access to water in the morning (9-10am) and 2 hours in the afternoon (1-3pm) to re-hydrate until morning intake stabilized. Conditioning. There was no CS pre-exposure day in this experiment. Each conditioning trial consisted of one taste-drug pairing using a 0.15% saccharin solution (CS) paired with a 10, 20, or 30 mg/kg dose of cocaine (US), or an equivalent volume of saline. All injections were administered intraperitoneally (ip). Groups (saline, 10 mg/kg, 20 mg/kg, and 30 mg/kg) were determined in a random manner and matched for average morning water intake (also see Table 1 above). Thus, each animal had access to the 0.15% saccharin CS for 1 hour in the morning and then was injected immediately thereafter. There were 5 taste-drug pairings occurring every other day. On the days between conditioning all mice were given 2 hr access to water in the afternoon.

Tastants and Drugs. Sodium Saccharin was dissolved in distilled water overnight and presented at room temperature. Cocaine HCl was dissolved in sterile saline and syringes were filled the morning of injections. Drug was generously provided by Dr. Gene Erwin of the UCHSC and by NIDA.

Experiment 1: Results

All data were analyzed using Statistica using a mixed factorial ANOVA. Post hoc tests were conducted, when appropriate, using Newman Keuls tests, with alpha set at 0.05.

Saccharin (CS) intake. The main effect of Replication was not significant, $F(1,37)=1.49$, $p>0.05$, following a multifactorial 2 x 3 x 3 x 5 repeated measures ANOVA varying replication (2), drug (saline, 10 mg, 30 mg), strain (C57, DBA, A/J), and trial (1-5). Therefore, the data from the first and second replications were combined, analyzed, and presented accordingly.

Mean saccharin intake data for each strain across trials 1-5 are shown in Figure 7.1. The data were first analyzed using a 4 x 4 x 5 overall ANOVA varying drug (saline, 10, 20, 30 mg), strain (C57, DBA, A/J, Sv129), and trial (1-5). Although the main effect of Strain was not significant, $p>0.05$, the results of this analysis did reveal a significant main effect of Drug, $F(3,74)=4.63$, $p<0.05$, and Trial, $F(4,296)=2.50$, $p<0.05$, indicating that saccharin intake decreased as a function of increasing dose and across trials, as expected. There were no significant two or three way interactions in this analysis, $p_s>0.05$. Next, intake data were analyzed separately for each strain using a 2-way ANOVA varying drug dose and trial. For the C57 strain, Newman Keuls post hoc comparisons of a significant Dose x Trial interaction, $F(12,100)=17.43$, $p<0.05$, showed that saccharin consumption by mice injected with a 10 mg/kg dose of cocaine did not differ from consumption by mice injected with saline, $p_s>0.05$. The 20 mg/kg dose resulted in a significant decrease in saccharin intake compared with both

the saline and 10 mg/kg groups by Trial 4 and remained different through Trial 5, $p < 0.05$. Finally, the 30 mg/kg dose resulted in a sharp and significant decrease in saccharin consumption by Trial 3 that remained significantly lower than that of all other groups through Trial 5, $p < 0.05$. The DBA strain demonstrated greater avoidance of the taste cue than the C57 strain when using the 20 and 30 mg/kg doses. This conclusion was supported by post hoc comparisons of a significant Drug x Trial interaction, $F(12,80)=12.51$, $p < 0.05$, that indicated a significant difference in saccharin intake by Trial 3 for the 20 mg/kg group and by Trial 2 for the 30 mg/kg group compared with both the saline and the 10 mg/kg groups, $p < 0.05$. The saline and 10 mg/kg groups did not differ on any of the Trials, $p > 0.05$. The 20 and 30 mg/kg groups were different only on Trial 3, $p < 0.05$, after which, responding by both groups fell to near zero ml. Differences between groups within the A/J strain did not attain significance nor was there a significant main effect of Trial, $p > 0.05$. The suppressive effects of cocaine occurred in mice from the Sv129 strain, however, when using the 20 and 30 mg/kg doses of the drug. Support for this conclusion was provided by post hoc comparisons of a significant Drug x Trial interaction, $F(12,36)=4.22$, $p < 0.05$. The results of the post hoc analyses showed that, again, there were no significant differences between the saline and the 10 mg/kg groups, $p > 0.05$. Saccharin intake dropped quickly for both the 20 and 30 mg/kg groups, becoming significantly different from the saline and 10 mg/kg groups by Trial 2, $p < 0.05$. Saccharin intake for the 30 mg/kg group was near zero ml by the Trial 2 and significantly lower than that of

the 20 mg/kg group on Trials 2 and 3, $p < 0.05$, until both groups demonstrated near complete avoidance of the taste cue on Trials 4 and 5.

Saccharin Intake (Intake:Weight Ratios). Body weights for the Sv129 mice tended to be higher than for the other strains. Sv129 mice also were the most sensitive to the suppressive effects of cocaine. This observation raises the possibility that body weight is somehow affecting drug-induced suppression of CS intake. To test this hypothesis, the intake data were converted to Intake:Body weight ratios and these data were subject to analyses. Figure 7.1b shows mean intake:body weight ratios for each strain of mice across Trials 1-5. The data were analyzed using a 4 x 4 x 5 overall ANOVA varying drug (saline, 10, 20, 30 mg), strain (C57, DBA, A/J, Sv129), and trial (1-5). The results of this analysis did not change the interpretation of the data. Thus, body weight did not significantly contribute to strain differences in cocaine-induced suppression of saccharin intake.

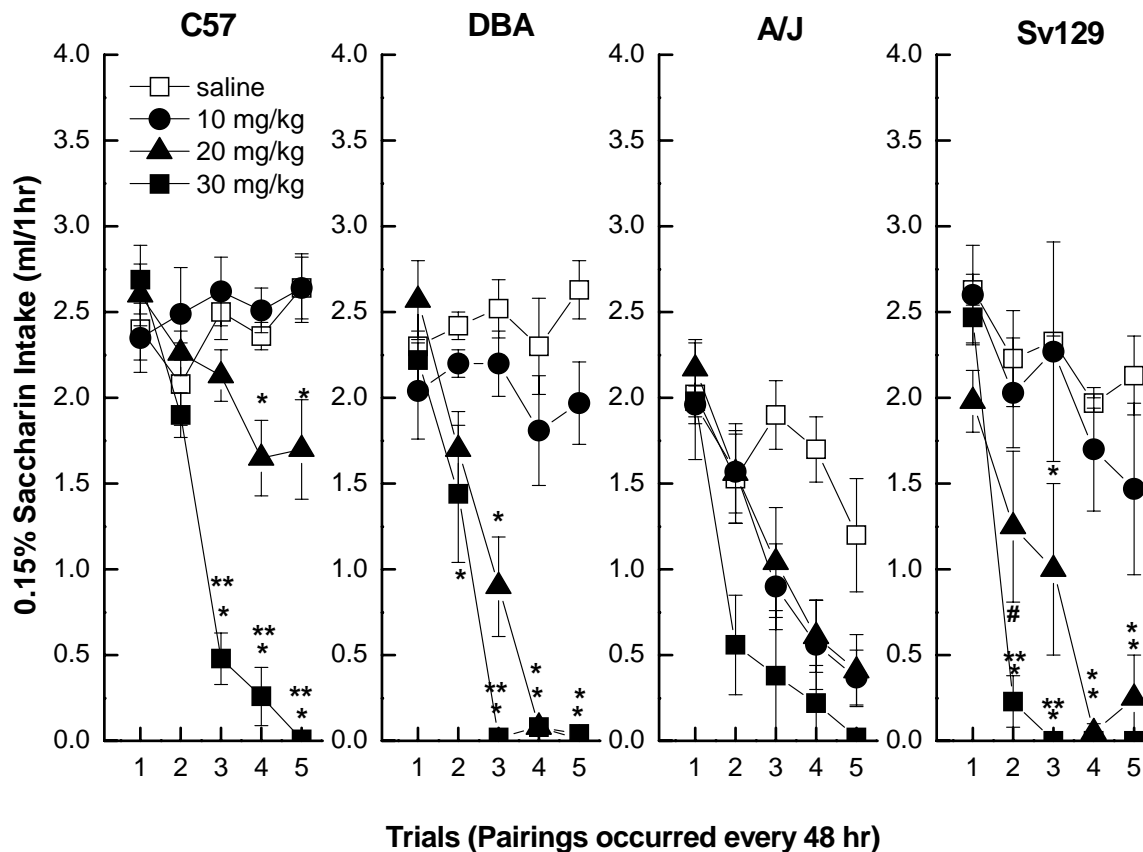


Figure 7.1a. Mean (\pm SEM) intake of 0.15% saccharin (ml/1 hr) across five pairings with either saline or cocaine (10, 20, or 30 mg/kg). Data for each of the four strains (C57, DBA, A/J, and Sv129) are shown separately in across the four panels. * Different from saline and 10 mg/kg, ** different from 20 mg/kg, # diff from saline only $p < 0.05$.

