THE DEVELOPMENT OF A NOVEL RODENT MODEL OF DRUG INDUCED DEVALUATION OF NATURAL REWARDS AND ITS RELEVANCE TO FEATURES OF DRUG ADDICTION

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ABSTRACT

Humans and animals learn to avoid pain and to seek pleasure. In fact, actions that are essential to the survival of the organism such as mating, eating, and drinking, are invariably pleasurable. The subjective experience of pleasure is typically perceived when engaging the sensory systems, but may even begin in anticipation of consumption of a given reward. These sensory systems send neural projections to areas of the brain that, when activated, produce euphoria in humans and reinforce behavior that is instrumental in obtaining rewards. To protect from over consumption, organisms also have evolved the capacity to stop seeking and consuming rewards. These feedback mechanisms reduce the sensation of pleasure with continued consumption. This is why turkey tastes far better at the beginning of Thanksgiving dinner than at the end.

Such is not the case with the consumption of drugs of abuse. Drugs of abuse directly and potently activate brain reward systems and they do not rely, at least initially, on the senses to produce their pleasurable effects. In fact, this potent activation turns ordinary sights, sounds, places, smells, and tastes into profound emotional experiences and coveted goals in and of themselves by mere association. In this way, abused drugs ‘hijack’ brain reward systems by eroding self-control and redirecting attention and motivated behaviors toward the drug and its associated cues. Furthermore, as drug taking escalates, brain reward substrates are repeatedly overstimulated and become hypersensitive in the presence of drug while becoming hyporesponsive in its absence. This hyposensitivity increases negative affect, and reduces the sensitivity to the relative value of less powerful natural rewards and increases drug craving and seeking. The result is a compulsive preoccupation with drug seeking and consumption, and a reduced motivation to pursue natural rewards (e.g., employment, child care, nutritional needs, sex). Despite the pervasive nature of this debilitating aspect of drug addiction there are no animal models that target it. Therefore, the basic research contained within this dissertation is organized under one theme: To create a rodent model of drug-induced devaluation of natural rewards and cue induced craving.
To that end, rats were given access to a saccharin cue that always predicted access to either passively delivered or self-administered drug (usually cocaine). Upon repeated pairings with the drug of abuse, the value of the saccharin cue decreased similar to when it predicts access to a much sweeter sucrose solution. In the first set of experiments, the parameters under which devaluation of a palatable taste cue occurs were determined, individual differences in the phenomenon were characterized, and this variation was compared to cocaine seeking and self-administration. Principally, it was found that the more sensitive a rat was to the reinforcing properties of the drug the less of the saccharin cue they consumed.

The next set of experiments tested manipulations that might protect against the acquisition of cocaine self-administration. Interestingly, relative to active administration, the unpredictable, uncontrollable yoked delivery of cocaine enhanced saccharin avoidance, reduced the motivation to work for cocaine, and reduced the preference for contextual cues associated with drug delivery. Additionally, it was found that a sudden unexpected loss or gain of sucrose reward had no effect on the motivation to self-administer cocaine; however, daily experience with a consistently high sucrose reward, relative to a low one, greatly reduced the motivation to work for cocaine.

The last two chapters investigated the role of the VTA and NAc, two brain regions critical for drug reward, in modulating the devaluation of saccharin by drugs of abuse. It was found that dopaminergic lesions of the VTA had little effect on the phenomenon. Nevertheless, the electrophysiological experiment showed that the activity of single neurons in the NAc (the target for VTA dopamine release) tracked this devaluation. The degree of devaluation was associated with a shift toward negative affect, and this shift predicted the rapidity with which rats would seek and take drug. Together, these data integrate several distinct theories of drug addiction, provide a behavioral model for the investigation of novel drug therapeutics to combat addiction, and reveal new insight on the solution to a nearly 40 year old paradox.
TABLE OF CONTENTS

LIST OF FIGURES ..............................................................................................xi

LIST OF TABLES ................................................................................................xvii

ACKNOWLEDGEMENTS ....................................................................................xviii

Chapter 1  Introduction ...................................................................................1
  1.1 The most important aspect of drug addiction .........................................1
  1.2 Contrast Effects ...................................................................................6
    1.2.1 As a general phenomenon ............................................................6
    1.2.2 Rodent Models of Contrast Effects ..............................................7
    1.2.3 Functional Significance of Contrast Effects ...............................10
  1.3 Contrast effects and a common neural substrate for reward ..................12
  1.4 The Model .........................................................................................23
  1.5 Summary ............................................................................................32

Chapter 2  Heroin-induced suppression of saccharin intake in water-deprived and non-deprived rats .................................................................35
  2.1 Introduction .........................................................................................35
  2.2 Method ...............................................................................................37
    2.2.1 Subjects .......................................................................................37
    2.2.2 Apparatus ....................................................................................38
    2.2.3 Procedure ....................................................................................38
  2.3 Results ..................................................................................................39
    2.3.1 Mean CS Intake ............................................................................39
    2.3.2 Mean CS Intake and Individual Differences ...............................41
    2.3.3 5 min dH2O intake ......................................................................42
    2.3.4 1 h dH2O intake ..........................................................................43
    2.3.5 Body weight ................................................................................45
  2.4 Discussion .............................................................................................47

Chapter 3  The role of dose and deprivation state in drug-induced suppression of saccharin intake: A comprehensive analysis ............................................51
  3.1 Introduction .........................................................................................51
  3.2 Methods ...............................................................................................53
    3.2.1 Subjects .......................................................................................53
3.2.2 Apparatus ..............................................................53
3.2.3 Procedure: Water-Deprived experiments .....................54
3.2.4 Procedure: Food-Deprived experiments ......................55
3.2.5 Procedure: Non-Deprived experiments ......................56
3.3 Results .................................................................57
  3.3.1 Experiment 1: LiCl ............................................57
    3.3.1.1 Experiment 1A: Water-Deprived .....................57
    3.3.1.2 Experiment 1B: Food-Deprived .......................59
    3.3.1.3 Experiment 1C: Non-Deprived .......................61
  3.3.2 Experiment 2: Cocaine .........................................63
    3.3.2.1 Experiment 2A: Water-Deprived .....................63
    3.3.2.2 Experiment 2B: Food-Deprived .......................65
    3.3.2.3 Experiment 2C: Non-Deprived .......................66
  3.3.3 Experiment 3: Morphine .......................................68
    3.3.3.1 Experiment 3A: Water-Deprived .....................68
    3.3.3.2 Experiment 3B: Food-Deprived .......................69
    3.3.3.3 Experiment 3C: Non-Deprived .......................71
3.4 General Discussion ....................................................73

Chapter 4  Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards ..................80
  4.1 Introduction ........................................................80
  4.2 Experiment 1 .....................................................83
    4.2.1 Introduction ...................................................83
    4.2.2 Method .........................................................84
      4.2.2.1 Subjects ...................................................84
      4.2.2.2 Self-administration catheter construction ...........84
      4.2.2.3 Catheter implantation ...................................85
      4.2.2.4 Coupling assembly .......................................86
      4.2.2.5 Apparatus ................................................87
      4.2.2.6 Procedure ................................................88
      4.2.2.7 Data Analysis ............................................89
    4.2.3 Results and Discussion ....................................90
      4.2.3.1 CS Intake (licks/5min) ..................................90
      4.2.3.2 Log 10 Latency (sec) to Lick the CS .................93
      4.2.3.3 US Infusions/h ...........................................96
      4.2.3.4 Log 10 Latency (sec) to First Infusion .............101
  4.3 Experiment 2 .....................................................103
    4.3.1 Introduction ...................................................103
    4.3.2 Method .........................................................104
      4.3.2.1 Subjects ...................................................104
      4.3.2.2 Apparatus ................................................105
      4.3.2.3 Procedure ................................................105
Chapter 4

4.3.3 Results and Discussion ............................................................106
4.4 Experiment 3 ......................................................................................109
  4.4.1 Introduction ...............................................................................109
  4.4.2 Method......................................................................................110
    4.4.2.1 Subjects ..........................................................................110
    4.4.2.2 Apparatus ........................................................................111
    4.4.2.3 Procedure ........................................................................111
  4.4.3 Results and Discussion ............................................................112
    4.4.3.1 Post-abstinence CS intake ..............................................112
    4.4.3.2 Post-abstinence infusion attempts.................................114
    4.4.3.3 Log latency (sec) to lick bottle-1 saccharin......................115
    4.4.3.4 Log latency (sec) to obtain the first infusion ....................115
  4.5 General Discussion ............................................................................116

Chapter 5  Non-contingent cocaine enhances the devaluation of a saccharin cue and appears aversive in rats. ............................................125

  5.1 Introduction ........................................................................................125
  5.2 Methods .............................................................................................129
    5.2.1 Subjects ....................................................................................129
    5.2.2 Catheter Construction...............................................................129
    5.2.3 Catheter Implantation and Maintenance ...................................130
    5.2.4 Apparatus .................................................................................130
    5.2.5 Procedure .................................................................................130
    5.2.6 Habituation................................................................................130
    5.2.7 Conditioning..............................................................................131
      5.2.7.1 Experiment 1 ...................................................................131
      5.2.7.2 Experiment 2 ...................................................................132
      5.2.7.3 Experiment 3 ...................................................................132
  5.3 Results and Discussion......................................................................133
    5.3.1 Experiment 1: CS Intake (Licks/5 min)......................................134
    5.3.2 Experiment 1: CS Intake and individual differences...............136
    5.3.3 Experiment 1: Cocaine Intake and individual differences .........138
    5.3.4 Experiment 2: Fixed and Progressive Ratio Responding .........144
      5.3.4.1 Introduction......................................................................144
      5.3.4.2 Results and Discussion ...................................................145
    5.3.5 Experiment 2: Fixed and Progressive Ratio Responding and individual differences .................................................................148
      5.3.5.1 Experiment 2: Fixed Ratio Days .................................149
      5.3.5.2 Experiment 2: Progressive Ratio Challenge .................151
    5.3.6 Experiment 3: Alternating-Side Choice Test .......................151
    5.3.7 Experiment 3: Alternating-Side Choice Test and Individual Differences ..................................................................................155
  5.4 General Discussion ............................................................................158
Chapter 6  Reward History, rather than current reward value, affects the motivation to acquire cocaine self-administration.................................164

6.1 Introduction ........................................................................................164
6.2 Methods .............................................................................................168
   6.2.1 Subjects ....................................................................................168
   6.2.2 Apparatus .................................................................................168
   6.2.3 Prior Experimental History ........................................................168
   6.2.4 Procedure .................................................................................169
6.3 Results ...............................................................................................170
   6.3.1 Sucrose Intake ..........................................................................170
   6.3.2 Cocaine Self-Administration on the Post Shift Day ...................171
6.4 General Discussion ............................................................................175

Chapter 7  Lesions of the ventral tegmental disrupt drug-induced appetite stimulating effects but spare reward comparison. .........................................178

7.1 INTRODUCTION................................................................................178
7.2 Experiment 1a (Saccharin-Morphine).................................................181
   7.2.1 Methods ....................................................................................182
      7.2.1.1 Subjects ..........................................................................182
      7.2.1.2 Surgery ............................................................................182
      7.2.1.3 Recovery .........................................................................183
      7.2.1.4 Apparatus ........................................................................184
      7.2.1.5 Solutions .........................................................................184
      7.2.1.6 Procedure .....................................................................185
      7.2.1.7 Analysis ...........................................................................185
      7.2.1.8 HPLC Analysis ................................................................186
   7.2.2 Results and Discussion ............................................................188
      7.2.2.1 HPLC Analysis ................................................................188
      7.2.2.2 Saccharin-CS Intake .......................................................189
7.3 Experiment 1b (Alanine-Cocaine) ......................................................190
   7.3.1 Methods ....................................................................................191
      7.3.1.1 Subjects ..........................................................................191
      7.3.1.2 Procedure........................................................................191
   7.3.2 Results and Discussion ............................................................192
      7.3.2.1 Alanine-CS Intake ...........................................................192
7.4 Experiment 1c (CDP-Induced Appetite) .............................................194
   7.4.1 Methods ....................................................................................196
      7.4.1.1 Subjects ..........................................................................196
      7.4.1.2 Apparatus ........................................................................196
      7.4.1.3 Procedure ........................................................................197
   7.4.2 Results and Discussion ............................................................197
7.5 Experiment 2a (Alanine-Cocaine) ......................................................200
LIST OF FIGURES

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.........31

Figure 2-1: Mean (± SEM) intake of 0.15% saccharin (ml/5min) in water-replete (left panel) and water-deprived (right panel) rats following 7 saccharin-saline or saccharin-heroin (8 mg/kg ip) pairings followed by one saccharin only test. Taste-drug pairings occurred at 48 h intervals...40

Figure 2-2: Mean (± SEM) intake of 0.15% saccharin (ml/5min) for the saline controls, small, and large suppressers across 8 pairings with either saline or heroin (8 mg/kg). *'s = statistically significant differences from saline controls, #’s from both saline controls and small suppressers, ps<0.05. .................................................................42

Figure 2-3: Mean (± SEM) afternoon intake (ml/1h) of distilled water (dH2O) in water-deprived rats on the days of, and between, 7 saccharin-saline or saccharin-heroin (8 mg/kg ip) pairings. Taste-drug pairings occurred on even-numbered days and asterisks indicate statistically significant changes in fluid consumption.................................44

Figure 2-4: Mean (± SEM) body weight (g) in water-replete (left panel) and water-deprived (right panel) rats throughout testing (days 1 - 15) for subjects in the saccharin-saline vs. the saccharin-heroin condition. Taste-drug pairings occurred on odd-numbered days..............................46

Figure 3-1: LiCl-induced suppression of saccharin intake in water deprived rats ........................................................................................................58

Figure 3-2: LiCl-induced suppression of saccharin intake in food deprived rats.................................................................................................................60

Figure 3-3: LiCl-induced suppression of saccharin intake in free-feeding rats.................................................................................................................62

Figure 3-4: Cocaine-induced suppression of saccharin intake in water deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 8 doses of cocaine (closed circles)........................................................................64
Figure 3-5: Cocaine-induced suppression of saccharin intake in food-deprived rats. .................................................................66

Figure 3-6: Cocaine-induced suppression of saccharin intake in free-feeding rats .................................................................67

Figure 3-7: Morphine-induced suppression of saccharin intake in water-deprived rats ............................................................69

Figure 3-8: Morphine-induced suppression of saccharin intake in food-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine (closed circles). .................................................................71

Figure 3-9: Morphine-induced suppression of saccharin intake in free-feeding rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine (closed circles). .................................................................72

Figure 4-1: **Left panel.** Mean (+/- SEM) intake (licks/5 min) of 0.15% saccharin following 13 pairings with the opportunity to self-administer either saline (n=17) or cocaine (0.33 mg/infusion, n=18) for 1 h using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small (n=8) and large suppressers (n=10) on the basis of the median split for saccharin intake. .................................................................91