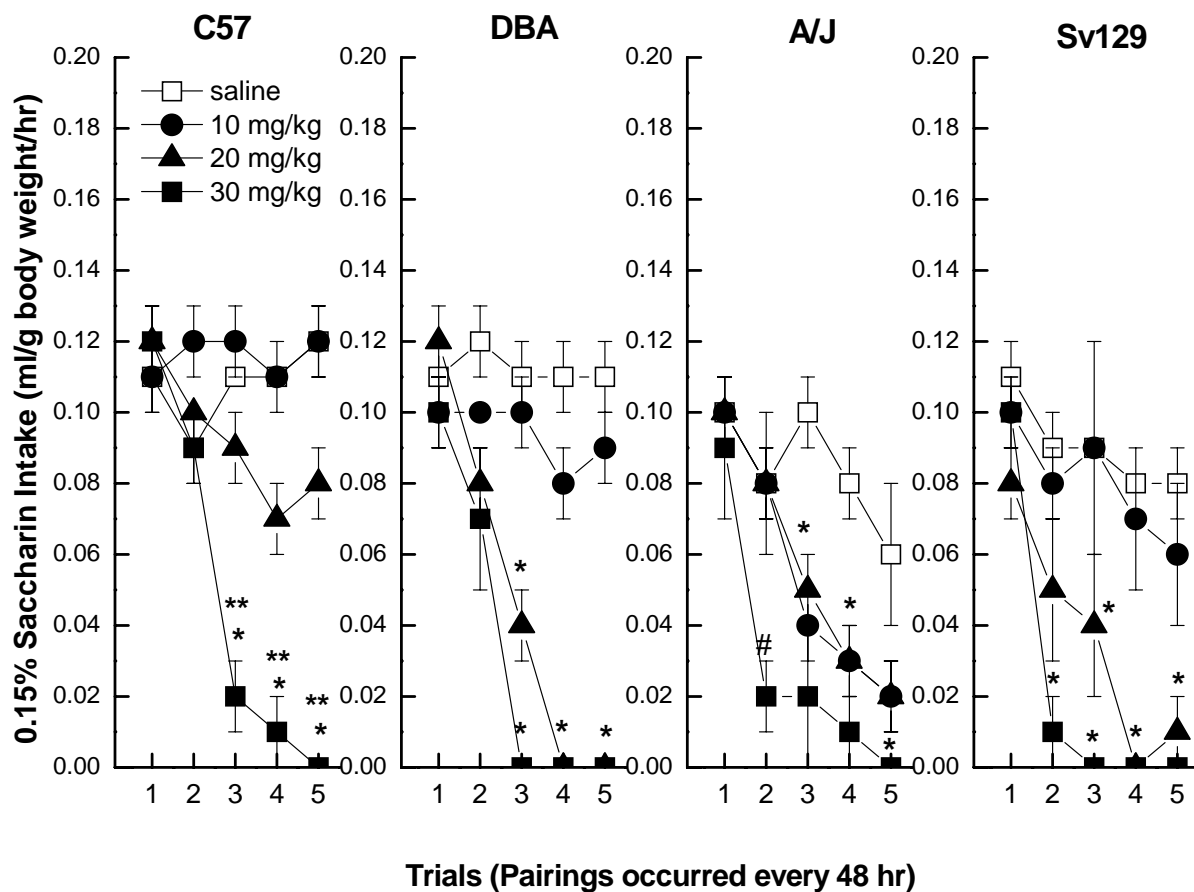


Figure 7.1 b. Mean (\pm SEM) intake of 0.15% saccharin (ml/g body weight/1 hr) across five pairings with either saline or cocaine (10, 20, or 30 mg/kg). Data for each of the four strains (C57, DBA, A/J, and Sv129) are shown separately in across the four panels. * Different from saline and 10 mg/kg, ** different from 20 mg/kg, # diff from saline, 10, and 20, $p < 0.05$. For A/Js * different from saline only.

The results of this experiment showed that mice, like rats, will suppress intake of a saccharin CS that is paired with the administration of cocaine. Suppression of CS intake was dose-dependent with strain differences occurring when using the 20 and 30 mg/kg doses but not when using the 10 mg/kg dose. The Sv129 strain was the most sensitive of all strains to the effects of cocaine, followed closely by the DBA strain, leaving the C57 strain as the least sensitive, at all doses. Saccharin intake by saline-treated A/J mice was reduced suggesting a generalized anxiety or stress response to the injection itself. Indeed, several studies have shown mice from the A/J strain to be one of, if not the most, anxious when placed on an elevated plus maze, tested in a light/dark exploration box, or in a novel object exploration task (Trullas and Skolnick 1993; Mathis, Paul et al. 1994; van Gaalen and Steckler 2000).

Originally, it was predicted that C57 mice would develop stronger cocaine-induced avoidance of saccharin than DBA mice. This prediction was based on findings showing that C57 mice are more sensitive to the rewarding properties of cocaine than DBA mice. The C57 mice were less sensitive, however, than both DBA and Sv129 mice to the suppressive effects of cocaine. Cocaine-induced suppression of CS intake, however, depends not only upon the response to the cocaine US, but also upon the response to the sweet-tasting CS (Grigson 1997). In Experiment 1, saccharin intake did not appear to differ across strains, but these mice were water deprived which can, augment intake. Experiment 2, then, revisited this issue under conditions of food-, rather than water-, deprivation.

Experiment 2

The goal of the next experiment was to characterize saccharin concentration-response functions across different strains of mice. Mice from the C57, DBA, and Sv129 strains were used in this experiment. Mice from the A/J strain were not available for us but given the observation that A/J mice suppress intake of saccharin when paired with saline, reasons for suppression in the A/J strain are probably not due to reward comparison effects. Body weight and water intake data also are presented for this experiment.

Methods: Experiment 2

Subjects. A total of 22 naïve, male, mice were run in this study. There were 9 DBA, 9 C57, and 4 Sv129 mice, weighing between 20 and 27 g at the beginning of the experiment. All mice were housed as described for Experiment 1.

Procedure. Handling. Weighing and handling began on the same day that mice were separated and individually housed. All mice were weighed and handled every afternoon throughout the course of the experiment. Food Deprivation. Food deprivation consisted of a once a day feeding in the late afternoon after weights were taken. Mice were gradually brought down to 85% of their free-feeding weight over the course of 2 weeks. Access to fluid was unlimited throughout the experiment. Saccharin Administration. The saccharin concentration response was taken over two separate series of presentations. In the first series, only the DBA and the C57 strains were used. Five concentrations

(0.05%, 0.10%, 0.15%, 0.20%, and 0.25%) of saccharin were presented in ascending order over five consecutive days. There were two 30 min access periods (one in the morning and one in the afternoon) for each concentration so that averages could be calculated for better accuracy. Solutions were presented in inverted graduated cylinders with stainless steel spouts protruding into the home cage. Intake was measured to the nearest one tenth ml. The second series was conducted like the first except for the following modifications. Sv129 mice were added to the experiment and all mice were limited to one 30 min access period a day that took place in the afternoon. Furthermore, concentrations were presented in ascending order in increments of 0.05% beginning with 0.10% up to 0.45%, after which, each concentration was presented once more in descending order.

Tastants. The tastants were the same as those described for Experiment 1.

Experiment 2: Results

Concentration Response Function. The data were initially analyzed using a 2 x 2 x 4 mixed factorial ANOVA varying replication (1 and 2), strain (C57 and DBA), and concentration (0.10%, 0.15%, 0.20%, and 0.25%). The results of this analysis showed that the main effect of Replication was significant, $F(1,32)=334.5$, $p<0.05$, but primarily because the C57 mice ingested more saccharin in the second, compared with the first, replication (see discussion below). The replication factor did not meaningfully interact with any other factor and as a result, the data were combined for the following analysis.

The combined intake data were analyzed using a 3 x 8 mixed factorial ANOVA varying strain (C57, DBA, and Sv129) and concentration (0.10%, 0.15%, 0.20%, 0.25%, 0.30%, 0.35%, 0.40%, 0.45%). Regardless of the concentration presented, mice from the C57 strain consumed greater amounts of saccharin than mice from the DBA or Sv129 strain (see Figure 7.2). This conclusion was supported by both a significant main effect of Strain, $F(2,18)=151.47$, $p<0.05$, and by post hoc tests of a significant Strain x Concentration interaction, $F(14,126)=3.78$, $p<0.05$. Specifically, post hoc comparisons of this significant interaction confirmed that saccharin intake for C57 mice was significantly greater than that for DBA and Sv129 mice at 0.10 - 0.45% saccharin, $p_s<0.05$. The results from a 2-sample t-test (Origin) confirmed that C57 mice consumed more saccharin than DBA mice at the 0.05% concentration as well, $p<0.05$ (this concentration was presented in the first round but not in the second).