Figure 4-2: **Left panel.** Mean (+/- SEM) log 10 latency (sec) to initiate licking the 0.15% saccharin solution following 13 pairings with the opportunity to self-administer either saline (n=17) or cocaine (0.33 mg/infusion, n=18) for 1 h using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small (n=8) and large suppressers (n=10) on the basis of the median split for saccharin intake. .......................94

Figure 4-3: **Left panel.** Mean (+/- SEM) number of infusions/h of either saline (n=17) or cocaine (0.33 mg/infusion, n = 18) following 13 saccharin-infusion pairings. The unconditioned stimulus (saline or cocaine) was infused using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small (n=8) and large suppressers (n=10) on the basis of the median split for saccharin intake. .......................97

Figure 4-4: A correlational analysis of each rat’s terminal intake (i.e., licks/5 min on the final two days of testing) of the saccharin
conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a strong negative relationship where low saccharin intake was highly correlated with high drug self-administration behavior. ..........................100

Figure 4-5: **Left panel.** Mean (+/- SEM) log 10 latency (sec) to obtain the first iv infusion of either saline (n=17) or cocaine (n=18, 0.33 mg/infusion) across 13 saccharin-drug pairings using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small (n=8) and large suppressers (n=10) on the basis of the median split for saccharin intake. ......................................................................................102

Figure 4-6: **Left panel.** Mean (+/- SEM) number of iv infusions/h across a range of cocaine doses (0.04, 0.08, 0.16, and 0.33 mg/infusion) administered in a descending order across days. **Middle panel.** Mean (+/- SEM) number of iv infusions/h across a range cocaine doses, depicted as a function of operant (fixed ratio 10 lick contingency on the spout, or fixed-ratio 1 contingency on a lever). **Right panel.** Mean (+/- SEM) number of iv infusions/h across a range of cocaine doses, collapsed across the operant, but depicted separately for the saline rats, small suppressers, and large suppressers in Experiment 1. ...............107

Figure 4-7: **Left panel:** Mean (+/- SEM) intake (licks/5 min) of the 0.15% saccharin solution for rats in the saline group (n=9), the small suppressers (n=5), and the large suppressers (n=6) in Experiment 1 when tested following a period of at least 30 days of abstinence. **Right panel:** Mean number of infusion attempts/h (i.e., completions of the 10 lick contingency) for the same subjects when given the opportunity to lick on the fixed ratio 10 lick contingency on the empty spout. This was essentially an extinction trial, however, during which time neither saline nor cocaine was delivered. ..............................................................................113

Figure 5-1: Mean (+/- SEM) intake (licks/5 min) of 0.15% saccharin following 18 pairings with either active or yoked cocaine (0.33 mg/infusion) for 1 h. * = significantly different from saline controls, # = significantly different from active cocaine and saline controls. ............................135

Figure 5-2: A depiction of the same data as in figure 5-1 but divided into small (left panel) and large (right panel) suppressers. Mean (±SEM) intake (licks/5 min) of 0.15% saccharin following 18 pairings with either saline or active vs yoked cocaine (0.33 mg/infusion) for 1 h. * = significantly different from saline controls, # = significantly different from active cocaine and saline controls ....................................................137
Figure 5-3: Mean (+/- SEM) number of infusions/h of either saline or active (left panel) vs. yoked cocaine (right panel) following 18 saccharin-cocaine (0.33 mg/inf) pairings. The data are depicted with the rats in the cocaine groups divided into small and large suppressers on the basis of the saccharin intake of the active cocaine rats. * = significantly different from saline controls and large suppressers; # = large suppressers significantly > saline and small suppressers .................................................................139

Figure 5-4: A correlational analysis of each rat’s average intake (i.e., licks/5 min) across trials 2-18 of the saccharin conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a strong negative relationship where low saccharin intake was highly correlated with high drug self-administration behavior (left panel) or high administration of yoked cocaine (right panel). ........................................................................................................................................141

Figure 5-5: A correlational analysis of each small suppresser’s terminal intake (i.e., licks/5 min on the final two days of testing) of the saccharin conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a negative relationship where lower saccharin intake was correlated with higher levels of cocaine administration in both the active (left panel) and yoked (right panel) small suppressers. However, this relationship attained statistical significance only when the drug was delivered non-contingently........................................................................143

Figure 5-6: Left Panel: Mean (± SEM) active cocaine intake (0.33 mg/inf) on a fixed (left of dashed line) and progressive (right of dashed line) schedule of reinforcement for rats with a history of either active or yoked cocaine in Experiment 1. Right Panel: Mean (± SEM) number of total contacts with the active empty spout on the progressive ratio day for the rats with a history of either active or yoked cocaine in Experiment 1..........................................................147

Figure 5-7: A depiction of the same data that appears in Figure 5-6 separated on the basis of saccharin intake in Experiment 1. Mean (± SEM) active cocaine intake (0.33 mg/inf) on a fixed (left of dashed line) and progressive (right of dashed line) schedule of reinforcement for large (left panel) and small (right panel) suppressers with a history of either active or yoked cocaine in Experiment 1..........................150
Figure 5-8: Mean (±SEM) choices on the left or right empty spout operant to gain access to either water for 20 sec or a cocaine infusion (0.33 mg/inf) in the 1 hour choice tests for the rats separated by their history of saline or active vs. yoked cocaine in Exp 1. ..........................................................154

Figure 5-9: A depiction of the same data as in Figure 5-8 but divided into large and small suppressers on the basis of saccharin intake in Exp 1. Mean (±SEM) choices on the left or right empty spout operant to gain access to either water for 20 sec or a cocaine infusion (0.33 mg/inf) in the 1 hour choice tests for the rats separated by their history of saline or active vs. yoked cocaine in Exp 1 ..........................................................156

Figure 6-1: Mean (+/- SEM) intake (licks/5 min) of 0.1 M or 1.0 M sucrose across preshift trials 1-10, followed by the post shift trial (11). .................171

Figure 6-2: Mean cocaine (0.33 mg/inf) intake on a progressive ratio test on trial 11 (shift day). Left Panel: Rats that had access to a consistently high (1.0 M) or low (0.1 M) concentration of sucrose for all 11 trials. Right Panel: Rats that had access to the high or low concentration of sucrose for 10 trials and were shifted to the opposite concentration for trial 11 (shift day) just prior to cocaine access. Red box indicates sucrose history over the 10 pre-shift trials.............................172

Figure 6-3: Brief (5 min) daily access to a high concentration of sucrose (1.0 M) for 10 (or 11 days for the Unshifted-High controls) reduced cocaine self-administration on a progressive ratio schedule relative to control rats that had similar access to a low concentration (0.1 M). The relative loss or gain of reward on the sift day (trial 11) did not override this effect. * = significance at the p<.01 level. ......................................................174

Figure 7-1: Mean (± S.E.M) intake (ml/5 min) of 0.15% saccharin in SHAM and VTA-lesioned (VTAx) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings. .......................189

Figure 7-2: Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTA-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings. ......................................193

Figure 7-3: Mean (± S.E.M) intake (licks/5 min) of 0.1 M sucrose in SHAM and VTA-lesioned (VTAx) rats. Baseline intake was assessed on trials 1-5. Intake on the remaining trials was assessed 35 min after an intraperitoneal injection of either saline (trials 6-8 &10) or a 10 mg/kg dose of chlordiazepoxide (trial 9). .................................................................198
Figure 7-4: Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTA-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings.

Figure 7-5: Mean (± S.E.M) intake (ml/5 min) of 0.15% saccharin in SHAM and VTA-lesioned (VTAx) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings.

Figure 7-6: Mean (± S.E.M) intake (g/h) of powdered chow in SHAM and VTA-lesioned (VTAx) rats. Baseline feeding was assessed on trials 1 & 2. Food intake on the remaining trials was assessed 30 minutes after an intraperitoneal injection of either saline (trials 3 & 5), a 4 mg/kg dose of morphine (trial 4), or after 24 h food deprivation (trial 7).

Figure 8-1: Behavioral responses to saccharin that predicts cocaine access. Rats exhibited predominantly appetitive orofacial reactions to infusion of the CS- (A) but aversive reactions to the CS+ (B). These responses were reflected in the patterns of EMG activity (C and D). An analysis of oromotor behavior during the session revealed appetitive taste reactivity expressed for the CS- (E) and aversive taste reactivity for the CS+ (F). Asterisks denote significant differences, p<.05. Aversive taste reactivity scores were highly correlated with the acquisition of stable cocaine self-administration (G) and Drug loading in the final training day before testing (H).

Figure 8-2: The activity of NAc neurons reflects hedonic devaluation. Infusions of orange (A) and grape (B) flavored saccharin solutions in naïve rats primarily elicited inhibitory responses. Infusions of the CS- at test primarily elicited inhibitory responses (C) while infusions of the CS+ primarily elicited excitations (D). Two populations of neurons were responsible for the altered response profile. Twelve neurons exhibited inhibitory responses for the CS- (E) and excitatory responses for the CS+ (F). Seven neurons exhibited no response to the CS- (G) but increased firing rate to the CS+ (H). Horizontal lines indicate the infusion period.
LIST OF TABLES

Table 3-1: Summary of suppressive effects across drugs and deprivation states .................................................................74

Table 7-1: Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines relative to SHAM Controls for the Rats in Experiment 1. .......................................................................................188

Table 7-2: Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines Relative to SHAM Controls for the Rats in Experiment 2. .................................................................202
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cure to existential angst - something I had in abundance. This search coexisted well with the pursuit of a psychological/philosophical education but did not serve to promote what one might call a "sane" existence. With my sanity in question (at least to me) I embarked on questioning just about everything I could and I became quite distressed in my search for the profound meaning of life. Doc was with me every step of the way and in such a manner that no one else on earth could have been. He demonstrated an almost superhuman patience and ability to comprehend what most would have called (and many did) the insane ramblings or rants of a crazy person. He helped me learn to corrall, structure, and interpret my rather intense and disorganized way of thinking. In so doing, Doc and I forged a classic mentor-mentee relationship that was like capturing lightening in a bottle. When I look back on my time as an undergraduate I can safely say that were it not for this pivotal relationship my life’s journey since then would not have been as rich or as meaningful. I carry with me the lessons I learned at the time - his witty and wise advice still fending off “demons” be they internal or external. While exploring life’s deeper issues, little did I know that he was helping me further mold myself into the scientist that I wanted to become.

Dr. Patricia Sue Grigson was my mentor while I was a graduate student. Beyond her contribution to the intellectual backbone of my graduate investigations, only some of which form the pages of this dissertation, Sue has been an outstanding mentor not just for my career development but also for my development as a human being. Sue has overseen and helped me achieve an
emotional maturity that I did not have before. Sue has taught me to be both intellectually ambitious and rigorous without abandoning a benevolent attitude toward colleagues and competitors alike. She does all this first by example, and then if you ask, she will tell you how she does it without hesitation, in great detail, and tailored to your ‘ear’. As a mentor, she does not hold back on sharing ideas and it is this quality that continues to amaze me. In the academic world of science, ideas are the currency upon which we ‘bank’. Many scientists cling to their ideas with a selfishness that would suggest they will never have another good one. Not Sue. Scientific discussions with her are synergistic for both parties as they always lead to both a greater quantity and quality of ideas for potential experiments (of which there are a seemingly endless supply). She is a fearless and creative contributor to the field and a fearless defender of her students and her family. Somehow, Sue was always able to inspire one to do yet even more grueling work without ever uttering a single threat or ever displaying anger of any degree – fear was never her tool. She, like my parents and the Doc, are true mentors. Only with humble pride do I carry forth pieces of their visions. I thank you all from the bottom of my heart and (hopefully) the top of my intellect.
Chapter 1

Introduction

1.1 The most important aspect of drug addiction

Evolution has instilled in both humans and animals an innate capacity to learn to seek pleasure from a variety of sources and to avoid pain. In fact, actions that are essential to the survival of either the organism or the species such as mating, eating, and drinking, are invariably pleasurable. The subjective experience of pleasure is typically perceived when engaging the sensory systems, but may even begin in anticipation of consumption of a given reward or goal object (Breiter et al., 1997; O'Doherty, Deichmann, Critchley, & Dolan, 2002). These sensory systems send neural projections to areas of the brain that, when activated, produce feelings of euphoria in humans (Rolls, 2006; Sem-Jacobsen, 1976) and reinforce actions aimed at obtaining these rewards in both humans and animals (Kornetsky & Esposito, 1979; Kornetsky, Esposito, McLean, & Jacobson, 1979; Olds, 1967). To protect the organism from damaging itself through over consumption, organisms also have evolved the capacity to stop seeking or consuming rewards. These feedback mechanisms reduce the sensation of pleasure with continued consumption of a reward (Kringelbach, O'Doherty, Rolls, & Andrews, 2003). This is why turkey tastes far better at the beginning of Thanksgiving dinner than at the end.
Such is not the case with the consumption of drugs of abuse. Drugs of abuse powerfully activate, and directly interact with, brain reward systems and they do not rely, at least initially, on the senses to produce their pleasurable effects (Wise, 2002b; Zittel-Lazarini, Cador, & Ahmed, 2007). In fact, drugs of abuse are so powerful that by mere association they can turn ordinary sights, sounds, places, smells, and tastes into profound emotional experiences and coveted goals in and of themselves (Berridge & Robinson, 2003; Childress et al., 1999; Ehrman, Robbins, Childress, & O'Brien, 1992; O'Brien, Childress, McLelan, & Erhrman, 1992; Robinson & Berridge, 2003; Volkow, Ding, Fowler, & Wang, 1996). In this way, abused drugs ‘hijack’ brain reward systems by eroding self-control and redirecting attention and motivated behaviors toward the drug and to the various cues associated with drug taking (Baler & Volkow, 2006; Goldstein, Alia-Klein et al., 2007; Goldstein, Tomasi, Alia-Klein et al., 2007; Goldstein, Tomasi, Rajaram et al., 2007; Vanderschuren & Everitt, 2005; Volkow et al., 2006). Unlike ingested ‘natural’ rewards, the mechanism for satiety for the consumption of abused drugs is not known (Cabanac & Duclaux, 1970; Zittel-Lazarini et al., 2007). Indeed, there is little evidence for satiety. In fact, as drug taking escalates, the incentive value of the drug increases despite tolerance to the drugs euphoric effects (Robinson & Berridge, 1993b). This has profound implications for the addict because each experience with the drug-taking ritual engenders the next with more motivational significance (e.g., “needing” the drug) even while stripping away at the experience of the pleasurable effects. Furthermore, as brain reward substrates are repeatedly overstimulated by drugs
of abuse they become hypersensitive in the presence of drug and hyporesponsive when the drug effects wear off (Ahmed & Koob, 2005; Koob, 2006). This hyposensitivity manifests behaviorally as depression (Carlezon et al., 2006; Carlezon, Duman, & Nestler, 2005; Nestler & Carlezon, 2006), and reduces the sensitivity to the relative value of less powerful natural rewards (e.g., sucrose, food, sex) and increases drug craving and seeking (Barr, Fiorino, & Phillips, 1999; Barr & Phillips, 1999). Therefore, as addicts compulsively preoccupy themselves with drug seeking and drug taking, they are less motivated to pursue, and less affected by, natural rewards. It is precisely this devaluation of natural rewards by drugs of abuse that is the primary focus of this dissertation.

This behavioral manifestation is the single worst, and most important, component of drug addiction. In fact, the problem is not that drugs of abuse directly and powerfully engage brain reward systems. The real problem is that the consequences of this indulgence, when habitually repeated, leads to profoundly dysfunctional behavior. The DSM-IV distinguishes between mere physical dependence, which is defined by the appearance of withdrawal effects upon drug abstinence, and addiction which may or may not include signs of overt physical dependence. Instead, it refers to substance abuse disorder as the compulsive use of the drug despite adverse consequences. Losing the capacity to respond appropriately to natural rewards is a highly adverse consequence by definition and it underlies all of the devastating effects that drug addiction has on the individual addict and the toll it takes on society. For humans addicted to
psychostimulants, opiates, and/or alcohol, this dysfunctional devaluation of natural rewards is particularly devastating. For instance, these drug addicts have difficulty maintaining employment (Jones, Casswell, & Zhang, 1995), their own health, nutritional requirements (S. W. French, 1993; Santolaria-Fernandez et al., 1995; Virmani, Binienda, Ali, & Gaetani, 2006), and caring for their children (Nair et al., 1997). Instead of taking care of themselves and their families, the National Institute on Drug Abuse (NIDA) cites a report from the Office of National Drug Control Policy (ONDCP) which shows that between 1988-1995, drug addicts spent $57.3 billion on illegal drugs of abuse. The most was spent on cocaine ($38 billion) and heroin ($9.6 billion), followed by marijuana ($7 billion). This is a significant amount of capital that otherwise could have been spent legitimately or even saved by the individual user. Since 1995, cocaine and heroin use has continued to rise in urban environments so the cost is even higher today.

Furthermore, the cost to society does not end with the direct cost of drugs. The indirect costs are substantially higher. In 1992, the total economic cost of alcohol and drug abuse was estimated by the National Institute on Drug Abuse (NIDA) to be $245.7 billion. More than half of this figure consisted of the cost of drug related crime (i.e., incarceration, property damage, corrective services). While, approximately 41% (~$100 billion) was lost due to drug-related illness, absenteeism in the workplace, health care, and even premature death.

The individual and social cost of this devastating feature of human drug addiction is staggering, yet it is the focus of very few drug treatment strategies. Instead, the primary method of rehabilitation is either to impose abstinence
through incarceration or wait for addicts to seek treatment on their own and voluntarily abstain. For addicts seeking treatment, detoxification, drug replacement therapy, and counseling are the most common options. For instance, methadone, a long-acting partial μ-agonist, was evaluated in human heroin addicts as a heroin replacement therapy more than 40 years ago (Dole & Nyswander, 1965) and is still in use today (Connock et al., 2007). While methadone and buprenorphine, a similar μ-agonist that also is a κ-receptor antagonist, are both vital adjuvants to therapy (Boothby & Doering, 2007), they do not cure heroin addiction as relapse remains a lifelong risk (van den Brink & van Ree, 2003). The same is true for anti-depressant treatment of nicotine or cocaine addiction (Hughes, Stead, & Lancaster, 2007). Moreover, for cocaine addicts the situation is even worse as there are still no effective pharmacologic interventions approved for treatment (van den Brink & van Ree, 2003). Taken together, it is clear that no available treatments are truly effective at preventing relapse. Therefore, addicts need more than the mere reduction of pain associated with drug withdrawal. It is noteworthy that the few drug treatment programs that offer incentives to addicts that successfully abstain report less recidivism in a greater proportion of patients and for longer periods of time compared to ones that do not (Budney, Higgins, Radonovich, & Novy, 2000; Budney, Moore, Rocha, & Higgins, 2006; Higgins, Badger, & Budney, 2000; Higgins et al., 1994; Van Etten, Higgins, Budney, & Badger, 1998). These findings demonstrate that responding to natural rewards is a key component in the development of addiction and in recovery. Despite this, there are no animal
models that target the mechanism of this critical feature. Therefore, the basic research contained within this dissertation is organized under one theme: To create a rodent model of drug-induced devaluation of natural rewards and cue induced craving. Before that research is outlined, however, the theoretical construct upon which it was built must be described.

1.2 Contrast Effects

1.2.1 As a general phenomenon

Contrast effects are among the most fundamental and pervasive perceptual phenomena in the animal kingdom yet they depend upon a complex convergence of processes including sensation, perception, learning, memory and behavioral output. They readily occur in humans (Zellner, Allen, Henley, & Parker, 2006) and in animals as diverse as the bumblebee (Wiegmann, Wiegmann, & Waldron, 2003). Simply put, they are the exaggeration of the perceived differences between stimuli as a result of the opportunity to compare those stimuli. A contrast effect occurs when a given stimulus is compared with another stimulus on a similar dimension that is of either greater or lesser magnitude or value. This causes the target stimulus to be perceived as though it were of lesser or greater magnitude than when it is perceived alone. In humans, contrast effects can occur along complex cognitive dimensions such as in the ratings of the level of “abstraction” of paintings (Specht, 2007) or along more
simple dimensions such as ratings of the level of sweetness of sugar solutions (Specht & Twining, 1999). However, in animals, contrast effects are traditionally observed only by evaluating motivated behaviors like ingestive behaviors or work output on different operandi (e.g., lever pressing, runway speed, nose poking). Therefore, contrast effects in animals are manifest in the form of either elated or depressed consumption of, or operant responding for, a target reward relative to the same reward when presented alone (L. P. Crespi, 1942; L P Crespi, 1944).

It is within the context of motivated behaviors that the functional significance of contrast effects is apparent. For instance, Crespi’s depression effect or successive negative contrast as it is called today, was first observed by O. L. Tinklepaugh in (1928) when a laboratory monkey refused a normally acceptable piece of lettuce after witnessing the researcher hide a more preferred banana under a cup from which the subject regularly retrieved food rewards. Unbeknownst to the monkey, the researcher switched the reward back to the piece of lettuce. This did not please the monkey who searched vigorously for the banana and “shrieked in apparent anger” at the researchers (from, Flaherty, 1996).

1.2.2 Rodent Models of Contrast Effects

The concept of contrast effects in instrumental performance following shifts in reward magnitude did not receive rigorous treatment until Leo P. Crespi discovered and then elaborated on it in the albino rat in the early 1940’s (L. P.
Crespi, 1942; L P Crespi, 1944). The terms “successive negative contrast” and “successive positive contrast” were coined by Zeaman (1949) who independently observed the same effects that Crespi did in his rats; Zeaman used these terms to avoid any emotional connotation that was inherent in the terms “depression effect” and “elation effect” (Flaherty, 1996).

There are essentially three major models of consummatory contrast that are studied extensively in rodents today. The first, a simultaneous contrast effect, occurs when rats are given repeated opportunities to compare two disparate levels of a reward within a single daily session. For example, rats with access to alternating high and low sucrose concentrations consume comparatively more of the high and less of the low than their respective control groups that have been maintained on only high or low sucrose (Flaherty, 1996; Flaherty & Rowan, 1986). This is the simplest procedure from which to produce contrast effects rapidly, in a single day, and is thought to rely on a short term memory process (P. S. Grigson, Kaplan, Roitman, Norgren, & Grill, 1997) rather than emotional processes (Flaherty, Lombardi, Kapust, & d'Amato, 1977). The second, successive negative contrast (mentioned above), has a long history of study and occurs when rats with a history of once-daily 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 each day (for review see, Flaherty, 1982). This effect occurs as a result of a retrograde comparison between the memory of the 32% solution on the day before with the currently
available 4% solution (Flaherty, 1996). This memory of the preshift solution, expressed as significant negative contrast upon exposure to the lesser solution, is quite robust lasting up to 70 hours upon a single 5 minute exposure (Flaherty, Ciszewski, & Kaplan, 1979) and up to 17 days after the typical 10-days of daily 5-min exposures (Ciszewski & Flaherty, 1977; Gordon, Flaherty, & Riley, 1973).

Finally, and of particular importance to this dissertation, is anticipatory negative contrast (ANC). In ANC, rats are given daily access to a sweet tasting reward such as a 0.15% saccharin solution that reliably predicts subsequent access to a more preferred reward such as 32% sucrose. The mechanism is thought to involve classical conditioning whereby the saccharin conditioned stimulus (CS) comes to predict access to the sucrose unconditioned stimulus (US) over repeated daily pairings. Indeed, the saccharin enters into an anterograde association with the imminent 32% sucrose solution and not a retrograde comparison with the memory of sucrose the day before (i.e., successive negative contrast). This was determined by implementing a within subjects design using contextual and temporal cues that accompanied the saccharin cue only on days that it predicted the sucrose solution (Flaherty & Rowan, 1985). In this case, an anticipatory negative contrast effect then occurred when intake of the otherwise rewarding saccharin solution was suppressed in anticipation of the impending, more preferred, sucrose reward (Flaherty & Checke, 1982a; Flaherty & Grigson, 1988; Flaherty & Rowan, 1985, 1986). In fact, groups of rats that consumed the least of the saccharin cue actually consumed the most sucrose (Flaherty, Turovsky, & Krauss, 1994). These data indicate that the motivation to consume
the US exerts an inhibitory effect on the consumption of the less preferred, predictive saccharin cue (Flaherty, 1996).

1.2.3 Functional Significance of Contrast Effects

At first glance a contrast effect may seem like little more than a perceptual anomaly and, as such, have little value for survival. However, successive negative contrast provides a great example to the contrary (e.g., the monkey’s response to the lettuce when he thought he was going to get a banana). Generally, rats and other animals in the wild do not live with the convenience of supermarkets as do humans. Instead, they are opportunistic because they are never certain of the constancy of their food supply and always foraging for new sources of nourishment. With this in mind, it does not seem as surprising that a change in the expected amount or quality (i.e., caloric density, hedonic value) of an ingested food item profoundly affects motivation and behavior. In fact, an exaggeration of that change could be quite beneficial. For example, experiencing a relative loss of reward such as a reduction in magnitude or quality of the current expected level of food reward, which occurs in successive negative contrast, could signify a need to forage in a new location (Flaherty, Grigson, Coppetelli, & Mitchell, 1996; Timberlake, Gawley, & Lucas, 1988). Importantly, the animal has to decide whether to accept this lesser reward or embark on a search to recover the superior reward or find a new one altogether. If this reduction in reward was of no significant consequence, then animals would
simply return to the typical level of responding that is appropriate for the lesser reward without experiencing stress or increased locomotion. However, the data support a different story.

Successive negative contrast effects occur in conjunction with behaviors that suggest a search strategy replete with conflict. Rats that had access to 32% sucrose and then were shifted to 4% sucrose immediately engage in increased ambulation and rearing that is correlated with the magnitude of contrast (Flaherty, Blitzer, Collier, 1978 exp. 2; Flaherty, 1996). Moreover, closer analysis of the intake pattern suggests that negative contrast is a product of less sustained licking, or longer departures from the spout during the access period (P. S. Grigson, Spector, & Norgren, 1993). However, when the rats were licking the reduced sucrose solution there was no change in the rate of licking (P. S. Grigson et al., 1993). Taken together, these findings suggest that as ambulation and rearing increase, the pattern of intake changes in important ways which ultimately result in consummatory negative contrast. This is a vexing circumstance for the rat that must weigh whether to accept and ingest the reduced reward (which is better than nothing) or avoid it in favor of expending energy on a search for the missing reward (Flaherty, 1996). This interpretation is supported by successive negative contrast experiments that were conducted in an eight-arm radial maze. It was demonstrated that the search pattern was, in fact, not random. Rather, the search was directed toward the location of the lost reward. Specifically, on the shift day, the rats would more frequently enter an arm that had at one time had a high level of sucrose reward as apposed to any other
arm (Flaherty, 1996). Furthermore, this decision is made under duress as it results in activation of the hypothalamic-adrenal-pituitary (HPA) axis as evidenced by increased secretion of the stress hormone corticosterone on the second post shift day (Flaherty, Becker, & Pohorecky, 1985; Mitchell & Flaherty, 1998). In fact, this hormone plays an important part in the decision to accept or reject the reduced reward. For instance, the administration of corticosterone immediately after the shift enhances SNC (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006) while manipulations designed to reduce anxiety like administration of anxiolytics agents (Becker & Flaherty, 1983; Flaherty, Grigson, & Rowan, 1986; Flaherty, Lombardi, Wrightson, & Deptula, 1980; Freet, Tesche, Tompers, Riegel, & Grigson, 2006; Genn, Barr, & Phillips, 2002) or even prior ejaculations (Freidin, Kamenetzky, & Mustaca, 2005) facilitates recovery on the second post-shift day. Taken together, these data indicate that the hedonic contrast effect is deeply embedded and serves as a vital component of animal behavior that facilitates adaptability to ever-changing circumstances. However, might this adaptive, ingrained perceptual anomaly which continues to serve animals so well today actually be a liability under some circumstances?

1.3 Contrast effects and a common neural substrate for reward

Although hedonic contrast effects appear beneficial under some circumstances they are detrimental when extreme. The devaluation of natural rewards by drugs of abuse is a perfect example of that detrimental effect, but
how this occurs is not entirely clear and underscores the need for a behavioral model. Conceptually, it is intuitive that smaller rewards when compared to large ones lose their value. However, to understand the mechanism by which drugs of abuse might devalue natural rewards, an understanding of the neural systems involved in reward is required.

Sex, feeding, drinking, sleep, and caring for young, are among the most reinforcing motivated behaviors. Animals regulate these motivations well probably because they have evolved with them for many thousands of years. The motivation to “approach” and/or “consume” these rewards is fixated on a single goal at a time and varies depending on a variety of factors (e.g., severity of hunger or deprivation, mating season). Animals do not typically 'consume' these rewards simultaneously. Instead they engage in these goals sequentially. For example, it is not possible to engage in reproductive behaviors, feed, and sleep simultaneously. Nevertheless, the opportunity to engage in these behaviors are often present simultaneously. Therefore, the animal must compare these rewards and obtain the one that it wants/needs the most. In order to compare these vastly disparate rewards the animal has to evaluate them along some common dimension. That dimension, not surprisingly, is its perceived reinforcing value. Therefore, despite the fact that rewards of different modalities are sensed and perceived in different ways and through separate neural circuits, there is believed to be a common neural substrate that all rewards must impact (Cabanac, 1971, 1992; Cabanac, Guillaume, Balasko, & Fleury, 2002; Ramirez & Cabanac, 2003).
Contrast, particularly cross modal contrast (e.g., between food and drugs of abuse), is evidence of this common substrate (P. S. Grigson, 2000, 2002).