The results showed that C57 mice consumed greater amounts of saccharin than DBA and Sv129 mice. This finding is consistent with those showing that C57 mice have relatively high preferences to sweet substances (Bachmanov, Tordoff et al. 1996; Bachmanov, Tordoff et al. 2001). On the other hand, the DBA and Sv129 mice consumed relatively little of the saccharin solution, regardless of concentration. Overall saccharin intake was lower in the present experiment than in the first experiment for all three strains. This reduced consumption is probably due to different deprivation states. The mice were fluid deprived in the Experiment 1 and food deprived in the present experiment. It is important to note the initial consumption of the novel saccharin CS was not only greater, overall, when fluid deprived but initial consumption also was similar among the 3 strains in the first experiment. When food deprived in the second experiment, overall consumption was not only lower, but also highly variable among the 3 strains. Fluid deprivation in the first experiment may have increased the motivation to drink a saccharin solution while unlimited access to water (24 hr) in the second experiment likely resulted in a decreased motivation to drink the saccharin solution. Even so, consumption of saccharin increased gradually with increasing concentration. A second possible explanation for strain differences in saccharin intake is body weight differences. This issue is addressed in the next section.

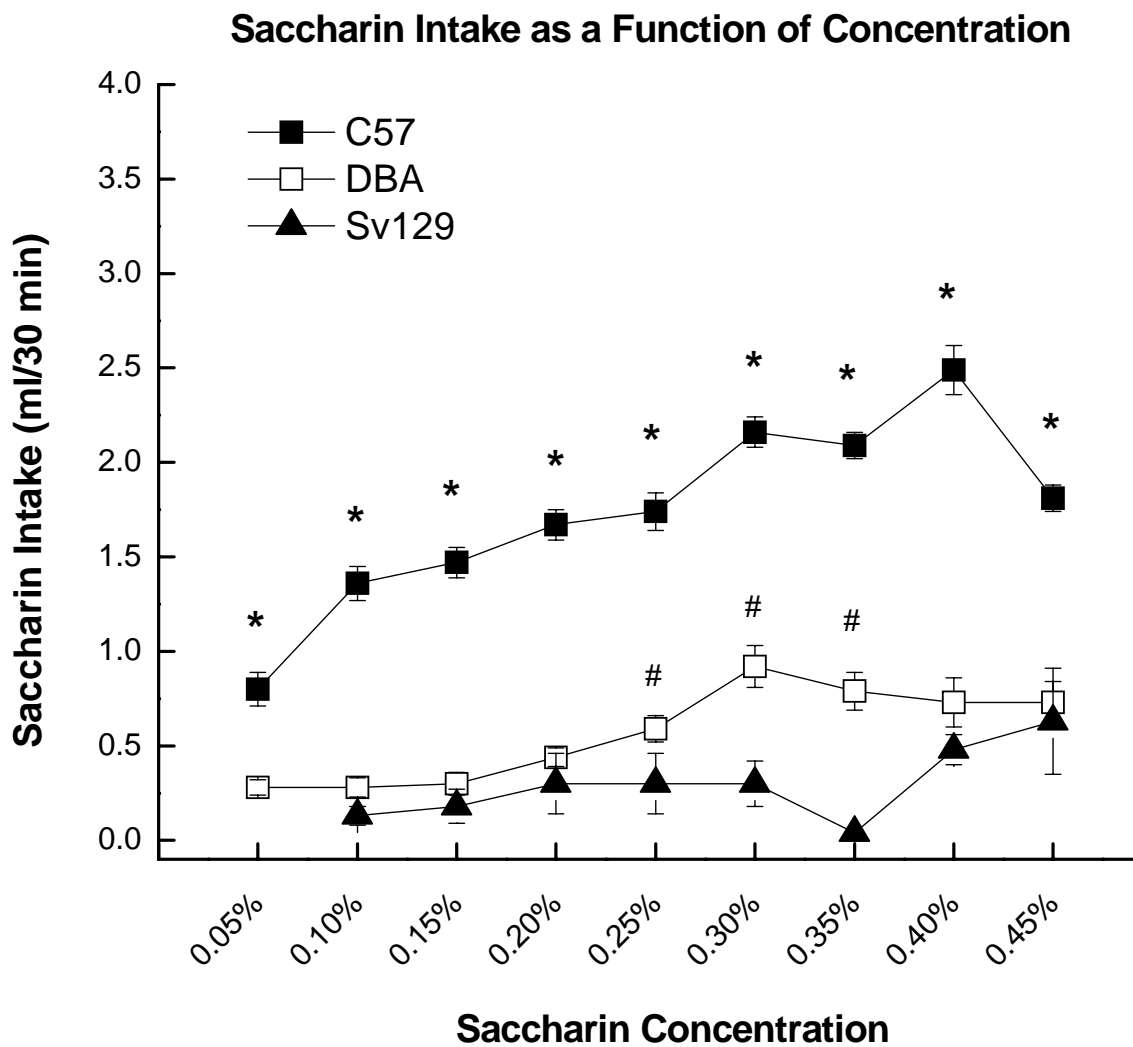


Figure 7.2. Mean (+/- SEM) intake of saccharin (ml/30 min) across nine concentrations of saccharin. Data are shown for three inbred mouse strains (C57, DBA, and Sv129). * Different from DBA and Sv129, #diff from Sv129 only $p < 0.05$.

24 hr Water Intake. The mean water intake (ml/24 hr) data for the C57, DBA, and Sv129 strains are shown in Figure 7.4. Measurements were taken for 10 days during the 2nd replication of this experiment and the data were analyzed using a 3 x 10 mixed factorial ANOVA varying strain and day (1-10). Twenty four hr water intake over trials was greatest for the DBA strain with little difference in intake between the C57 and the Sv129 strains. This conclusion was supported by a significant main effect Strain, $F(2,19)=25.6$, $p<0.05$, and a significant Strain x Day interaction, $F(18,171)=3.89$, $p<0.05$. Post hoc comparisons of this interaction revealed that water intake by the DBA mice was significantly greater than that by C57 and Sv129 mice on days 1-10, $p<0.05$. Intake by the latter two strains differed only on days 4, 8, 9, and 10, with C57 mice drinking slightly more than Sv129 mice, $p<0.05$.

There are two possible explanations for why 24 hr water intake was greater in DBA mice than in C57 or Sv129 mice. First, the differences may reflect differences in body weight. This issue is addressed in the next section. Second, the DBA mice may have been consuming more water to compensate for their low saccharin intake. If this were the case, it would be expected that 24 hr water intake for Sv129 mice, who also consumed very little saccharin, would be similar to that of DBA mice, given there are no weight differences among the three strains.

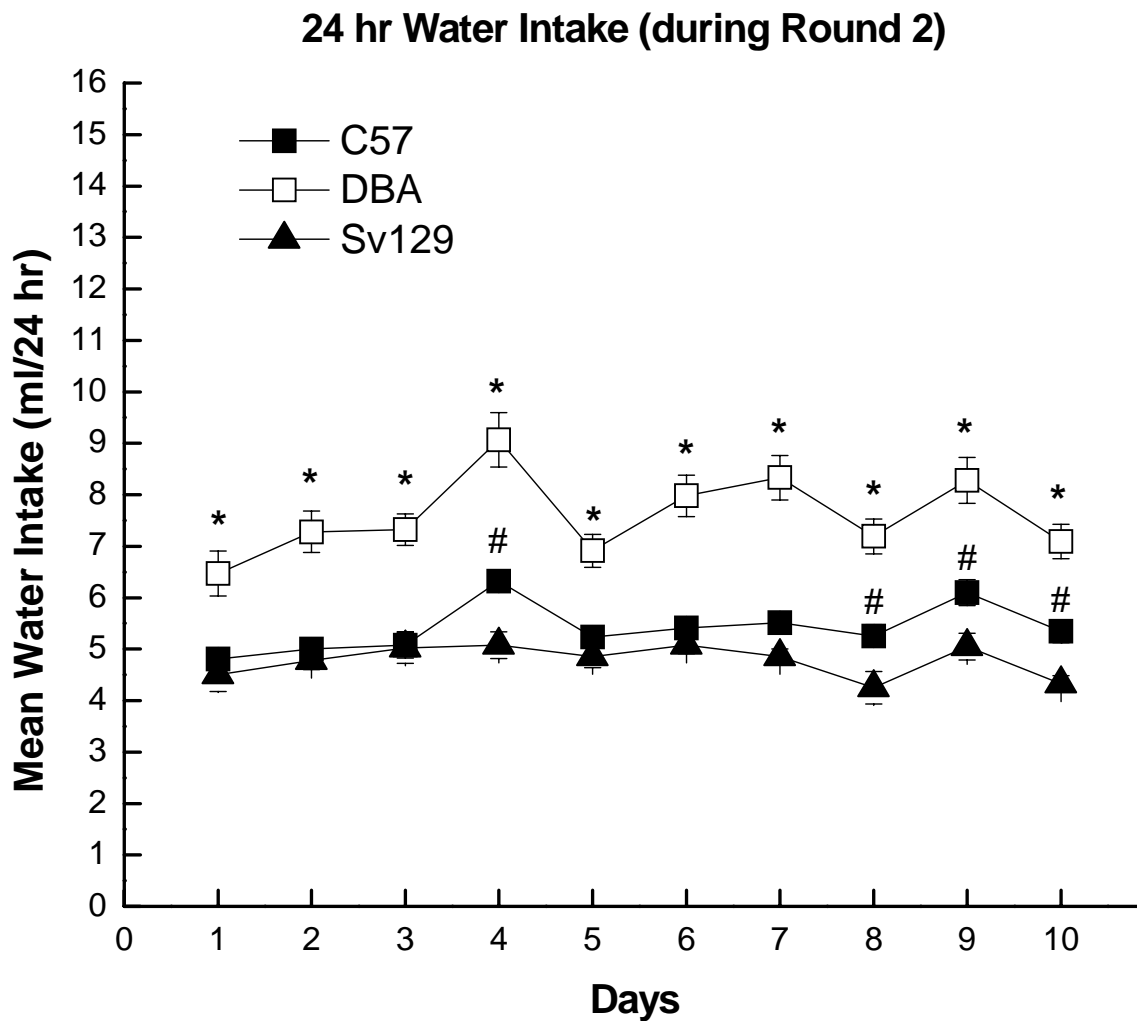


Figure 7.3. Mean (\pm SEM) intake of water (ml/24 hr) across 10 days during the 2nd replication of saccharin presentations. Data are shown for three inbred mouse strains (C57, DBA, and Sv129). * Different from C57 and Sv129, #diff from Sv129 only $p < 0.05$.

Weights. Body weight was analyzed separately for the 1st and 2nd replication of Experiment 2 (see Figures 7.5 and 7.6). In the 1st replication, there was no overall difference in body weight between C57 and DBA mice. This conclusion was supported by a nonsignificant main effect of Strain, $p > 0.05$, following a 2 x 19 repeated measures ANOVA varying strain (C57 and DBA) and day (1-19). Post hoc comparisons of a significant 2-way interaction, $F(18,288)=3.59$, $p < 0.05$, showed that although DBA mice weighed more than C57 mice on Days 1-8, $p < 0.05$, this difference went away by Day 9 and weights for the 2 groups remained the same through the end of the 1st replication of this experiment on Day 19, $p > 0.05$. The main effect of Day was significant, $F(18,288)=270.4$, $p < 0.05$, indicating that weights decreased as a function of time. This decrease in weight took place during the first 11 days (before access to saccharin began on Day 15) and reflects the process of bringing weights down to 85% of free feeding body weight through food deprivation.

The weight data from the 2nd replication also were analyzed using a 2-way mixed factorial ANOVA varying strain (C57, DBA, and Sv129) and day (1-18). The main effect of Strain was, again, not significant, $p > 0.05$, indicating that overall weights were not different among the 3 strains. The main effect of Day, however, was significant, $F(17,323)=143.4$, $p < 0.05$, indicating that, like in the 1st replication, weights gradually decreased over time. Post hoc comparisons of a significant Strain x Day interaction, $F(34,323)=29.5$, $p < 0.05$, revealed that although C57 mice weighed more than both DBA and Sv129 mice on Days 1-4, $p < 0.05$, these differences went away on Day 5, $p > 0.05$, with patterns switching

on Day 6. On Days 7 and 8, weights decreased for DBA and C57 mice leaving Sv129 mice weighing the most, $p < 0.05$. By Day 12, patterns changed again and weights became greatest for the DBA strain through the end of the experiment on Day 18, $p < 0.05$. Again, weights were reduced to 85% of free feeding weight over time. Body weights for C57 and DBA mice were brought down to target by the time access to saccharin began on Day 7. The Sv129 mice, however, were brought into the experiment late. Consequently, body weights on Days 1-3 reflect free feeding weight during acclimation to the new environment with food deprivation beginning on Day 4 and continuing throughout the experiment.

Although slight differences in body weight may account for differences in 24 hr water intake, they did not account for differences in saccharin intake. The mice that tended to weigh the most were not the same mice that consumed the most saccharin. The C57 mice consumed the most saccharin, but the DBA mice were the heaviest. Thus, differences in consumption of sweet saccharin solutions during the concentration response function likely reflect differences in sweet taste preferences.

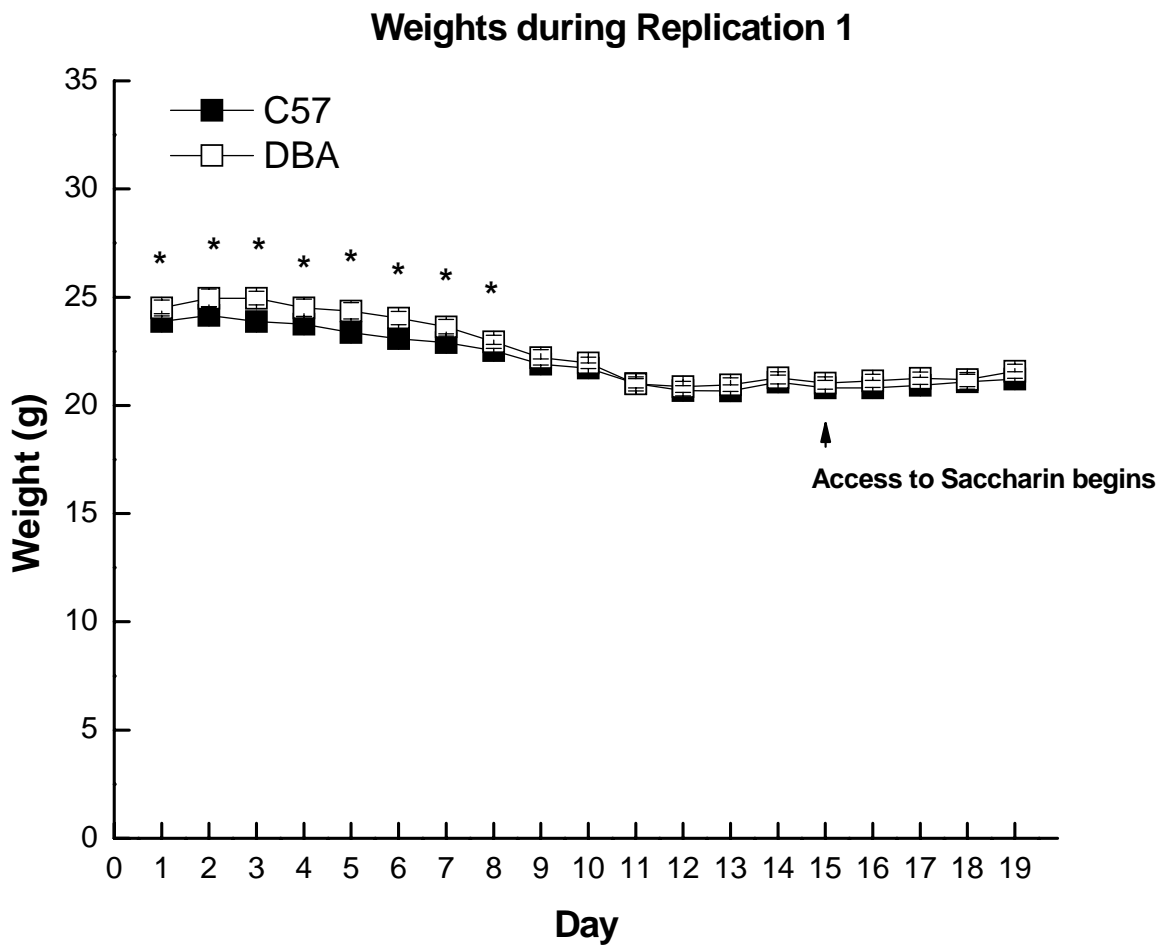


Figure 7.4. Mean (+/- SEM) weight (g) across 19 days during the 1st replication of saccharin presentations. Data are shown for the C57 and DBA strains. * Different from C57, $p < 0.05$.