There is a tremendous body of literature that describes the important role of the mesolimbic dopamine system for responding to rewards. The discussion of this system mainly centers on the release of dopamine from ventral tegmental area (VTA) neurons that synapse on medium spiny neurons in the nucleus accumbens (NAc) (Schultz, 2001; Wise & Bozarth, 1984). The precise role for dopamine in the subjective experience of reward has been hotly debated and is a controversial topic to this day (Baldo & Kelley, 2007; Berridge, 2006; Hajnal & Norgren, 2005; Koob, 2006; Nestler & Carlezon, 2006; Ungless, 2004; Wise, 2004; Young, 2004). It was believed for some time that dopamine mediates the subjective experience of pleasure inherent to all rewards and this stems largely from the very early work conducted in this area (Bozarth & Wise, 1983; Fouriezos & Wise, 1976; Wise, 1978, 1984; Wise & Bozarth, 1985; Wise, Spindler, deWit, & Gerberg, 1978; Wise, Spindler, & Legault, 1978). However, today this has been largely replaced by the view that dopamine mediates important aspects of motivation and reward processing, not reward per se (Baldo & Kelley, 2007; Berridge, 2006; Salamone, 2007).

The evidence of dopamine’s involvement in reward processing is staggering and comes from a wide array of techniques and several decades of intense research. For instance, brain regions that are rich in dopamine support lower levels of intra cranial self stimulation (ICSS) than other brain regions indicating an increased efficacy of these areas to support positive reinforcement.
(Olds, 1967). Furthermore, electrodes monitoring neuronal activity in the VTA and the NAc reveal phasic changes in firing rates when water deprived rats consume water and when normally sated rats either self-administer or are passively injected with abused drugs such as cocaine, ethanol, or Δ⁹-tetrahydrocannabinol (Δ⁹-THC - the active agent in marijuana and hashish) (Brodie, Pesold, & Appel, 1999; Carelli & Deadwyler, 1994b; E. D. French, Dillon, & Wu, 1997; Wu & French, 2000). As a direct result of VTA neuronal excitation, in vivo microdialysis probes implanted into the NAc detect significant increases in extracellular dopamine when rats are injected with, or are self-administering, any drug of abuse that is self administered by humans (Di Chiara et al., 1999). Moreover, dopamine in the NAc increases as a function of sucrose concentration under sham feeding conditions with no post ingestive feedback (Hajnal, Smith, & Norgren, 2004) or when rats consume a neutral, non-rewarding taste that is paired with nearly simultaneous intragastric infusion of nutritive polycose (G. P. Mark, Smith, Rada, & Hoebel, 1994). A similar increase in DA is observed when rats are presented with sexual opportunities (Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Pfaus, Damsma, Wenkstern, & Fibiger, 1995), or when food-, or water-, deprived rats are allowed to alleviate deprivation respectively (Martel & Fantino, 1996a, 1996b; Young, Joseph, & Gray, 1992).

Interestingly, while accumbens DA is increased in naïve animals when a palatable saccharin CS is presented, if that taste is devalued by association with an aversive agent such as LiCl, via conditioned taste aversion (CTA; see below for a description) DA tracks that devaluation (G.P. Mark, Blander, & Hoebel,
1991). Though not as strong, this effect is also seen with successive negative contrast. When rats were shifted from 32% to 4% sucrose, the normal dopamine efflux for 4% sucrose was blunted compared with the DA response elicited by the group maintained on 4% sucrose (Genn, Ahn, & Phillips, 2004). Since animals readily work for both ‘natural’ and drug reinforcers and this results in increased DA in the NAc it was concluded that increased DA was the defining neural element of pleasure or euphoria (i.e., the subjective experience of reward).

Furthermore, it was thought that by blocking or depleting NAc DA it would eliminate the euphorogenic properties of rewarding stimuli and, thus, eliminate behavioral reinforcement and induce a general “anhedonic” state (Wise, Spindler, deWit et al., 1978). For instance, the dopamine antagonists pimozide and haloperidol alter operant responding to ICSS or psychostimulant self-administration in a manner proportional to the degree of blockade. Partial antagonism leads to a rightward shift in the dose or current response curve indicative of the need for an increased dose or current to achieve the same level of reinforcement. Total antagonism leads to extinction of the operant response (Fenton & Liebman, 1982; Zarevics & Setler, 1979). The same is true for self-administration of psychostimulants (Corrigall & Coen, 1991; Pilla et al., 1999; Weissenborn, Deroche, Koob, & Weiss, 1996; Wise & Bozarth, 1985).

On the other hand, depleting food of its ‘goodness’ proved more problematic. Interestingly, extensive depletion of ascending dopamine via microinjection of the DA selective neurotoxin 6-hydroxydopamine (6-OHDA) into the nigrostriatum, lateral hypothalamus, and ventrolateral striatum, caused rats
with plentiful food supplies to die of starvation (Berridge & Robinson, 1998b; Marshall, Richardson, & Teitelbaum, 1974). These starvation deaths were probably due to severe motor/performance deficits (Bakshi & Kelley, 1991; Salamone & Correa, 2002). However, severe 99% dopaminergic depletion in the NAc, which leaves motor faculties in tact (e.g., grooming, chewing, licking etc), does not effect consumption unless work is required to obtain the meal (Aberman & Salamone, 1999). This is a critical finding as it reveals that NAc DA depletion does not cause food to lose its inherent rewarding properties. Nevertheless, the food still loses value as a behavioral reinforcer and fails to energize lever pressing while leaving general consumption in tact.

Indeed, the evidence for a selective appetitive motivational deficit in rats with compromised NAc dopamine is quite compelling. Dopamine depletions do not prevent rats from appropriately choosing 4 food pellets over 2; however, it renders them significantly more likely than control rats to select 2 pellets over 4 if there is an obstacle to climb over to get the larger reward (Salamone, Cousins, & Bucher, 1994). Furthermore, these lesioned rats obtain as many food pellets as control rats on low fixed ratio requirements but significantly fewer when having to perform on even a modest fixed ratio (Aberman & Salamone, 1999; Salamone, Wisniecki, Carlson, & Correa, 2001) or progressive ratio and/or force requirements (Hamill, Trevitt, Nowend, Carlson, & Salamone, 1999; Ishiwari, Weber, Mingote, Correa, & Salamone, 2004).

While the motivation to work for food may be mediated by dopamine, it appears that the subjective experience of pleasure inherent to feeding may be
more closely attributed to the opiates that bind at the µ-receptor. There are very few non-confounding procedures to test this hypothesis since eliminating or enhancing the pleasurable effects of food items would tend to have a corresponding effect on motivation as well. For example, when work requirements are high, motivation and pleasure are conflated with mere µ or dopamine agonist treatment. For example, either D-Ala2, NMe-phe4, Glyol5-enkephalin (DAMGO) or amphetamine directly into the NAc enhances performance on a progressive ratio task for sugar pellets (Zhang, Balmadrid, & Kelley, 2003).

One solution to this problem is to keep work requirements low and measure the general consumption of food while manipulating dopaminergic signaling. To that end, microinjections of D1 and D2 antagonists directly into the NAC have no effect on the general consumption of food in deprived rats (Baldo, Sadeghian, Basso, & Kelley, 2002). Likewise, dopamine deficient mutant mice actually consume slightly more sucrose than heterozygous controls (Cannon & Palmiter, 2003). Another solution to the problem is to measure involuntary behaviors that are correlated with ingestion of hedonically pleasing foods (i.e., components of appetitive taste reactivity such as lateral tongue protrusions, see Grill & Norgren, 1978b) while manipulating the dopaminergic system in ways that enhance the motivation to work for rewards (Berridge & Robinson, 1998b). To that end, neither rats with enhanced dopaminergic signaling via NAc amphetamine microinjections nor mutant mice that are hyperdopaminergic (by dopamine transporter knockdown) exhibit altered appetitive taste reactivity during
the consumption of water, food or sucrose (Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003; Wyvell & Berridge, 2000). Finally, neither dopaminergic antagonists nor extensive depletion of dopamine by 6-OHDA reduce appetitive taste reactivity to intraoral sucrose (Berridge, Venier, & Robinson, 1989; Pecina, Berridge, & Parker, 1997; Treit & Berridge, 1990). Thus, when there is no work requirement, it is clear that manipulations of dopamine do not impact the perception, or responding for, reward.

On the other hand, both systemic morphine pretreatment or direct microinjection into the NAc of morphine or the µ-agonist, DAMGO, into the NAc increase general consumption, and conditioned (i.e., cue or context induced) consumption of high fat foods (Bakshi & Kelley, 1993; Kelley, Bakshi, Fleming, & Holahan, 2000). Moreover, only the opiate drugs (or benzodiazepines via their actions on the endogenous opiate system) enhance both appetitive taste reactivity to, and the general consumption of, sucrose or food (Pecina & Berridge, 2000, 2005; Pecina, Smith, & Berridge, 2006, and see Chapter 7; D. K. Richardson, Reynolds, Cooper, & Berridge, 2005). Interestingly, microinjections of D1 and D2 antagonists into the NAc do nothing to reduce this opiate driven, enhanced intake (Baldo et al., 2002; Kelley et al., 2000; Kelley et al., 2002). Taken together, both dopamine and opioid signaling in the ventral striatum are required for functional approach and consumption of natural rewards; however, they mediate somewhat distinct aspects of natural reward processing. Specifically, dopamine appears to mediate the willingness to approach or to work
for natural rewards while the opiates appear to mediate the hedonic sensory qualities of reward consumption.

Conversely, by a similar analysis it appears that both the appetitive motivational and subjective rewarding properties of drugs of abuse are primarily mediated by the mesolimbic DA system. This is especially true for cocaine. For example, whether work requirements are high or low, rats with dopaminergic lesions of the VTA do not acquire cocaine self-administration and, if the lesion occurs after acquisition, they undergo extinction (Roberts & Koob, 1982; Roberts, Koob, Klonoff, & Fibiger, 1980). This is not surprising given that cocaine’s reinforcing action occurs primarily through blocking dopamine reuptake and not at all via other proposed mechanisms like blockade of the serotonin or norepinephrine transporters (despite earlier controversies, see Chen et al., 2006 for a review). There are some data to suggest that the endogenous opiate system within the mesolimbic system also is involved in cocaine reward. Administration of an irreversible μ-receptor antagonist, beta-funaltrexamine, directly into either the VTA or NAc had no effect on cocaine self-administration on a fixed ratio schedule but reduced the motivation to work for cocaine on a progressive ratio schedule to a similar degree (Ward, Martin, & Roberts, 2003). Since μ-receptor activation inhibits both the GABA containing interneurons in the VTA, and GABA containing efferent neurons in the NAc that project back to the VTA (mu activation disinhibits DA release, Guttenberg, Klop, Minami, Satoh, & Voorn, 1996), it was ultimately concluded that the μ-antagonist blocked μ-receptor mediated dopamine release (Ward et al., 2003). Therefore, μ-receptor
activation in the mesolimbic dopamine system by cocaine-induced release of endogenous opiates modulates dopamine release independently (i.e., a characteristic shared by all systemically administered µ-agonists such as heroin and morphine) from its indirect agonist properties at the dopamine transporter. By doing so, these µ-receptors contribute to cocaine reward by enhancing dopamine release and may explain the synergy of both the motivational- and dopamine releasing- characteristics of heroin and cocaine mixtures (i.e., “speedball”) when compared to either drug alone (Hemby, Co, Dworkin, & Smith, 1999; J. E. Smith, Co, Coller, Hemby, & Martin, 2006).

Although it is intuitive to think that the rewarding effects of opiate drugs such as morphine and heroin are mediated by the endogenous opiate system, the evidence for this has been mixed and, more recently, the evidence points toward a mediating role for dopamine in opiate reinforcement. For example, while heroin self-administration was left in tact following DA depletion (Ettenberg, Pettit, Bloom, & Koob, 1982) morphine self-administration was not (J. E. Smith, Guerin, Co, Barr, & Lane, 1985). These and other findings have led many to propose distinct dopamine dependent and dopamine independent mechanisms for opiate reward which requires expanding the reward pathway to include NAc efferent projections to the ventral pallidum (VP) and back to the VTA (Bechara, Nader, & van der Kooy, 1998; Laviolette & van der Kooy, 2001; K. Nader, Bechara, Roberts, & van der Kooy, 1994). Indeed, lesions of the ventral pallidum (VP) and inhibition of the GABA containing NAc efferent neurons projecting to the VP (i.e., the proposed DA independent mechanism of opiate reward) were both
found to block heroin self-administration (Hubner & Koob, 1990; Xi & Stein, 2000). These NAc efferents to the VP and to the VTA, however, are inhibited directly by either dopamine or µ-receptor agonists. Furthermore, the NAc efferents to the VTA, when inhibited, cause VTA DA neurons to release more dopamine. Therefore, until the exact role of the NAc efferents to the VP are determined, the proposed dopamine independent mechanism in opiate reward remains controversial (Xi & Stein, 1999, 2000). Moreover, there is no denying that dopamine has a substantial contribution to the reinforcing effects of opiates. For instance, the dopamine D2 antagonist, haloperidol, selectively reduced the reinforcing efficacy of heroin in a runway task while leaving cue-induced enhancement of runway performance intact (Ettenberg & McFarland, 2003; McFarland & Ettenberg, 1995). Moreover, highly selective D3 receptor antagonists that have been shown to block cocaine-induced relapse, conditioned place preference, and enhancement of electrical brain stimulation (Vorel et al., 2002) also block the acquisition and expression of heroin induced conditioned place preference (Xi et al., 2004). Clearly there is a crucial role for NAc dopamine in responding to even opiate reward. Whether it is even possible to fully disentangle its role from that of the endogenous opiate system is a question for the future.

Taken together, these data demonstrate that the mesolimbic dopamine system tracks the value of all natural and drug reinforcers, modulates appetitive motivation (i.e., “work”) of natural rewards, and plays a significant role in both motivational and subjective reinforcing properties of drugs of abuse. Moreover,
disruption of this reward substrate reduces the motivation to work for natural rewards, abolishes the drive for cocaine self-administration, and severely limits opiate mediated reinforcement. So, how does the mesolimbic DA system become altered in addicts to simultaneously reduce the motivation for natural rewards but *enhance* the motivation for drugs of abuse? Does this reward substrate respond to natural rewards in a different way after comparison? Why do some people become addicted while others who try these drugs turn away? Is the propensity to consume drugs of abuse related to, perhaps caused by, the devaluation of natural rewards? To answer these questions, a rat model whereby drugs of abuse directly devalue natural rewards was developed and honed.