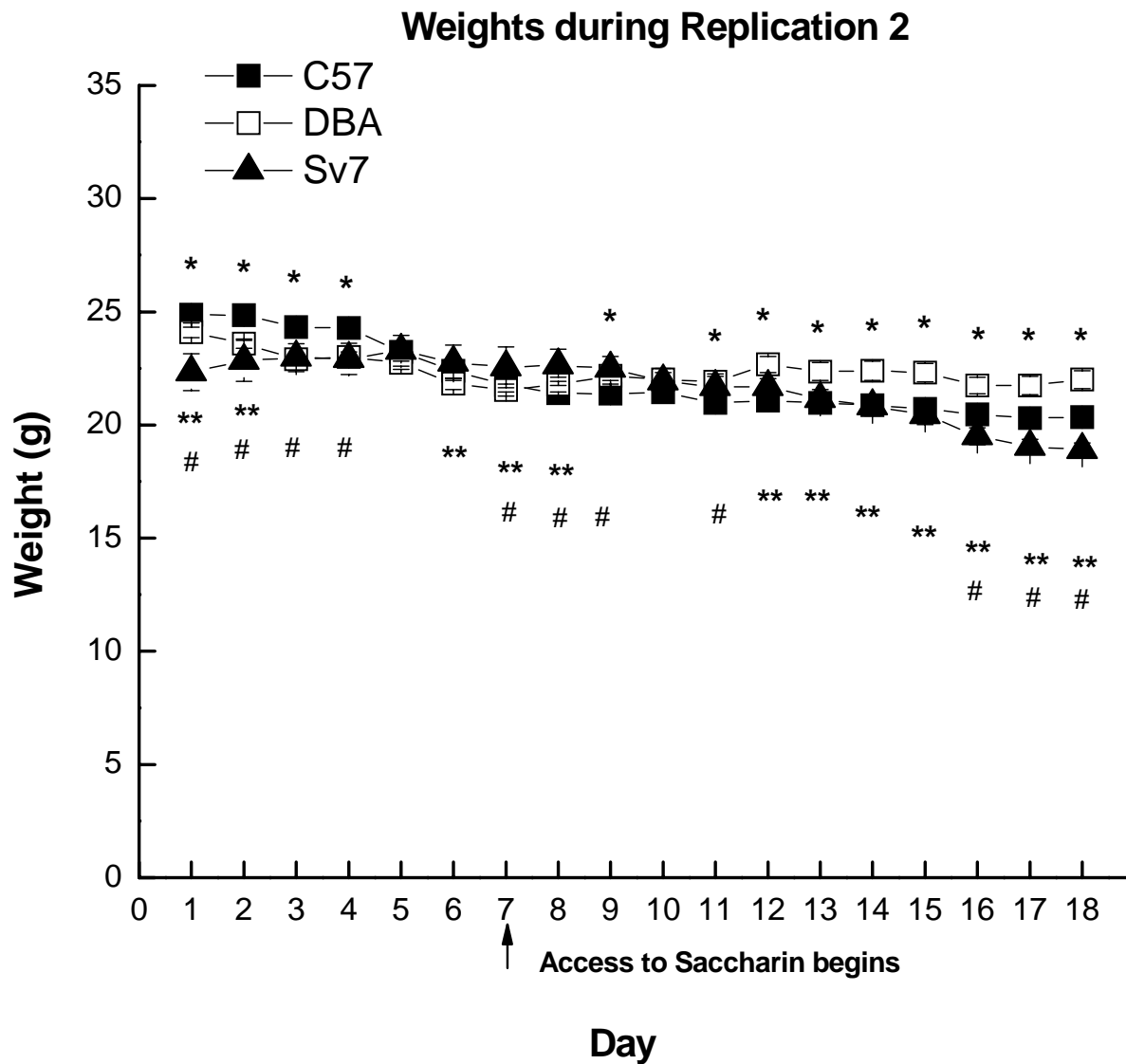


Figure 7.5. Mean (+/- SEM) weight (g) across 18 days during the 2nd replication of saccharin presentations. Data are shown for the C57, DBA, and Sv129 strains. * DBA different from C57, ** Sv129 different from C57, # Sv129 different from DBA, $p < 0.05$.

Discussion

The results from Experiment 1 show that mice, like rats, will avoid intake of a saccharin CS when paired with cocaine. The Sv129 strain was the most sensitive to the suppressive effects of cocaine, followed closely by the DBA strain, then C57, leaving the A/J strain as the least sensitive. It was predicted that C57 mice would develop stronger cocaine-induced avoidance of saccharin intake than DBA mice. This prediction was based on findings described above in the Introduction that suggest C57 mice are more sensitive to the rewarding properties of cocaine than DBA mice.

According to the reward comparison hypothesis, rats reduce intake of a saccharin CS that is paired with a drug of abuse such as cocaine because the rewarding properties of the sweet saccharin solution pale in comparison to the rewarding properties of the drug. If this hypothesis is true, then greater sensitivity to the rewarding properties of a drug should result in greater sensitivity to the suppressive effects of the drug (i.e., greater devaluation of the saccharin cue). The findings presented are relevant to this hypothesis but still admit two possible interpretations. First, if 'reward', per se, is at the root of the strain differences, then in this particular paradigm DBA and Sv129 mice are more sensitive to the rewarding properties of cocaine than C57 mice. The second possibility is that reward does not lie at the root of these strain differences. Then again, reward comparison accounts may account for some of the behavioral responses while other undefined mechanisms account for the rest.

If cocaine's rewarding properties do not account for cocaine-induced avoidance of saccharin intake, then several other possible explanations may account for the strain differences observed in Experiment 1. First, there could be strain differences in the ability to learn or remember CS-US associations in taste-drug pairing designs. Second, suppression of saccharin intake may occur in response to the aversive, rather than rewarding, properties of the drug. Third, the strain differences in drug-induced avoidance of saccharin intake may reflect differences in the reward produced by saccharin.

Relative to C57 mice, DBA mice demonstrate poor performance in several complex learning paradigms, the Morris water maze hidden platform task, contextual fear conditioning, and conditional spatial alternation (Upchurch and Wehner 1989; Paylor, Baskall et al. 1993; Owen, Logue et al. 1997). The relationship is not simple however. In some studies, C57 mice perform better than DBA mice in complex learning paradigms, but others report the reverse. For example, although C57 mice were shown to develop greater cocaine-induced CPP than DBA mice by Seale and Carney (1991), C57 mice were shown to demonstrate weaker ethanol and morphine-induced CPPs than DBA mice by Cunningham et al. (1992). Furthermore, a recent study by Cunningham et al. (1999) revealed no differences between C57 and DBA mice in cocaine-induced CPP when given longer conditioning trial durations. DBA mice also perform better than C57 mice in avoidance learning paradigms that give the choice to avoid a location associated with footshock (Henderson 1989; Weinberger, Koob et al. 1992). These conflicting findings raise the possibility that, in the present

study, DBA mice acquired faster and greater CTA because they learned or remembered the CS-US association better than C57 mice.

DBA mice also perform better than C57 mice in the conditioned taste aversion paradigm when using lithium chloride, a drug well known for its aversive properties. This paradigm closely resembles the reward comparison paradigm because, in both, the behavior relies on associative learning whereby a tastant serves as the CS and the administration of drug serves as the US. Risinger and Cunningham (2000) found that DBA mice develop stronger LiCl-induced conditioned taste and place aversions than C57 mice. The authors suggest that strain differences in the LiCl CTA paradigm is an issue of lithium pharmacokinetics, with drug concentrations being higher or persisting longer in critical tissues of mice from the DBA strain. It should be noted that a salty solution served as the CS in this experiment and when the same two strains were tested using a highly preferred 15% sucrose solution as the CS, strain differences failed to occur (Ingram 1982). Thus, the nature of the CS must be considered when interpreting results from taste conditioning experiments. It remains unknown whether strain differences in LiCl CTA learning would occur when a 0.15% saccharin solution is used as the CS. Further evaluation is needed to determine whether differences in associative learning are responsible for the strain differences observed in taste conditioning paradigms.

Unlike LiCl, cocaine is known to have both rewarding and aversive properties. This raises the question of whether it is the rewarding or the aversive properties of cocaine driving avoidance of the saccharin solution in this

paradigm. If it is an aversive experience that results in taste avoidance, there are at least two possible situations to consider. One situation would occur in the acute response to the unconditioned pharmacokinetic effects of cocaine. A second possible situation is that presentation of the saccharin cue induces withdrawal and intense craving for the drug

A third possible explanation for these strain differences is the reward value of the CS. In the present experiment, C57 mice were the most resistant to the suppressive effects of cocaine (Again, A/J mice are not being considered because they suppressed intake of saccharin even when receiving saline as the US). The saccharin concentration response functions in Experiment 2 showed that C57 mice consume greater amounts of saccharin than DBA and Sv129 mice at any concentration. This finding is consistent with other reports showing that C57 mice have greater sweetener preferences and consume more of a saccharin or sucrose solution than DBA and 129/J mice (Belknap et al., 1993; Bachmanov et al., 1996; Bachmanov et al., 2001). Thus, it is possible that C57 mice experience more difficulty suppressing intake of saccharin when it is paired with cocaine because they perceive it as more rewarding than the other strains. In support of this idea, water-deprived rats will not forego ingestion of a sucrose CS when paired with morphine (Grigson, Lyuboslavsky et al. 1999). Moreover, food-deprived rats will not suppress intake of sucrose when paired with LiCl, cocaine, or morphine, showing that the suppressive effects of any US can be offset by the use of a CS that is highly rewarding in a given situation (Gomez and Grigson 1999).

In contrast to the C57 and DBA strains, less is known about the Sv129 strain in terms of its sensitivity to drugs of abuse and learning. Miner (1997) found that, although 129/SvJ mice are sensitive to the locomotor activating effects of cocaine, they are not as sensitive to the rewarding effects of the drug. In this study, 129 mice failed to develop a CPP under the same conditions that resulted in significant CPP by C57BL/6J mice. Consistent with this report, Kuzmin and Johansson (2000) found that 129 mice show little response when given the opportunity to SA cocaine under the same conditions that C57 and DBA mice SA the drug. A more recent study showed, however, that 129 mice do develop a CPP for similar doses of cocaine (Zhang, Mantsch et al. 2002). The authors suggested that the discrepancy is due to the fact that Miner (1997) used only four conditioning trials whereas Zhang et al. (2002) used eight. The authors also point out that 129 mice perform poorly in memory-related tasks (Crawley, Belknap et al. 1997; Balogh, McDowell et al. 1999). Nevertheless, poor performance was not an issue for 129 mice in the present experiment.

Given the wide range of both learning and drug-related responses that have been described for DBA, C57, and 129 mice, there are likely different reasons for suppression following saccharin-cocaine pairings in each strain. These reasons include but are not limited to differences in learning, sensitivity to cocaine's rewarding and/or aversive properties, and sweetener preferences. The strain differences found here should prove useful for future studies designed to investigate the genetic basis of drug abuse. More research is needed, however,

to further characterize how cocaine-induced avoidance of saccharin intake is similar to devaluation of natural rewards by drugs of abuse.

Chapter 8

GENERAL DISCUSSION

Summary

The primary goal of the present set of investigations was to determine what factors mediate individual differences in drug-induced avoidance of a naturally rewarding saccharin solution. This question was addressed by testing the extent to which behavioral (saccharin intake, cocaine SA), physiological (CORT), environmental, and genetic factors, all contribute to these individual differences. Using a multidimensional approach helped to build a behavioral, hormonal, and genetic profile that previously did not exist. The establishment of such a profile will help to predict which drug naïve rats will become large or small suppressors. The ability to predict one's propensity to become addicted raises the potential to prevent. Indeed, there is a growing shift, in drug addiction research, from the need to understand the neurobiology of addiction to the need to understand why some individuals become addicted and others do not.

The first few sets of experiments evaluated the nature of the CORT elevation in the phenomenon and also searched for a predictive tool using a self-administration design. The first important finding was that the CORT response is a conditioned response of the drug US being paired with the saccharin CS. Moreover, greater elevations in CORT following presentation of the saccharin

cue at test was correlated with greater drug-induced devaluation of that same saccharin cue. The results of these experiments also showed that greater elevations in CORT following the first exposure to cocaine (the unconditioned response) are correlated with greater suppression in saccharin intake. Thus, the CORT elevation that occurs in response to stimuli such as cocaine can indicate individual predisposition to cocaine-induced devaluation of a naturally rewarding saccharin solution. The preference for sweets was also measured for its ability to predict which rats would be more sensitive to the suppressive effects of cocaine. While this measure was not associated with cocaine-induced avoidance of saccharin intake, certain modifications in this experiment including experience with the highly rewarding sucrose appears to have modified subsequent reward comparison behavior. That is, the search for a predictive tool resulted in the discovery of potential ways to prevent rats from becoming large suppressers.

While the present findings provide a much needed foundation on which to continue building a profile, being able to lower the propensity for a drug naïve rat to become a large suppresser may prove to be equally important. Specifically, certain simple environmental manipulations such as extra handling and exposure to naturally rewarding stimuli attenuated drug-taking behavior across the board. Moreover, these manipulations resulted in smaller CORT elevations at test and made rats more resistant to drug-induced devaluation of a saccharin reward. Although we do not know which rats were more prone than others, the effect was protective in that there were fewer large suppresser rats compared with other

studies. This finding suggests that there were several rats whose biological 'fate' was altered as a result of simple environmental manipulations.

The next few experiments were designed to determine whether genetic background would influence responsiveness to cocaine as measured by subsequent devaluation of the saccharin reward. The results of the selective breeding study together with the results from testing different inbred mouse strains reveal that genetic make-up does influence behavior in the reward comparison paradigm. Collectively, these data demonstrate that not just one, but several factors play important roles in influencing responsiveness to cocaine in a reward comparison paradigm.

Final Discussion and Conclusions

In both the passive and the active design, the saccharin CS serves as a cue to predict the coming of the drug US and becomes 'devalued' by the large suppressers. As described in earlier chapters, exposure to conditioned cues is a leading cause for relapse in drug addiction. While addiction is very complex disease, conditioning appears to be central to the vicious cycle of abuse, abstinence, and relapse. Indeed, understanding how the presentation of a drug-associated cue leads to relapse is crucial for breaking the cycle.

While researchers often refer to individual differences in sensitivity to the rewarding properties of drugs, they less often consider individual differences in the conditioning, or the learning, of drug and cue associations. At the heart of individual differences observed in the present investigation, one common and perhaps unifying theme is conditioning. All the individual differences relate to how salient, or powerful, the saccharin CS is perceived to be. Presentation of this cue consistently brings about the greatest response (behaviorally and physiologically) in the large suppressers. Thus, it is possible that the strength of the taste-drug association, by itself, greatly influences vulnerability to addiction-like behavior.

For the sake of illustration, imagine a group of people exposed to cocaine for the first time in the same environment. Some may like it, some may not. Regardless, certain individuals will take more notice than others of their surroundings (e.g., objects, colors, energy, smells). After the drug experience,

everyone leaves and goes about their separate lives. Some time later, contextual stimuli from that same environment are presented to the same group of people. Certain individuals will have a stronger 'response' to these stimuli than others. These responses are conditioned effects of the drug experience. Perhaps these are the individuals, through no choice of their own, that experience what is called 'craving' for the drug in response to the cue despite how euphoric or aversive the drug experience actually was. These individuals, as a consequence of having 'learned' the association more quickly or with greater strength than others, are now more vulnerable to becoming addicted than the others. The individuals who enjoyed the drug experience may be more vulnerable to using the drug again, but not necessarily to becoming addicted.

It is known that, in drug-dependent individuals, presentation of drug-associated cues can induce withdrawal symptoms and intense craving for the drug. Although there is still some confusion over what constitutes cocaine withdrawal or craving, *per se*, there is no debate that conditioned cues can and often do lead to reinstatement of drug seeking behavior. On the contrary, there is much less known about how contextual stimuli might actually facilitate the transition from use to abuse and eventually, the vicious cycle of addiction.

Two very important questions regarding cue-induced craving are *how* and *who*. In our model, it is proposed that the large suppressers are *who* and the facilitation of conditioning, or learning, by activity of the HPA axis (i.e., CORT) is *how*.

The reward comparison paradigm provides a unique and promising model for drug addiction, especially for cue-induced craving. In the present set of studies, the paradigm was applied to two different experimental designs, passive or active administration of cocaine. Although the passive design does not model the human condition as well as the active design, the passive design offers the advantages of saving time, costs, and materials. Despite a few differences between the designs, certain features hold true to both designs. First, individual differences in the suppressive effects of cocaine occur whether the drug is administered passively or actively. Second, greater drug-induced suppression of saccharin intake by the large suppressers is associated with greater saccharin cue induced elevations in CORT at test in both designs. Finally, the mechanisms underlying cue-induced craving (i.e., conditioning) are fundamental to, and can be elucidated in, both situations.

As stated above, it is proposed that the large suppresser rats are more prone to addiction-like behavior than the small suppresser rats. When using the active design, the large suppresser rats demonstrate greater cocaine SA than the small suppresser rats. Moreover, greater avoidance of the saccharin cue is highly correlated with greater cocaine SA, as shown in Grigson and Twining (2002), and in Chapter 3. Finally, a saccharin cue is immensely effective in eliciting reinstatement of drug-seeking behavior in large suppresser rats even after six months of abstinence (unpublished results). Thus, the paradigm serves as a model for not only cue-induced craving, but also cue-induced relapse.

While controversy surrounds the issue of whether pathological drug use (e.g., addiction) can accurately be studied in non-human species, there are data to suggest that addiction can indeed be studied in rats. In a recent *Science* report, Deroche-Gamonet et al. (2004) show that rats exhibit three behaviors resembling those currently considered the hallmarks of substance dependence in humans in the DSM-IV. Like humans, rats (1) have difficulty stopping drug use or limiting drug intake, (2) have an extremely high motivation to take the drug, with activities focused on its procurement and consumption, and (3) continue to abuse substances despite their harmful consequences. Most relevant to the present set of studies is that not all the rats exhibited these 'addiction-like' behaviors. Only the rats with a higher propensity to relapse were found to exhibit these 'addiction-like' behaviors following extended access to the drug. These 'High Reinstatement' rats were assigned the label only if they demonstrated significantly high levels of responding for cocaine induced by the drug itself or by a cocaine-associated CS. One very important question, then, is whether or not our large suppresser rats are the same as their addiction prone, High Reinstatement rats.

Interestingly, Deroche-Gamonet et al. (2004) found that addiction-like behaviors were not present after a short period of SA but developed only after a prolonged daily exposure to the drug (about 3 months). While reinstatement was higher in rats that demonstrated addiction-like behavior than in rats that did not, overall intake of cocaine was not different between the two groups. They also found that a higher sensitivity to the unconditioned effects of cocaine on

locomotion did not seem to be involved with vulnerability. At first glance, these observations do not parallel the data presented in Chapter 3.