1.4 The Model

Humans are accomplished at the task of extracting from rewards that component which is most reinforcing. The food industry has taken advantage of this and, thereby, contributed to the obesity epidemic in America by making sure that calorically dense foods high in fat and sugar are readily available. Eating these foods under certain schedules can lead to compulsive eating habits in rats (Buda-Levin, Wojnicki, & Corwin, 2005; Corwin, 2006; Wojnicki, Roberts, & Corwin, 2006) and alter brain reward systems in a manner much like drugs of abuse (Colantuoni et al., 2002). Nevertheless, even “obese” humans and animals care for their young, and go to work (i.e., except for the morbidly obese which are rare exceptions). However, this tendency when applied to substances containing
drugs of abuse is even more pronounced. For example, when humans
discovered that they could extract the reinforcing psychoactive compounds out of
the coca leaf or the opium poppy and chemically alter and purify them, it resulted
in cocaine (Erythroxylon coca) and heroin. These are two of the most reinforcing
substances on the planet. Of course, neither one of these substances is required
for survival yet once in the blood stream, as stated above, they tap directly into
brain reward systems. Fortunately, humans and animals do not encounter these
powerful rewards frequently. However, of those humans that do encounter them
at least 15-17% become “addicted” and have difficulty stopping or limiting drug
intake, have an extremely high motivation to take the drug, and focus their
activities on the procurement and consumption of the drug despite its harmful
consequences (Deroche-Gamonet, Belin, & Piazza, 2004). These behaviors
have been modeled in rats and a very similar percentage (17%) exhibit persistent
drug seeking during signaled nonavailability or even when drug delivery was
associated with punishment in the form of an electric shock (Deroche-Gamonet
et al., 2004). Importantly, these behaviors were reliably predictive of each other
and also predicted which rats would exhibit increased motivation to work for the
drug on a progressive ratio schedule of reinforcement and drug-induced
reinstatement (an animal model of relapse) (Deroche-Gamonet et al., 2004).

Finally, the sheer magnitude of reward inherent in drugs of abuse, if
compared, would tend to overshadow any natural reward. Importantly, this
implies that rewards of different modalities are compared. While it is easy to
accept that two similar stimuli are compared along the same stimulus dimension,
such as the sweetness of sugar solutions, it is harder to accept that disparate stimuli that depend largely on different neural circuits (e.g., sex vs. food intake) can be compared at all. Nevertheless, every time we decide what to do on a Saturday night, or go to the store or even the kitchen refrigerator, we make these cross modal comparisons. These disparate objects of our affection can all be evaluated along the same, simple stimulus dimension (i.e., appetitive motivation, see above). Contrast effects are quite robust and occur independent of the context in which the stimuli are presented (Flaherty, Hrabinski, & Grigson, 1990).

Moreover, as mentioned above, rats can remember the value of the preshift solution (i.e., 32% sucrose) well enough to exhibit statistically significant negative contrast effects for up to 17 days with the typical exposure (Ciszewski & Flaherty, 1977; Flaherty et al., 1977; Gordon et al., 1973). This implies that contrast effects are occurring anywhere, and virtually at any time. This is a potentially frightening scenario for the drug user. The more regular the use the greater the potential for direct reward comparisons anywhere, at any time. Furthermore, the fact that this drug reward is particularly long lived in the memories of drug users means that there is always a potential for it to be compared to any less powerful reward, every second, of every day, and especially when provoked by drug associated cues which may exert an inhibitory effect on drives for alternative reinforcers.

As explained above, contrast effects between disparate levels of sucrose reward form the theoretical backbone of the model of devaluation of natural rewards featured in this dissertation. It is important that this model pit a natural reward against a drug reward. However, the presentation of these rewards
should not be concomitant and force a choice because this will tell us little about
the nature of devaluation by contrast. It is not surprising that any species will
choose a larger reward over a smaller one when they are simultaneously
presented (and this is not to say that concomitant access is not useful for other
models of addiction). Instead, access to the smaller reward should be given at a
time when motivation for the larger one is reliably maximal but not immediately
available, so as to allow for attention to be directed completely at the smaller
reward. Maximal motivation for drug reward is associated with cue induced
craving/withdrawal (Kenny, Chen, Kitamura, Markou, & Koob, 2006) and is
stronger if the access to the drug is reliably predictable (i.e., in anticipation of the
drug access, McBride, Barrett, Kelly, Aw, & Dagher, 2006). Furthermore, in
humans the strength of this negative anhedonic state is predictive of subsequent
cigarette smoking (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004) and the
magnitude of the euphorigenic effects of cocaine (Field, Santarcangelo, Sumnall,
Goudie, & Cole, 2006). Therefore, in order to model predictable, maximal
motivation for reward consumption in rodents, there must be a cue that informs
the subject that access to the larger one is forthcoming. In anticipatory contrast,
this smaller saccharin reward serves as the cue and informs the rat that the
preferred sucrose reward is imminent.

Using the anticipatory contrast model as a theoretical guide one does not
have to look far for a drug of abuse model that resembles it, as one already
exists and, in fact, predates it (Le Magnen, 1969). Instead of using sucrose as
the US, a drug of abuse will be injected by the experimenter. As such, it will
serve as the first stage in the development of a model of drug induced
devaluation of natural rewards and cue induced craving and makes up the first
block of experiments in this dissertation (Chapters 2 & 3). Specifically, it has
been well documented that rats will avoid intake of the normally preferred
saccharin solution when paired with the passive administration of just about any
drug of abuse such as alcohol, morphine, cocaine, amphetamine, or nicotine
(Cappell & LeBlanc, 1971; Le Magnen, 1969; LeBlanc & Cappell, 1975;
Nachman, Lester, & Le Magnen, 1970). Like anticipatory contrast, this avoidance
behavior involves classical conditioning where the saccharin solution serves as a
gustatory CS that is repeatedly paired with a drug of abuse US. After repeated
pairings, the saccharin CS serves as a cue that is associated with, and is
predictive of, the administration 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
dissertation’s assertions notwithstanding).

A significant challenge for this model is that it has been misinterpreted for
some 35 years. This phenomenon has long been interpreted as a conditioned
taste aversion (Cappell & LeBlanc, 1971; Goudie, Dickins, & Thornton, 1978;
Nachman et al., 1970). The probable reason for this interpretation is that the
same behavior, avoidance of a gustatory CS, was first observed when a tastant
was paired with aversive stimuli such as lithium chloride (LiCl) or gamma
radiation (Carroll & Smith, 1974; Garcia, Kimelford, & Koelling, 1955; Nachman &
Ashe, 1973). As a result, it is thought by many, despite the well known rewarding
properties of drugs of abuse (see van Ree, 1979 for review), that aversive drug properties are responsible for avoidance of the gustatory CS. The finding that drugs of abuse induce CTAs has been viewed as highly paradoxical and has initiated a number of relatively unsuccessful attempts to resolve the paradox. Explanations have focused upon: the nature of the drug, the dose of the drug, the time course of drug action, the nature of drug action (e.g., whether the drug induces nausea, toxicity, adipsia, or suppression of appetite), the familiarity or novelty of drug action, the route of drug administration, and even the active (i.e., use of a runway to receive passive injections) or the passive nature of drug administration (Carey, 1978; Gamzu, 1977; for review see, Goudie, 1979; Goudie & Dickins, 1978; Hunt & Amit, 1987; Stolerman & D'Mello G, 1978; Vogel & Nathan, 1975). As mentioned above, this paradoxical interpretation has predominated.

Fortunately, despite these unsuccessful attempts to solve the paradox differences have become evident between the conditioned taste aversion induced by LiCl and the conditioned taste avoidance induced by drugs of abuse. Specifically, although it has long been known that rats exhibit aversive orofacial responses (i.e. aversive taste reactivity, e.g., “gapes,” and “chin rubs”) to gustatory stimuli that have been paired with LiCl, they fail to do so when similar gustatory stimuli are paired with the passive delivery of morphine or cocaine even at very high doses (Grill & Norgren, 1978c; Parker, 1988, 1991, 1993, 1995). Furthermore, rats will decrease both instrumental and consummatory responding for a gustatory CS that has been paired with LiCl, but will increase
instrumental responding for, and decrease ingestion of, a gustatory CS that has been paired with a drug of abuse (Reicher & Holman, 1977; White, Sklar, & Amit, 1977). Specifically, rats will decrease ingestion of a novel food or sapid stimulus that is paired with a drug of abuse and simultaneously exhibit a conditioned place preference (Reicher & Holman, 1977), increased speed in a runway to get to the drug (White et al., 1977), and even self-administer the drug (Wise, Yokel, & DeWit, 1976).

In light of such findings, Grigson (1997) 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 evidence is accumulating to show that this phenomenon is mediated by the drug’s rewarding properties in spite of the evidence that these rewarding drugs have aversive side effects (Bechara & van der Kooy, 1985; D. C. Blanchard & Blanchard, 1999; R. J. Blanchard, Kaawaloa, Hebert, & Blanchard, 1999; McFarland & Ettenberg, 1997). First, while LiCl-induced CTAs occur with all CSs tested, the suppressive effects of a rewarding sucrose solution and drugs of abuse can be reduced or eliminated when using sucrose or salt, rather than saccharin, as the CS (Bevins, Delzer, & Bardo, 1996; Flaherty, Grigson, Checke, & Hnat, 1991; Gomez & Grigson, 1999; P. S. Grigson, 1997; P. S. Grigson, Lyuboslavsky, Tanase, & Wheeler, 1999). Second, bilateral lesions of the gustatory thalamus prevent the suppressive effects of sucrose and morphine, but have absolutely no impact upon the development of a LiCl-induced CTA (P. S.
Grigson, Lyuboslavsky, & Tanase, 2000; Reilly & Pritchard, 1996b; Scalera, Grigson, & Norgren, 1997; Schroy et al., 2005). Finally, the suppressive effects of a rewarding sucrose solution and cocaine, but not LiCl, are exaggerated in reward-preferring Lewis rats and in Sprague-Dawley rats following chronic morphine treatment (Glowa, Shaw, & Riley, 1994; P. S. Grigson, 2000; P. S. Grigson & Freet, 2000; for a discussion, see P. S. Grigson, Wheeler, Wheeler, & Ballard, 2001). Taken together, these data suggest that rats suppress intake of a saccharin CS following daily pairings with a drug of abuse because they are anticipating the rewarding properties of the drug. The mechanistic confusion is not surprising given the striking similarity between the three phenomena. All three agents (morphine, LiCl, and sucrose) similarly suppress intake when paired with an otherwise palatable tastant such as saccharin (see Figure 1-1). 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.
1.5 Summary

To summarize, one of the most important aspects of addiction to drugs of abuse is the severe lack of interest by the addicted individual to pursue naturally reinforcing goals that are essential for survival and contribute meaningfully to society. No current animal models of addiction exist that target this debilitating cardinal feature of addiction. Therefore, the goal of the experiments in this dissertation is not only to create the first and only model of drug induced devaluation of natural rewards and cue induced craving but also to explain the theoretical mechanisms that mediate this phenomenon. To that end, the basic phenomenon of contrast already has provided a framework for the creation of the model and its interpretation. However, significant challenges remain and serve as the impetus for these experiments. The experiments outlined herein will create that model, hone it, and add to this interpretation.

The first 4 chapters evaluated the parameters under which devaluation of a palatable taste cue occurs, characterized individual differences in the phenomenon, and related this variation in intake of the taste cue to drug-seeking behavior by incorporating the drug self-administration technique. More specifically, both Chapters 2 & 3 employed a passive model where the saccharin CS comes to predict the drug US injections delivered by the experimenter. In Chapter 2 the suppressive effects of heroin on saccharin intake were investigated in both non-deprived and water deprived rats because it was reported to be ineffective in the CTA design whereas morphine was highly
effective (Switzman, Hunt, & Amit, 1981). Since heroin is more potently addictive than morphine, the reward comparison hypothesis predicts that rats should avoid a saccharin CS that is paired with heroin administration. The results of this experiment revealed that rats avoid intake of a saccharin cue when paired with heroin but do so with great individual variation if they are deprived of water. This led to Chapter 3, which is a large scale analysis of the suppressive effects of a range of doses of cocaine, morphine and LiCl across deprivation states (food vs. water vs. non deprived). Chapter 4 abandoned the passive model and incorporated the drug self administration technique to investigate whether the individual differences observed in the avoidance of the saccharin CS would predict drug self-administration behavior and drug-seeking after a period of forced abstinence. As such it represents the next step in the development of the model of drug induced devaluation of natural rewards and cue-induced craving.

The next two chapters tested manipulations that might protect against the acquisition of cocaine self-administration. In Chapter 5, this was accomplished by testing whether the yoked delivery of drug would affect saccharin avoidance, the subsequent motivation to work for cocaine on a progressive ratio schedule, and the preference for contextual cues associated with drug delivery. Chapter 6 employed the successive negative and positive contrast paradigm to test whether a sudden unexpected loss or gain of reward would alter the motivation to self-administer cocaine on a progressive ratio schedule.

The last two chapters investigated the role of the VTA and NAc in modulating the suppressive effects of drugs of abuse on saccharin intake. In
Chapter 7, rats were given dopaminergic lesions of the VTA and then evaluated in the passive model (rats with this type of lesion do not acquire drug self-administration, Roberts & Koob, 1982) using both cocaine and morphine. Implications for the role of dopamine in reward are discussed. Finally, Chapter 8 investigated the activity of single neurons in the NAc in response to the saccharin cue as it comes to predict cocaine self-administration. This final experiment was particularly revealing due in large part to the necessary modifications to the procedure to accommodate the electrophysiological investigation (i.e., the use of intraoral delivery of the CS and taste reactivity measures). Together, these new manipulations led to a novel discovery. Indeed, the implications of that discovery may tie together and integrate several distinct theories of drug addiction, provide a behavioral model for the investigation of novel drug therapeutics to combat addiction, and open the door for a series of experiments that may finally solve a nearly 40 year old paradox.
Chapter 2

Heroin-induced suppression of saccharin intake in water-deprived and non-deprived rats

2.1 Introduction

Chapter 2 is intended to rectify an anomalous report where heroin, a highly reinforcing drug of abuse, did not suppress intake of a saccharin CS when paired with a range of doses of heroin (Switzman et al., 1981). This is odd because all other drugs of abuse tested do suppress intake of a palatable gustatory CS, including morphine, which is a less potent drug in the same class as heroin. Switzman et al. (1981) interpreted this finding as evidence that heroin was more reinforcing than morphine and, therefore, does not support CTA learning. This interpretation is contradictory to the reward comparison hypothesis which states that rats avoid a drug-associated saccharin CS because they are anticipating the reinforcing properties of the drug (P. S. Grigson, 1997). As such, this hypothesis would predict even greater avoidance of a heroin associated saccharin cue.

As with all hypotheses the usefulness of this reward comparison account depends both upon its accuracy and upon its generality to all drugs of abuse. In this regard, it has been demonstrated that intake of a saccharin CS is reduced following pairings with all other drugs of abuse tested including morphine, cocaine, amphetamine, alcohol, amobarbital, chlordiazepoxide, flurazepam, and
nicotine (Cappell & Le Blanc, 1977; Cappell & LeBlanc, 1971; Etkind, Fantegrossi, & Riley, 1998; Goudie et al., 1978; Le Magnen, 1969; Riley & Freeman, 2004; Shoaib & Stolerman, 1995; Vogel & Nathan, 1975). Furthermore, manipulations that influence the suppressive effects of one drug of abuse often exert a similar impact upon the suppressive effects of another. For example, use of a salt rather than a saccharin CS prevents the suppressive effects of both morphine and cocaine in water-deprived rats (Bevins et al., 1996; P. S. Grigson, 1997). Use of a caloric sucrose CS can reduce or eliminate the suppressive effects of morphine in water-deprived rats and the suppressive effects of morphine and cocaine in food-deprived rats (Gomez & Grigson, 1999; P. S. Grigson et al., 1999). Finally, food-deprivation has been found to attenuate the suppressive effects of morphine, cocaine, amphetamine, and chlordiazepoxide when using a saccharin CS (Bell, Thiele, Seeley, Bernstein, & Woods, 1998, AND CHAPTER 2).