In Chapter 3, the large suppresser rats took more drug than the small suppresser rats. If these large suppresser rats are more 'addiction-prone' than the small suppresser rats then overall drug intake, according to the Deroche-Gamonet et al. (2004) findings, should not differ between small and large suppressers. Overall drug intake in the present, however, was measured over a short period of time (about 2 weeks). It remains unknown whether overall drug intake still would differ between small and large suppressers if measured over a longer period of time and given longer SA periods. The observations in Chapter 2 and 3 that CORT tends to rise later (at 45 min) or remain elevated (at 120 min) for small suppressers suggest, if given more time, these rats would self-administer more cocaine.

The second finding from Deroche-Gamonet et al. (2004) that does not seem to parallel findings in Chapter 3 relates to the unconditioned effects of the drug. Deroche-Gamonet et al. (2004) found no differences between groups in the unconditioned effects of cocaine on locomotor activity, whereas in Chapter 3, greater unconditioned effects of cocaine on CORT were observed for the large suppresser group. Locomotor activity was not measured for small and large suppresser rats, however, and CORT was not measured for Deroche-Gamonet's high and low reinstatement rats. Thus, it is possible that, like our large suppresser rats, their high reinstatement, addiction-prone rats would

demonstrate a greater unconditioned effect of cocaine on CORT and vice versa with respect to locomotor activity.

As discussed in earlier chapters, rats that demonstrate increased locomotor activity and prolonged CORT secretion by High Responder rats in response to a novel environment, compared with Low Responder rats, demonstrate greater amphetamine SA (Piazza et al. 1991). Interestingly, CORT levels were different at the 120 min timepoint, but not at the 30 min timepoint. Moreover, locomotor activity was different at a timepoint (30 min) when CORT levels were identical. For this reason, it is possible that if locomotor activity is identical between two groups at a given timepoint, then CORT levels would not necessarily be identical. Using this logic, it remains to be determined whether the addiction-prone rats in the Deroche-Gamonet et al. (2004) study would demonstrate a higher unconditioned CORT response to cocaine even though they did not demonstrate higher locomotor activity.

At the outset of the present set of investigations, it was proposed that our large suppresser rats would turn out to be Piazza's High Responder rats. The CORT data presented in Chapter 3, however, show that our large suppresser rats do not demonstrate prolonged secretions of CORT at the 120 min time point. While this finding does not parallel Piazza's, the findings in Deroche-Gamonet (2004) help explain why rats that demonstrate greater drug SA in one study are not necessarily the same rats that demonstrate greater drug SA in another study. A common reason for such a discrepancy is that different measures often are used to determine the 'profile' of the rat. Another reason is that, sometimes,

acquisition of drug SA is not distinguished from overall drug intake, especially over short vs long periods of time. Other reasons include methodology, handling, genetic background, etc.. Interestingly, the High Reinstatement, addiction-prone rats in the Deroche-Gamonet et al. (2004) study, also are not High Responder rats, as indicated by their average locomotor response to cocaine. Thus, while our large suppresser rats do not appear to have the same hormonal profile as Piazza's High Responder rats, it is possible that our large suppresser rats have the same profile as the High Reinstatement, addiction-prone rats described by Deroche-Gamonet et al.(2004). By the same token, it is possible that Deroche-Gamonet's addiction prone rats would be sensitive to the suppressive effects of cocaine if tested in our model.

Although we have yet to fully establish why rats suppress intake of a saccharin CS when paired with the availability of a drug of abuse, we do know that greater avoidance of the saccharin cue is associated with greater cocaine SA. As described in the Introduction, the data are most consistent with the conclusion that a reward comparison, or contrast effect occurs as the saccharin reward is devalued in anticipation of the opportunity to self administer a more rewarding drug of abuse. An equally important question is why some rats, as with humans, are more vulnerable than others to not only drug-taking behavior but also drug-induced devaluation of natural rewards.

As mentioned above, two very important questions regarding cue-induced craving and eventual relapse are *who* and *how*. The same two questions apply to

drug-induced devaluation of natural rewards and this model, the same answers are proposed, the large suppressers being *who* and CORT being *how*.

Although glucorticoids play an important role in drug sensitivity (Piazza, Maccari et al. 1991; Goeders and Guerin 1996; Deroche, Marinelli et al. 1997; Marinelli, Rouge-Pont et al. 1997; Mantsch, Saphier et al. 1998; Goeders and Clampitt 2002; Marinelli and Piazza 2002), it is not clear what role they play in drug-induced devaluation of natural rewards. CORT may facilitate drug-induced devaluation of natural rewards and reward comparison by modifying learning and memory. Increased CORT levels following acute stress are known to enhance learning and memory through its actions in both the hippocampus and the mesolimbic dopamine system (McEwen and Sapolsky 1995; Barrot, Abrous et al. 2001; Shors 2001). In the beginning of these investigations, it was proposed that CORT mediates reward comparison behavior, in part, through its actions on dopaminergic transmission in the VTA/NAc pathway. A recent discovery suggests, however, that the mesolimbic DA pathway need not be intact for reward comparison to occur (Twining, Hajnal et al. 2005). Thus, further investigation is needed to elucidate what neural pathways mediate reward comparison and how those pathways are affected by CORT.

In addition, other segments of the HPA axis should be investigated for their potential role in reward comparison behavior. For example, corticotrophin releasing factor (CRF) may mediate reward comparison behavior. As described earlier, CRF is involved with activation of the HPA axis by triggering the production of ACTH that subsequently causes the release of CORT by the

adrenal cortex. Thus, it is not surprising that both the unconditioned CORT response to cocaine and the conditioned CORT response to cocaine-associated cues are mediated ultimately by CRF (Sarnyai, Biro et al. 1992; DeVries, Taymans et al. 1998). CRF also exerts its actions in brain structures other than the hypothalamus and the pituitary. For example, CRF in the bed nucleus of the stria terminalis (BNST) mediates stress-induced cocaine seeking behavior (Erb, Shaham et al. 1998; Erb and Stewart 1999). Likewise, enhanced CRF activity in the central nucleus of the amygdala (CNA) is associated with craving and withdrawal symptoms following the chronic administration of cocaine (Richter and Weiss 1999). Furthermore, there is also some evidence for the role of glucocorticoids in memory modulation in the basolateral amygdaloid complex (Cahill and McGaugh 1998). These findings support the possibility that the magnitude of the CORT elevation seen at test parallels the magnitude of CRFs actions in the brain where it may contribute to individual differences in not only learning and memory, but also the drug experience.

While CORT and CRF likely act in many areas of the brain (e.g., mesolimbic DA pathway, amygdala, hippocampus, gustatory thalamus, prefrontal cortex) to mediate several drug responses in our paradigm, little is known about how their effects on learning and memory influence reward comparison. It is possible that the greater unconditioned effect of cocaine on CORT in the large suppresser rats enhances the strength of the CS-US association. Thus, the saccharin cue may be more salient and as a result, compared with the drug more efficiently in the large suppresser rats. Logic would support that a stronger

association between two stimuli and greater retention would facilitate a stronger contrast, or reward comparison effect. In turn, rats that learn the CS-US association better would be more vulnerable to greater cue-induced craving for the drug. In support for this conclusion, elevations in CORT following exposure to conditioned stimuli have been shown to occur specifically during reinstatement (Goeders and Clampitt, 2000). Thus, CORT and CRF may facilitate, through conditioning, not only the process of becoming a large suppresser but also reinstatement of drug seeking behavior.

One goal of the present set of investigations was to find a predictive tool that could determine which drug naïve rats would most likely become small or large suppressers. The unconditioned CORT response was predictive in the reward comparison paradigm but the intake of granulated sucrose was not.

While greater intake of granulated sucrose was not associated with subsequent drug-induced devaluation of CS intake, experience with the highly rewarding sucrose seems to have had a strong impact on the large suppresser group. Indeed, compared with the large suppressers in other experiments, those in this experiment were more resistant to the suppressive effects of cocaine, were slower to acquire cocaine SA, and demonstrated lower conditioned elevations in CORT at test. There are data to suggest that the mere exposure to sweets may have a 'protective' effect on drug self-administration. This 'protective' effect may not be that different from the protective effect it might have had on drug-induced avoidance of saccharin intake. Experience with the natural sucrose reward may have induced changes in the mesolimbic dopamine system and

other reward mediating brain systems so that the perceived value of other rewards was different than if there were no experience with the sucrose reward. It is also possible that exposure to the sucrose attenuated the CORT response to cocaine. Although it is difficult to conclude that exposure to sucrose had a protective effect on overall cocaine SA in Chapter 3, exposure to a high concentration of sucrose prior to the opportunity to work for cocaine (without pairing it with saccharin) has been shown to decrease the motivation to work for the drug (Twining and Grigson, 2004). In this study, rats given access to 1.0 M sucrose worked less for cocaine than rats exposed to 0.1 M sucrose. Furthermore, rats given 5 min access to a glucose plus saccharin mixture following 3 months of abstinence from cocaine were less likely to relapse than are rats that were not given the sucrose (Liu and Grigson 2005). These findings suggest that, in some situations, sucrose may have a protective effect on rats, making them less vulnerable to drug-taking behavior by lowering the incentive value of the cocaine. Even so, in the studies where sucrose had a protective effect, the rats had to work for the drug, whereas in Chapter 3, the rats did not have to work for the drug. In any case, it is important to distinguish the potential for exposure to sweet stimuli to protect an individual from drug-taking behavior and the potential for higher preferences for sweet stimuli to predict greater sensitivity to the reinforcing properties of drugs of abuse.