Despite this general finding that rats suppress intake of a saccharin CS when paired with a range of drugs of abuse, they fail to do so when paired with a 0.5, 1, 2, 4, 8, or 12 mg/kg dose of heroin (Switzman et al., 1981). As mentioned above, this glaring exception poses a challenge for the reward comparison hypothesis because heroin is known to be a highly potent reinforcer (Best et al., 1999; Hutto & Crowder, 1997). Moreover, morphine readily suppresses intake of a saccharin CS (P. S. Grigson, 1997) and, while heroin is known to exhibit unique receptor binding characteristics, it is quickly converted into high concentrations of morphine in the brain following injection (Inturrisi et al., 1983).
Given the importance of these data to the reward comparison hypothesis, the following experiment was designed to revisit this issue by testing whether heroin will suppress intake of a saccharin CS using procedures that are known to sustain clear morphine- and cocaine-induced suppression of saccharin intake (P. S. Grigson, 1997). Furthermore, because recent data indicate that the suppressive effects of both morphine and cocaine are most robust when evaluated in rats that have free access to both food and water-, heroin-induced suppression of saccharin intake was tested in both water-deprived and non-deprived subjects. Finally, in an effort to maximize our chances of obtaining heroin-induced suppression, we selected one of the higher doses (i.e., 8 mg/kg) from the dose-response function evaluated by Switzman et al. (1981). Clearly, a single instance of heroin-induced suppression of saccharin intake will prove that heroin, like all other drugs of abuse tested, reduces intake of a saccharin CS following taste-drug pairings.

2.2 Method

2.2.1 Subjects

The subjects were 32 naive, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 275 and 300 g at the start of the experiment. All rats were individually housed in stainless steel cages in a colony room where temperature (21°C), humidity, and lighting (12:12 h light:dark
cycle) were controlled automatically. All experimental manipulations began 2 h into the light phase of the cycle.

2.2.2 Apparatus

The experiment was conducted using inverted Nalgene graduated cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Fluid intake was recorded to the nearest 0.5 ml.

2.2.3 Procedure

The protocol was approved by the Institutional Review Committee for the use of animal subjects and is in compliance with the National Institutes of Health Guide for the Care and Use of laboratory Animals (Publication No. 85-23, revised 1985). The animals were given approximately two weeks to adapt to the colony room and then were handled for three days. Access to water was then restricted to 5 min in the morning and 1 h in the afternoon to encourage drinking at the front of the home cage. Once intake stabilized (9 days), the subjects were divided into two groups. Half of the rats (n=16) continued on the water-deprived condition described above and were given free access to food. The other half of the rats (n=16) were given free access to food and water. The water was always provided at the back of the home cage for these subjects. Morning (5 min) and afternoon (1 h) dH2O intake continued to be recorded at the front of the home cage for 4
additional days for both the water-deprived and the non-deprived subjects. The rats were then matched on the basis of mean 5 min dH2O intake during the final 2 days of baseline and were assigned to one of two US conditions: saline (n=8/cell) or 8.0 mg/kg heroin (n=8/cell). During testing, all rats were weighed and given 5 min access to a 0.15% saccharin solution. After a 5 min interstimulus interval they were injected intraperitoneally (ip) with either saline or heroin. One such CS-US pairing occurred every other day for a total of 7 trials, followed by one CS-only test. Sodium saccharin was obtained from Sigma Chemical Co., St. Louis, MO and was presented at room temperature. Heroin was provided by the National Institute on Drug Abuse and was dissolved in sterile saline immediately before testing.

2.3 Results

2.3.1 Mean CS Intake

The data were analyzed using 2 x 2 x 8 repeated measures analysis of variance (ANOVA) varying drug (saline or heroin), deprivation state (non-deprived or water-deprived), and trials (1-8). The results revealed a significant main effect of drug, $F(1,28) = 48.63, p < .0001$, indicating that the rats injected with heroin consumed less saccharin than the saline injected controls overall (see Figure 2-1)
The main effect of deprivation state was significant, $F(1,28) = 33.45, p < .0001$. This finding showed that, as a group, the non-deprived subjects consumed significantly less saccharin than the water-deprived subjects. The Drug x Trials interaction also was significant, $F(7,196) = 20.51, p < .0001$. Post hoc Newman-Keuls tests revealed that, relative to the saline injected controls, heroin suppressed intake of the saccharin CS following a single CS-US pairing and intake remained suppressed throughout, $p_s < .05$. Finally, the Drug x

Figure 2-1: Mean ($\pm$ SEM) intake of 0.15% saccharin (ml/5min) in water-replete (left panel) and water-deprived (right panel) rats following 7 saccharin-saline or saccharin-heroin (8 mg/kg ip) pairings followed by one saccharin only test. Taste-drug pairings occurred at 48 h intervals.
Deprivation State x Trials interaction was not significant, $F < 1$, confirming that the 8 mg/kg dose of heroin suppressed intake of the saccharin CS and that the magnitude of this effect did not differ significantly as a function of deprivation state.

### 2.3.2 Mean CS Intake and Individual Differences

While heroin-induced suppression of saccharin intake was robust and occurred in all rats in the non-deprived condition, the response was far more variable in the water-deprived condition. Upon closer inspection of the saccharin intake data, it was clear that two distinct populations exist. These individual differences have been observed before with morphine and analyzed following a median split (Gomez, Leo, & Grigson, 2000). Consequently, the water-deprived, heroin-treated rats were divided into two groups (large and small suppressers) based on a median split of the average intake per rat across 8 trials (Figure 2-2). The data were then re-analyzed using a mixed factorial 3 x 8 ANOVA varying group (saline, small suppressers, large suppressers), and trials (1-8). The results of this analysis revealed a significant Group x Trials interaction, $F(14, 91)=4.64$, $p<.0001$. Newman-Keuls post hoc tests of this significant two-way interaction revealed that the large suppressers consumed significantly less saccharin than the saline controls after a single taste-drug pairing. On the other hand, the small suppressers only significantly suppressed intake of saccharin after 5 taste-drug pairings (trials 6 & 8).
2.3.3 5 min dH₂O intake

A 2 x 8 ANOVA varying drug (saline or heroin) and day (1-8) was conducted on the 5 min dH₂O data for the water-deprived rats on the days...
between injections. Neither the main effect of drug, $F < 1$, nor day, $F < 1$, attained statistical significance. Thus, morning dH$_2$O intake was not significantly affected by the injection of heroin overall and the function for dH$_2$O intake was flat across days. The Drug x Day interaction, however, did approach statistical significance, $F(7,98) = 2.09$, $p < .052$. This finding reflects a non-significant tendency for the heroin treated rats to consume less dH$_2$O than the saline treated controls on the mornings between injections (data not shown).

2.3.4 1 h dH$_2$O intake

A 2 x 14 ANOVA was performed on 1 hour afternoon dH$_2$O intake for the water-deprived animals varying drug (saline or heroin) and days (1-14). The results of the analysis revealed that, while the main effect of drug was not statistically significant, $F < 1$, there was a significant main effect of day, $F(13,182) = 8.78$, $p < .0001$, and Drug x Day interaction, $F(13,194) = 3.70$, $p < .0001$. Post hoc analysis of the Drug x Day interaction revealed a biphasic or “saw-toothed” pattern of intake for the heroin treated rats (see Figure 2-3).
Specifically, the water-deprived heroin treated rats increased 1h dH2O consumption during the daily hydration periods on the day of each saccharin-heroin pairing (significant elevations occurred on days 6, 8, 10, & 14, ps < .05) and then subsequently decreased 1h dH2O on the days between injections (significant reductions in intake occurred on days 5, 7, 9, & 13, ps < .05). Similar

Figure 2-3: Mean (± SEM) afternoon intake (ml/1h) of distilled water (dH2O) in water-deprived rats on the days of, and between, 7 saccharin-saline or saccharin-heroin (8 mg/kg ip) pairings. Taste-drug pairings occurred on even-numbered days and asterisks indicate statistically significant changes in fluid consumption.
increases in water intake have been obtained following saccharin-morphine pairings (P. S. Grigson et al., 1999) and following the administration of heroin (Park, Hirst, & Gowdey, 1980). Finally, rats in the saccharin-saline group did not change 1h dH₂O intake over trials and the intake of these subjects was neither significantly above nor below that of the heroin treated subjects, ps > .05.

2.3.5 Body weight

Changes in body weight were analyzed using an ANOVA varying drug (saline or heroin), deprivation state (non-deprived or water-deprived), and days (1 - 15). The results of this analysis indicated that the main effects of drug, \( F(1,28) = 4.30, p < .05 \), deprivation state, \( F(1,28) = 12.41, p < .002 \), and days, \( F(14,392) = 45.14, p < .0001 \), were significant (see Figure 2-4).
Thus, rats injected with heroin weighed significantly less than rats injected with saline, water-deprived rats weighed significantly less than the non-deprived rats, and all rats generally increased body weight over days. Further statistical analysis revealed a significant Drug x Day interaction, $F(14,392) = 11.60$, $p < .0001$. Post hoc tests indicated that the heroin treated rats weighed less than the saline treated rats on days 3 and 5 - 15, overall, $ps < .05$. Finally, the Drug x Deprivation State x Day interaction was not significant, $F < 1$, demonstrating that heroin reduced body weight gain relative to the saline treated controls, whether

Figure 2-4: Mean (± SEM) body weight (g) in water-replete (left panel) and water-deprived (right panel) rats throughout testing (days 1 - 15) for subjects in the saccharin-saline vs. the saccharin-heroin condition. Taste-drug pairings occurred on odd-numbered days.
the rats were tested in a water-deprived or a non-deprived state. A similar pattern has been obtained using the same procedures with morphine, but not with cocaine, suggesting that the reduction in body weight gain is related to repeated opiate treatment rather than to a simple reduction in CS intake.

2.4 Discussion

Contrary to the finding of Switzman et al. (Switzman et al., 1981), an 8 mg/kg dose of heroin suppressed intake of the saccharin CS following a single taste-drug pairing in both the water-deprived and the non-deprived rats. Indeed, the magnitude of the suppressive effect was comparable to, in fact slightly greater than, that found in water-deprived rats using the same testing conditions and a standard 10 mg/kg dose of cocaine or 15 mg/kg dose of morphine. Under these circumstances, intake of the saccharin CS was not reduced until the 4th and 6th CS-US paring, respectively (P. S. Grigson, 1997). Both drugs, however, can suppress intake following a single CS-US pairing when using higher doses (Chapter 3). Thus, given the dose-dependent nature, one trial learning can no longer serve as a distinguishing feature for CTA learning. Together, these data confirm that heroin, like morphine, cocaine, amphetamine, alcohol, and nicotine, for example, (Cappell & Le Blanc, 1977; Cappell & LeBlanc, 1971; Etkind et al., 1998; Goudie et al., 1978; Le Magnen, 1969; Shoaib & Stolerman, 1995; Vogel & Nathan, 1975), also reduces intake of a saccharin CS following taste-drug pairings.
The discrepancy between the current report and that of Switzman et al. may be attributed to a number of procedural differences. For example, Switzman et al. used Wistar rats and we used Sprague-Dawley rats. Their heroin was dissolved in Ringer's solution and ours in saline. Their animals were given a 10, rather than a 5, min access period to a 0.1% saccharin CS and they used a 1, rather than a 5, min inter stimulus interval. Switzman et al. also used only a single CS-US pairing and a 5 day inter trial interval. Both of these latter manipulations are likely to have contributed to the absence of conditioned suppression following saccharin-heroin pairings. Of more relevance, however, may be the fact that Switzman et al. used a very rigorous water-deprivation regimen in which the animals were restricted to 20 min access to fluid a day. It appears that this regimen often is associated with greater CS intake, presumably because the rats have only one opportunity to hydrate each day and a failure to do so on a given day can lead to 48 h fluid deprivation. Finally, while the procedures employed by Switzman et al. may not have been conducive to heroin-induced suppression of CS intake, it must be noted that they were sufficient to sustain a significant reduction in saccharin intake following pairings with either an 8 or a 12 mg/kg dose of morphine. These effects, however, were small suggesting that the testing conditions were adequate, but not optimal.

Although water-deprivation has been found to reduce the expression of morphine- and cocaine-induced suppression of saccharin intake at lower doses (Chapter 3), heroin- induced suppression of saccharin intake occurred following a single taste-drug pairing in both the water-deprived and the non-deprived rats.
The similar magnitude of effectiveness of heroin in the water-deprived and the non-deprived condition likely reflects the use of what now appears to be a relatively potent dose of heroin. That is, while water-deprivation reduces the suppressive effects of standard doses of cocaine and morphine, it cannot offset the suppressive effects of higher doses of these drugs (Chapter 3).

Finally, although the 8 mg/kg dose of heroin was clearly potent, it is noteworthy that heroin-induced suppression of saccharin intake was subject to individual differences (i.e., variability), and that these effects were only evident in the water-deprived animals. Individual differences of this nature have been reported previously in water-deprived rats when using other drugs of abuse (Riley, Jacobs, & LoLordo, 1978; Turenne, Miles, Parker, & Siegel, 1996). They have, however, never been investigated with heroin and never with rats maintained on food and water ad libitum. Thus, the present account serves as the first indication that individual differences in the sensitivity to heroin (perhaps to the rewarding properties of heroin) can be exposed by investigating this phenomenon in water-deprived rats.

In sum, the results demonstrate that rats will reduce intake of a saccharin CS following pairings with all drugs of abuse tested including heroin (Cappell & Le Blanc, 1977; Cappell & LeBlanc, 1971; Etkind et al., 1998; Goudie et al., 1978; Le Magnen, 1969; Shoaib & Stolerman, 1995; Vogel & Nathan, 1975). Thus, the evidence suggests that rats suppress intake of a saccharin cue following saccharin-morphine or saccharin-cocaine pairings because they are anticipating the availability of the preferred drug of abuse (P. S. Grigson, 1997).
The present data demonstrate that heroin is no exception. Finally, heroin’s suppressive effects were uniform in the non-deprived condition and much more variable in the water-deprived condition. This variability may speak to the mechanism of saccharin avoidance when using reinforcing drugs like heroin, morphine, and cocaine as opposed to aversive agents like LiCl. The next chapter will investigate the relative efficacy of these drugs to induce saccharin suppression across a range of doses and deprivation states.
Chapter 3

The role of dose and deprivation state in drug-induced suppression of saccharin intake: A comprehensive analysis

3.1 Introduction

In chapter 2 it was found that heroin, the only drug of abuse that allegedly did not support CTA learning, causes a robust avoidance of the saccharin cue when tested under both non-deprived and water-deprived conditions. Furthermore, it was discovered that heroin-induced suppression of saccharin intake was more variable in the water-deprived condition. A median split was performed on the data and it was apparent that two phenotypes emerged. One phenotype, referred to as the large suppressers avoided the saccharin cue in one taste-drug pairing, while the other phenotype did not exhibit saccharin avoidance until the 5th taste drug pairing, and then did so only transiently. Water-deprivation may have weakened the drug-induced suppression of saccharin intake by increasing the value of the saccharin cue.