A second factor that appeared to have a 'protective' effect on the rats was handling. As mentioned in Chapter 4, other data suggest that environmental factors such as postnatal rearing conditions and experimenter handling can

attenuate stress-induced activation of the HPA axis (Nunez, Ferre et al. 1996; Vallee, Mayo et al. 1997; Gariépy, Rodriguiz et al. 2002; Brake, Zhang et al. 2004). For example, early handling in rats promotes a more rapid post-stress recovery in CORT levels and significant attenuation of amphetamine-induced conditioned place preference (Campbell and Spear 1999). Moreover, variations in maternal licking and grooming behavior influence the development of stress reactivity in rats (Liu, Diorio et al. 1997; Caldji, Diorio et al. 2000; Champagne and Meaney 2001). Specifically, a high level of maternal licking and grooming and arched-back nursing correlates with reduced CRF mRNA expression, enhanced glucocorticoid negative feedback, and lower stress responses in the adult (Champagne and Meaney 2001). These findings raise the possibility that variations in maternal behavior also influence individual differences in reward comparison behavior. Future studies in our paradigm should incorporate measurable handling in a separate group of rats to replicate and confirm the observations described for Chapter 4.

The final objective of the present set of investigations was to determine whether genetic factors contribute to individual differences in the reward comparison paradigm. The results of the selective breeding study and the strain differences found among inbred mice show that there is a potentially strong genetic source for variation in the reward comparison paradigm. This conclusion, however, holds true only if there were no differences in maternal rearing behavior in small and large suppresser rats. Regardless, even if there were differences in maternal behavior, it is unlikely that genetic make-up had no impact on behavior.

The classic question of nature vs. nurture ultimately lies at the heart of all variation in behavior. Collectively, the results from these studies support that both nature and nurture work together in determining a phenotype that is unique to every individual. Clearly, there are certain factors that make one individual more prone to addiction than others and these data provide hope that there will someday be tools to predict vulnerability. Importantly, these data provide hope that there are ways to prevent the transition from use to abuse.

Through this investigation, many factors were found and are proposed to contribute to individual variation in reward comparison behavior. These factors are listed below.

Genetic sources for variation:

- 1) Differential sensitivity to the rewarding properties of both natural rewards and drugs of abuse
- 2) Differential sensitivity to the aversive properties of cocaine
- 3) Differential sensitivity in the HPA axis
- 4) Learning & memory during conditioning
- 5) Gender

Environmental sources for variation:

- 6) Stress
- 7) Experimenter handling and/or maternal rearing
- 8) Experience with, and availability of, other naturally rewarding stimuli
- 9) Exposure to drug

A great deal is known about how these factors affect drug-taking behavior. The findings from the present investigation show how these factors affect a less understood facet of addiction, that is, how experience with drugs of abuse come to devalue experiences with natural rewards. While all of these factors are influential in determining vulnerability to addiction, learning is a factor that deserves more attention. The present findings suggest that, at least in the reward comparison paradigm, better learning facilitates becoming a large suppresser. The factors that would be most helpful in finding predictive tools, on the other hand, are a hormonal profile or a 'DNA thumbprint'. Deroche-Gamonet et al. (2004) presented promising data that may have established addiction-prone profiles, but there is not yet a well established predictive index of addiction-like behavior. Regardless of not being able to predict, there still is hope for prevention by using precautionary measures such as experimenter handling and maternal care.

Champagne and Meaney (2001, see for review) propose that for neurobiologists, the function of the family is an important level of analysis that influences the development of the stress response. Indeed, a maladaptive, overreactive stress response makes an individual more vulnerable to not only drug abuse, but many other psychiatric and somatic 'illnesses'. They also suggest that a critical question is that of how environmental events regulate neural systems that mediate the expression of parental care. This line of logic is pivotal to the issue of nature vs nurture. Regardless of one's genetic predisposition to a disease such as addiction, the probable outcome of addiction can be lowered through proper nurturing.

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AFTERWORD

I have been the biggest critic of my own work and the state of the biomedical research field I chose to enter. The first time I had to sacrifice an animal with my own hands, I was overcome with nausea and sadness. As a consequence, I had to stop and try again the next day. My first thought in response to killing a mouse was, "Why?" I got through that experiment but it was my last one and then I made the decision to not sacrifice any more animals, even if it would cost me my degree. Around the same time, I had my first experience with the death of a loved one and a few months later, the death of another loved one. My first few thoughts in reaction to such shocking news, was, "Why and where, where did they go?" From those moments on, everything was different. Everything felt different, everything looked different. The next big question I had to ask myself was, "What am I doing with my life?" I then forced myself to become the biggest critic of my own work.

I decided that regardless of what I can or cannot find that is meaningful in my data, they must be published. For even if I could not find meaning in them, there would be the hope that someone else could. One of the greatest challenges in my life was trying to extract something meaningful from my thesis. While the task was difficult, there is now hope where there once was none. I thank my advisor, Sue, for this hope. I learned from her the value of being optimistic with any given set of data. To offer a theory for why some individuals are more vulnerable to addiction than others is not only hopeful, but also very

exciting. The idea that something so basic, that is, conditioning, may be more critical in addiction than we realize is one meaningful message. Another is that there likely are ways to predict for the purpose of prevention. Importantly, these data support the idea that experience with natural rewards, and better care and handling may prevent the transition from use to abuse.

Perhaps the importance of proper nurturing is the message that should be louder than all others. It is sad to think that pharmaceutical and genetic interventions are the best solutions for some of the worst human conditions. Indeed, it is sad to think of the thousands of animals that are sacrificed each day for the purpose of facilitating the search for a new prescription when there are other ways to make a difference. Unfortunately, until love and compassion can really be 'prescribed', we must continue to collect data to convince the scientific community and to educate the public on the importance of nurture over nature. Granted, there are many individuals that cannot be helped through nurturing and these individuals will benefit most from a better understanding of all the neurochemical and neuronal pathways underlying addiction. Thank biomedical researchers for having discovered ways to intervene pharmaceutically for those who have no other hope. We must, however, be careful to not forget that there are countless ways to 'intervene' without the use of pharmaceutical or genetic intervention, but with love and compassion. These are tools that can and will stand the test of technology and time and they do not cost a single dime.

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ACADEMIC BACKGROUND

Pennsylvania State University, Hershey, PA, Ph.D., Neuroscience, 2006
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PUBLICATIONS

Schroy, P.L., Wheeler, R.A., Davidson, C., Scalera, G., Twining, R.C. and Grigson, P.S (2005). The Role of the Gustatory Thalamus in the Anticipation and Comparison of Rewards over Time in Rats. *Am J Physiol Regul Integr Comp Physiol*, Apr, 288(4):R966-80. Epub 2004, Dec 9.

PAPERS IN PREPARATION

Schroy, P.L., & Grigson, P.S. (in prep). Cocaine-induced avoidance of saccharin intake is associated with elevated circulating corticosterone levels at test when using a 5 min or a 30 min interstimulus interval. The Penn State University College of Medicine, Hershey, PA.

Grigson, P.S., Schroy, P.L., and Leuenberger, E. (in prep). Female rats, like male rats, exhibit individual differences in cocaine-induced avoidance of saccharin intake and greater avoidance of the tastant is associated with greater conditioned elevations in circulating corticosterone levels at test.

Grigson, P.S., Schroy, P.L., and Wardrop, K. (in prep). Selective Breeding: Evidence for heritability in cocaine-induced suppression of saccharin intake in rats.

Schroy, P.L., and Grigson, P.S. (in prep). Individual differences in cocaine-induced CORT elevations predict greater avoidance of saccharin intake when paired with the opportunity to self-administer cocaine.

Schroy, P.L., Wardrop, K., and Grigson, P.S. (in prep). Individual differences in sucrose intake are not predictive of reward comparison behavior involving saccharin and cocaine.

Schroy, P.L., Grigson, P.S., Radcliffe, R, and Wehner, J. (in prep). DBA/2J mice show greater cocaine-induced avoidance of saccharin intake than C57BL/6J mice.

AWARDS

Cocaine, Reward Comparison, and Individual Differences. Principal Investigator. National Institute on Drug Abuse, National Research Service Award, F31 DA 15261. Total Cost: \$70,110
Dates: 03/04/02 – 03/03/05.