This weakening effect of deprivation state on avoidance of a palatable gustatory CS induced by drugs of abuse may dissociate the suppressive effects of LiCl from drugs of abuse and, thereby, reveal a different mechanism. For example, when using a sucrose CS, food-deprivation exerts a greater disruption on the suppressive effects of a rewarding sucrose US and a drug of abuse than those induced by LiCl (Flaherty et al., 1991; Gomez & Grigson, 1999). Similarly,
water-deprivation also prevents the avoidance of a sucrose CS when paired with morphine, but not LiCl (P. S. Grigson et al., 1999). Finally, when using a saccharin CS, food-deprivation disrupts the suppressive effects of amphetamine and chlordiazepoxide, while leaving those of a standard dose of LiCl intact (Bell et al., 1998). Food- and water-deprivation, then, can attenuate avoidance of a taste cue and these effects might be greater when a drug of abuse, rather than the aversive agent, LiCl, serves as the US. If so, this would lend support to the reward comparison hypothesis.

Since drugs of abuse and LiCl can vary in intensity, it is critical that the reduction in CS intake is compared across a range of doses. However, even dose effects can vary greatly across laboratories using slightly different testing regimens. For example, some have reported no differences in the magnitude of morphine-induced suppression of saccharin intake across a range of doses (9, 10, 20, 17, 50, and 80 mg/kg) of the drug (LeBlanc & Cappell, 1975; Riley et al., 1978). Others have reported greater suppressive effects with the lower doses (Farber, Gorman, & Reid, 1976), while others have reported greater suppressive effects with the higher doses of the drug (Siegel, Parker, & Moroz, 1995). Given conflicting reports of this nature, and the marked effect that deprivation state can have on the willingness to avoid intake of an otherwise palatable gustatory cue (P. S. Grigson, Cornelius, & Wheeler, 2001; P. S. Grigson, Twining, & Carelli, 2000), a full, systematic investigation is warranted. To this end, the current series of experiments was designed to evaluate suppression of intake of a
saccharin CS following pairings with morphine, cocaine, and LiCl across a range of doses in non-deprived, food-deprived, and water-deprived rats.

3.2 Methods

3.2.1 Subjects

The subjects were 695 male Sprague-Dawley rats (Charles Rivers Laboratories) weighing between 250 and 350 grams at the beginning of the experiment. All animals were housed individually in stainless steel hanging cages in a temperature-controlled (21°C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 a.m.). All experimental manipulations were conducted approximately 3 h into the light phase of the cycle. The rats were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water, except where noted otherwise. In order to limit the already large number of experimental subjects required for these experiments, only select doses were tested across all deprivation states.

3.2.2 Apparatus

All experimental manipulations were conducted in the home cages using inverted Nalgene graduated cylinders and stainless steel spouts affixed to the front of the cage. Total intake was recorded to the nearest ml. All LiCl and morphine injections were administered ip while the cocaine injections were
administered subcutaneously (sc). A stock solution of 1.5 mg cocaine-HCl/ml saline was used to prevent skin necrosis typical of higher concentrations. Sodium saccharin and LiCl were obtained from the Sigma Chemical Company, St. Louis, MO and NaCl was obtained from Fisher Chemical, Pittsburgh, PA. Morphine sulfate and cocaine hydrochloride were generously provided by the National Institute on Drug Abuse and dissolved in sterile saline on the morning of each injection.

3.2.3 Procedure: Water-Deprived experiments

Rats were water deprived and trained to come to the front of the cages to drink for 4 days. During this time the rats received 5 min access to dH$_2$O in the morning and 1h in the afternoon to maintain hydration. Baseline water intake (5 min morning dH$_2$O) was conducted over 8 days. Groups were matched on the basis of their water intake on the final two days of baseline (LiCl: saline (n=16), 0.002 M LiCl (n=8), 0.004 M LiCl (n=8) 0.009 M LiCl (n=8), 0.018 M LiCl (n=8) 0.037 M LiCl (n=8) 0.075 M LiCl (n=8), and 0.15 M LiCl (n=8); Morphine: saline (n=18), 0.5 mg/kg (n=8), 1.5 mg/kg (n=8), 3 mg/kg (n=8), 5 mg/kg (n=10), 15 mg/kg (n=10), 30 mg/kg (n=10), and 60 mg/kg (n=8); Cocaine: saline (n=33), 1.25 mg/kg (n=8), 2.5 mg/kg (n=8), 5.0 mg/kg (n=15), 7.5 mg/kg (n=15), 10 mg/kg (n=15), 15 mg/kg (n=10), 20 mg/kg (n=10), and 40 mg/kg (n=9)). During testing, all rats were given 5 min access to a 0.15% saccharin solution, and after a 5 min wait, were injected with their assigned drug and dose or saline. Each
dose of LiCl had an appropriate amount of NaCl added to bring the total salt molarity to 0.15 M, which is isotonic to physiological saline. There was one pairing every other day for a total of 8 pairings. All subjects received 1h access to dH2O each afternoon and 5 min access each morning between conditioning trials.

3.2.4 Procedure: Food-Deprived experiments

Rats were food-deprived to 82% of their body weights prior to testing. They were fed 45 minutes after testing each day. All rats were water-deprived for 4 days in order to train them to come to the front of the cages to drink. During this time, the rats received 5 min dH2O in the morning and 1h in the afternoon to maintain hydration. Once free access to water was restored, baseline intake (5 min morning dH2O) was evaluated over 8 additional days and groups were matched on the basis of their water intake on the final two days (LiCl: saline (n=11), 0.002 M LiCl (n=7), 0.004 M LiCl (n=7) 0.009 M LiCl (n=4), 0.018 M LiCl (n=5) 0.037 M LiCl (n=4) 0.075 M LiCl (n=5), and 0.15 M LiCl (n=5); Morphine: saline (n=22), 0.5 mg/kg (n=12), 1.5 mg/kg (n=12), 3 mg/kg (n=11), 5 mg/kg (n=10), 15 mg/kg (n=10), 30 mg/kg (n=10), and 60 mg/kg (n=10); Cocaine: saline (n=22), 5.0 mg/kg (n=15), 7.5 mg/kg (n=14), 10 mg/kg (n=15), 15 mg/kg (n=8), 20 mg/kg (n=10), and 40 mg/kg (n=8)). During testing, all rats were given 5 min access to a 0.15% saccharin solution, and after a 5 min wait were injected with their assigned drug or saline. Drug was prepared and administered as described
above. There was one pairing every other day for a total of 8 pairings. All subjects received 1h access to dH₂O each afternoon and 5 min access each morning between conditioning trials.

3.2.5 Procedure: Non-Deprived experiments

Rats were water-deprived for 4 days in order to train them to come to the front of the cages to drink. Once free access to water was restored, baseline intake (5 min morning dH₂O) was evaluated over 8 additional days. The animals were then matched on the basis of their average 5 min intake during the final two days of baseline and were placed into their appropriate treatment group (LiCl: saline (n=13), 0.002 M (n=7), 0.004 M (n=7) 0.009 M (n=6), 0.018 M (n=6) 0.037 M (n=6) 0.075 M (n=6), and 0.15 M (n=6); Morphine: saline (n=20), 0.5 mg/kg (n=12), 1.5 mg/kg (n=11), 3 mg/kg (n=11), 5 mg/kg (n=8), 15 mg/kg (n=8), 30 mg/kg (n=8), and 60 mg/kg (n=8); Cocaine: saline (n=8), 5.0 mg/kg (n=8), 7.5 mg/kg (n=8), 10 mg/kg (n=8), and 20 mg/kg (n=8)). During testing, all rats were given 5 min access to a 0.15% saccharin solution and, after a 5 min wait, were injected with their assigned drug dose or vehicle. Drug was prepared and administered as described above. There was one pairing every other day for a total of 8 pairings. All subjects received 24h access to dH₂O; in addition, 5 min access to dH₂O was presented at the front of the cage each morning between conditioning trials.
3.3 Results

3.3.1 Experiment 1: LiCl

3.3.1.1 Experiment 1A: Water-Deprived

LiCl induced CTA varied as a function of dose. This conclusion was supported by the results of an 8 x 8 mixed factorial ANOVA varying drug (sal, .00225, .0045, .009, .018, .037, .075, .15) and trials (1-8). Newman-Keuls post hoc tests of a significant Drug x Trials interaction, \( F(49, 448) = 11.493, \ p < 0.0001 \), indicated that avoidance of the saccharin CS occurred in the later trials at lower doses and in the earlier trials at higher doses (Figure 3-1).
Specifically, at the three lowest doses, significant suppression began at the 6th, 5th, and 3rd pairing, respectively, and persisted to trial 8, ps<.05. At the remaining four higher doses, 0.15 M being the standard dose, significant suppression began after a single sac-lithium pairing and persisted to trial 8, ps<.05.

Figure 3-1: LiCl-induced suppression of saccharin intake in water deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of LiCl (closed circles).
3.3.1.2 Experiment 1B: Food-Deprived

Relative to the water-deprived state, the suppressive effect of lithium on saccharin intake is not as potent at the lowest dose tested when examined in the food-deprived state (Figure 3-2). This was indicated by the results of an 8 x 8 mixed factorial ANOVA varying drug (saline, .00225, .0045, .009, .018, .037, .075, .15) and trials (1-8), and confirmed by Newman-Keuls post hoc tests of the significant Drug x Trials interaction, $F(49,280) = 2.09$, $p < .0001$. 
Specifically, the 0.009, 0.037, 0.075, and 0.15 M doses of LiCl suppressed intake of the saccharin solution after a single taste-drug pairing, ps<.05. Although, the 0.018 M dose significantly suppressed intake by trial 3, p<.05, there was a strong tendency for this effect after only a single taste drug pairing. The only real
deviation from this robust effect occurred at the two lowest doses. The 0.004 M
dose did not suppress intake until trial 4, \( p < .05 \), and the lowest dose (0.002 M),
which was effective in the water-deprived state, was fully ineffective at
suppressing intake of the saccharin solution when evaluated in the food-deprived
condition, \( ps > .05 \).

3.3.1.3 Experiment 1C: Non-Deprived

The results of an 8 x 8 mixed factorial ANOVA varying drug (saline,
.00225, .0045, .009, .018, .037, .075, .15) and trials (1-8) showed that the
suppressive effects of LiCl on saccharin intake in the non-deprived condition are
similar to those in the water-deprived condition (Figure 3-3). This finding was
confirmed by post hoc tests of a significant Drug x Trials interaction, \( F (49,343) = 3.06, p < .0001 \). Specifically, the 0.009, 0.018, 0.037, 0.075, and 0.15 M doses
of LiCl suppressed intake of the saccharin solution after a single taste-drug
pairing, \( ps < .05 \). The 0.004 M dose suppressed intake by the 3\(^{rd}\) pairing, and the
lowest dose (0.002 M) suppressed intake by the 4\(^{th}\) pairing, \( ps < .05 \).
Taken together, these data clearly show that LiCl-induced suppression of saccharin intake (i.e., CTA), which has been traditionally run in the water-deprived state, is an extremely robust, orderly, and dose-dependent phenomenon regardless of deprivation state. Indeed, even at the lowest dose tested (0.002 M LiCl) a significant reduction in saccharin intake was observed by the 6th taste-drug pairing in the standard water-deprived condition. The non-

Figure 3-3: LiCl-induced suppression of saccharin intake in free-feeding rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of LiCl (closed circles).
deprived condition actually enhanced the suppressive effects of LiCl on saccharin intake, despite driving lower overall intake. In contrast, the food-deprived condition, which also reduced overall intake relative to the water-deprived condition, diminished the suppressive effects of LiCl on saccharin intake but only at the lowest dose tested. Therefore, the 0.002 M LiCl dose, while effective in the water-deprived and non-deprived conditions, was ineffective at reducing saccharin intake. Manipulating the deprivation state, then, had no effect on the efficacy of LiCl to support CTA at each of the other 6 doses tested.

3.3.2 Experiment 2: Cocaine

3.3.2.1 Experiment 2A: Water-Deprived

There appeared to be an orderly dose response function where lower doses of cocaine were less effective than higher doses to induce saccharin avoidance. To verify this a 9 x 8 mixed factorial ANOVA varying drug (saline, 1.25, 2.5, 5, 7.5, 10, 15, 20, 40 mg/kg cocaine) and trials (1-8) was conducted. Indeed, post hoc tests of a significant Drug x Trials interaction, $F(56,798) = 7.73$, $p < .0001$, revealed an orderly dose response function (Figure 3-4). Specifically, the 15, 20, and 40 mg/kg doses of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing and persisted to trial 8, $ps<.05$. Suppression occurred by trial 3 for the 10 mg/kg dose, and on trials 4 and 6-8 for
the 7.5 mg/kg dose. The three lowest doses (1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg) were ineffective at suppressing intake of the saccharin solution, p > .05.

Figure 3-4: Cocaine-induced suppression of saccharin intake in water-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 8 doses of cocaine (closed circles).
3.3.2.2 Experiment 2B: Food-Deprived

Similar to the water-deprived state food-deprivation appeared to reduce the efficacy of cocaine to induce saccharin avoidance at lower doses (Figure 3-5). To verify this, a 7 x 8 mixed factorial ANOVA varying drug (saline, 5, 7.5, 10, 15, 20, 40 mg/kg cocaine) and trials (1-8) was conducted. Indeed, post hoc tests of a significant Drug x Trials interaction, $F(42,595) = 7.79$, $p < .0001$, indicated that the 40 mg/kg dose of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing while the 15 mg/kg and 20 mg/kg doses suppressed intake by trial 3, $ps > .05$. The 10 mg/kg dose suppressed intake by trial 5, while the 7.5 mg/kg dose suppressed intake by trial 4, $ps > .05$. Deviating slightly from the water deprived condition at the 5 mg/kg dose, a significant but transient suppression of saccharin intake occurred on trials 5 – 7, $ps > .05$. 
3.3.2.3 Experiment 2C: Non-Deprived

Relative to the deprived conditions, the non-deprived condition appeared to enhance the suppressive effects of cocaine on saccharin intake (Figure 3-6). To verify this, a 5 x 8 mixed factorial ANOVA varying drug and trials was conducted. Indeed, post hoc tests of a significant Drug (sal, 5, 7.5, 10, 20 mg/kg cocaine) x Trials (1-8) interaction, $F_{(28,245)} = 10.61, p < .0001$, indicated that

Figure 3-5: Cocaine-induced suppression of saccharin intake in food-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 6 doses of cocaine (closed circles).
the suppressive effects of cocaine on saccharin intake are robust in the free feeding state. Specifically, the 5 mg/kg, 7.5 mg/kg, 10 mg/kg, and 20 mg/kg doses of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing.

Figure 3-6: Cocaine-induced suppression of saccharin intake in non-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 4 doses of cocaine (closed circles).

Cocaine-induced suppression of saccharin intake was reliably observed in the water deprived state in the doses ranging between 10-40 mg/kg. Although
the effect was intermittent across trials at the 7.5 mg/kg dose, it was completely eliminated at the lower 1.25 to 5 mg/kg doses. Although, suppression of saccharin intake was evident at each of the cocaine doses tested in the food-deprived condition the effect was no longer significant by trial 8 at the 5 mg/kg dose. In contrast, cocaine-induced suppression of saccharin intake was observed at all doses tested when rats were free-feeding. This is most evident at the 5 mg/kg dose that was totally ineffective in the water deprived state.

3.3.3 Experiment 3: Morphine

3.3.3.1 Experiment 3A: Water-Deprived

Morphine-induced avoidance of saccharin was either attenuated or abolished at several doses across the dose response function (Figure 3-7). To verify this, an 8 x 8 mixed factorial ANOVA varying drug (saline, .5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8) was conducted. Newman-Keuls post hoc tests of a significant Drug x Trials interaction, $F(49,504) = 3.44$, $p < .0001$, indicated that only the 15 mg/kg and 60 mg/kg doses of morphine suppressed intake of the saccharin solution following a single taste-drug pairing. Suppression by the 30 mg/kg dose occurred on the 5th, 7th and 8th pairings and the 0.5 mg/kg, 1.5 mg/kg, 3 mg/kg, and 5 mg/kg doses were ineffective at suppressing intake of the saccharin solution.
3.3.3.2 Experiment 3B: Food-Deprived

When evaluated in the food-deprived state, morphine-induced avoidance was attenuated at almost every dose tested. An 8 x 8 mixed factorial ANOVA varying drug (saline, .5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8) was

Figure 3-7: Morphine-induced suppression of saccharin intake in water-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine (closed circles).
conducted on the data and revealed an unusual dose response profile (see Figure 3-8). Specifically, post hoc tests of a significant Drug x Trials interaction, $F(49, 623) = 4.03, p < .0001$, indicated that the 0.5 mg/kg dose of morphine, the lowest dose tested, suppressed intake of the saccharin solution by trial 5, while the next two higher 1.5 mg/kg and 3 mg/kg doses were without effect. At the 5 mg/kg dose, intake of the saccharin solution was significantly suppressed on trial 2, and then began to increase on trials 3-7 at a rate on par with the saline controls until trial 8 where the morphine-injected rats actually consumed significantly more saccharin (i.e., a reinforcement effect). This type of transient suppression effect also was evident for both the 15 and 30 mg/kg dose, where suppression was established after a single taste-drug pairing and gradually waned to insignificance by the 5th trial. Even at the highest dose tested, 60 mg/kg, there was a tendency for weakened suppressive effects. Although rats at this high dose of morphine suppressed intake relative to saline controls on trials 2-7, they drank statistically similar amounts on trial 8.
3.3.3.3 Experiment 3C: Non-Deprived

Morphine induced avoidance of the saccharin cue was similar and robust across the dose response function (Figure 3-9). This was verified by the results of an 8 x 8 mixed factorial ANOVA varying drug (saline, .5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8). Newman-Keuls post hoc tests of a significant
main effect of drug, $F(7, 78) = 5.11, p < .0001$, indicated that all of the morphine doses were significantly different than the saline controls ($p < .02$). The fact that the Drug x Trials interaction was not significant, $F(49, 546) = 1.07, p = 0.3511$, speaks to the symmetry of morphine’s suppressive effects at each dose.

Figure 3-9: Morphine-induced suppression of saccharin intake in non-deprived rats. Mean (±SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine (closed circles).
The impact of deprivation state on morphine-induced suppression of saccharin intake was less straightforward than was the effect of deprivation state on either cocaine or LiCl induced suppression of CS intake. In the water-deprived state, lower doses (0.5 to 5 mg/kg) were ineffective at reducing intake of saccharin; in addition, the 15 mg/kg and 60 mg/kg doses appeared to be the most effective while the intermediate 30 mg/kg dose was less effective. In the food-deprived condition, the highest and lowest doses appear to be the most effective (suppression by trial 2 with 60 mg/kg and by trial 4 with 0.5 mg/kg) while the doses in between appear to be transiently effective or not effective at all. Finally, as mentioned above a significant Drug x Trials interaction was not significant in the morphine treated, non-deprived condition. This lack of significance was probably due to the very similar pattern of saccharin avoidance at each dose of morphine. Nevertheless, the post hoc analysis of the significant main effect of drug demonstrated that all morphine doses were significantly different than their saline controls. From this result, it is reasonable to conclude that avoidance of a saccharin CS following saccharin-morphine pairings is enhanced in free-feeding rats relative to both the water- and food- deprived condition.

3.4 General Discussion

The current set of experiments was designed to compare the efficacy of LiCl, morphine, and cocaine to produce a conditioned avoidance of a normally
preferred 0.15% saccharin solution in naïve rats that are water-deprived, food-deprived, or non-deprived using an extensive dose-response analysis. Importantly, all of the doses employed for each of the three drugs produced a significant conditioned avoidance of the saccharin CS after taste-drug pairings when the rats were evaluated in a non-deprived state. However, this avoidance was disrupted when many of these very same doses were evaluated after depriving the rats of either food or water. Of the three drugs tested, LiCl appeared to be the most resistant to the disruptive effects of either water- or food-deprivation (Table 3-1).

Table 3-1: Summary of suppressive effects across drugs and deprivation states

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WD = water deprived, FD = food deprived, FF = free feeding. (+) = suppression, (-) = disrupted suppression, (+/-) = transient suppression.
Importantly, the suppressive effects of LiCl, morphine, and cocaine, at every dose tested, induced a significant avoidance of the saccharin CS after just one or two taste-drug pairings when evaluated under non-deprived conditions. Thus, there were no differences in the suppressive effects across the compounds unless a deprivation state was imposed. As such, the disrupted suppressive effect of morphine, and cocaine cannot be attributed to learning deficits nor to dose effects, per se, because these interpretations would predict a similar disruption in the non-deprived state as well. Clearly, the deprivation states enhanced the drive to consume saccharin. This enhanced drive was sufficient to override the suppressive effects of the reinforcing drugs of abuse at lower doses but not even extremely low doses of the noxious LiCl.

More specifically, relative to the non-deprived condition, the water-deprived condition reduced the suppressive effects of all three drugs on saccharin intake, particularly at the lower doses. However, of the three drugs tested, LiCl was more resistant to this reduction in efficacy than either morphine or cocaine. In fact, even at doses that were highly effective in the non-deprived state (see Table 3-1), both morphine and cocaine were rendered either totally ineffective or marginally effective when evaluated in the water-deprived state. LiCl, on the other hand, retained its potency at even the lowest dose tested which resulted in at least a 50% reduction in saccharin intake under both non-deprived and water-deprived conditions by trial 8.

This rightward shift in the dose response curve of both morphine and cocaine, but not LiCl, by water deprivation may suggest that the drugs of abuse
mediate avoidance of a saccharin CS by a different mechanism than LiCl. Therefore, the current data are consistent with findings reported by Parker and colleagues (1988) who have reported that drug-of-abuse-mediated avoidance of a gustatory CS is not accompanied by aversive taste reactivity (i.e., gapes) whereas the avoidance produced by LiCl actually renders the CS aversive tasting (i.e., causes rats to gape). The illness induced by LiCl may inherently be a qualitatively stronger US needing only to reach a detectable threshold to induce avoidance of a taste-CS that has been paired with it. In contrast, morphine and cocaine need not only be “detectable” but also sufficiently rewarding to overpower the reinforcing properties of the saccharin CS that predicts its delivery. That is, a little nausea goes a long way toward reducing the value of the preceding CS, whereas, a comparatively much larger amount of “rewarding effect” is needed to result in a measurable amount of devaluation of the saccharin cue (particularly when in need of food or water). While intuitively appealing, it may still be possible that saccharin avoidance induced by higher doses of morphine and cocaine may be triggered by the onset of aversive properties as is true of high doses of nicotine, apomorphine, or amphetamine. These reinforcing drugs support both CTA learning and aversive taste reactivity at high doses (Parker, 1991; Parker & Brosseau, 1990). However, this is not likely to be true of the current data set because even repeated pairings of a palatable taste with doses as high as 80 mg/kg morphine and 40 mg/kg cocaine were insufficient to produce aversive taste reactivity while supporting robust taste avoidance learning (Parker, 1991, 1993).
The food-deprived state resulted in the most complicated pattern of responding across the dose response function for each drug and was not as effective as water deprivation at differentiating between the compounds. While saccharin is sweet, it contains no calories. Without calories, the saccharin may not be as appealing to a food-deprived rat as it is to a water-deprived rat. Nevertheless, similar to the water-deprived and relative to the non-deprived states, the efficacy of each drug compound to support conditioned avoidance of the saccharin CS was reduced by food-deprivation and LiCl was the most resistant to this reduction. It is unclear why food-deprivation was sufficient to disrupt avoidance of saccharin at the lowest dose of LiCl tested but simultaneously enhanced the efficacy of the lowest dose of morphine. However, this odd effect of food deprivation on morphine induced suppression was not consistent as the next two intermediate low doses (1.5 and 3 mg/kg) were totally ineffective, and 5 mg/kg actually produced a reinforcement effect by trial 8. In fact, in the food-deprived condition morphine did not suppress intake of a saccharin CS consistently except at the lowest and highest doses. This pattern of transient suppression for the 5, 15, and 30 mg/kg doses (i.e., the establishment of suppression that gradually dissipates) was unique to the food-deprived, morphine-injected rats and, therefore, discounts tolerance as a likely explanation. While tolerance to drugs of abuse does occur, it cannot account for the waning efficacy of morphine because it would have occurred in both the non-deprived and water-deprived conditions as well. Conversely, if it was merely an effect of food-deprivation then a similar reduction in efficacy would have been evident in
the food-deprived rats that were injected with cocaine. Clearly, there was a unique interaction between food-deprivation and morphine.

Indeed, perhaps the simplest explanation for the potent avoidance of saccharin intake when the rats are not deprived of either water or food is that the hedonic value of the saccharin solution and, therefore, the motivation to consume saccharin is reduced. It follows that depriving a rat of water or food increases the hedonic valence of a saccharin solution and that the increase in value is enough to override the expression of saccharin avoidance induced by reinforcing drugs. On the other hand, the relative motivational apathy inherent to the non-deprived state facilitates devaluation of saccharin induced by pairings with either a more rewarding consequence such as morphine or cocaine induced euphoria (i.e., ACE; Grigson, 1997) or an aversive consequence like LiCl induced nausea (i.e., CTA), regardless of the valence.

In conclusion, it is clear that suppression of saccharin intake by LiCl and drugs of abuse is mediated by different mechanisms. Both the suppressive effects of morphine and cocaine, two highly reinforcing drugs of abuse in distinctly different drug classes, were attenuated or abolished by making the rats either hungry or thirsty. On the other hand, LiCl, an unequivocally aversive agent, remained a potent suppresser of saccharin intake even at very low doses under both food-deprived and water-deprived conditions. Despite decades of research on conditioned taste avoidance induced by morphine, cocaine and LiCl, it was not possible to evaluate the effect of deprivation state on the individual dose response functions of these drugs let alone compare them directly to each other.
This is mostly because laboratories invariably use different procedures and testing parameters which is not always ideal for comparative analysis. In fact, one laboratory, using less than ideal parameters, falsely concluded that heroin did not support CTA learning (see Chapter 2). This conclusion was overturned by Chapter 2. In the current chapter, the experiments were conducted “under one roof” with standard procedures and parameters that only differed with respect to the experimental manipulations. This allowed for a reasonable comparative analysis across drug compounds. As such, this experiment (which employs nearly 700 rats) not only provides support for a different mechanism of suppression of saccharin intake by drugs of abuse and LiCl, but also provides important fundamental data.

Nevertheless, the degree to which the reinforcing properties of these drugs of abuse contributes to saccharin avoidance is still a mystery. This is because the drug injections are delivered passively to the rats by the experimenter. Therefore, the next chapter represents the next step in the development of the reward comparison model by incorporating the i.v. drug self-administration technique. This advance will allow the rats to directly control their consumption of both the saccharin CS and the cocaine US.
Chapter 4

**Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards**

### 4.1 Introduction

So far, this dissertation has investigated the devaluation of a saccharin cue induced by anterograde association with a drug of abuse consequence that is delivered exclusively by the experimenter. In chapter 2, it was discovered that water deprivation modulated the suppressive effects of heroin on saccharin intake but not uniformly across subjects. Instead, water deprivation effectuated reliable individual differences that resulted in two distinct phenotypes. The large suppressers greatly avoided the saccharin CS while the small suppressers did not avoid the saccharin CS convincingly. In chapter 3, it was confirmed that deprivation states modulate the suppressive effects of drugs of abuse such as morphine and cocaine by rendering doses that are effective in a non-deprived state impotent. An aversive agent like LiCl, on the other hand, was resistant to this modulation. While the data in these prior chapters support the predictions of the reward comparison hypothesis, they raise further questions. For instance, are these large suppresser rats more responsive to the reinforcing properties of the drug? Despite the fact that drugs of abuse are indisputably rewarding, they are not without aversive consequences and it remains possible that these rats were more sensitive to them. Therefore, rather than using experimenter delivered
cocaine, the current experiment used self-administration to allow the rats to control their cocaine intake. This is an important advance in the reward comparison model as it more closely resembles the paradigm of anticipatory contrast where the rat controls intake of the CS and the US. Furthermore, the drug self-administration technique more closely models human drug addiction and affords the opportunity to evaluate the relationship between the devaluation of the saccharin cue and the motivation to self-administer cocaine.

The reward comparison hypothesis predicts that, unlike LiCl-induced CTAs which are associated with a reduction in instrumental responding for LiCl (White et al., 1977), avoidance of the saccharin cue following taste-drug pairings should be associated with an increase in instrumental responding for the drug of abuse. There are some data to support this hypothesis. For instance, Reicher and Holman (1977) found that rats that avoided a flavor paired with amphetamine preferred the location in which the stimuli had been paired. Moreover, White et al. (1977) reported that the rats that consumed the least of a saccharin-adulterated chow following pairings with morphine, actually exhibited the fastest running speed to the goal box where the two stimuli had been associated. Finally, Wise, Yokel, and DeWit (1976) showed that while rats avoided intake of saccharin that preceded apomorphine self-administration, they still exhibited self-administration behavior.

The seminal data from Wise et al. (1976), in particular, go a long way to suggest that drug-induced suppression of CS intake is not associated with a decrease in instrumental responding for the drug US. Even so, the testing
parameters used by Wise et al. (1976) were not ideal. That is, in an effort to insure that intake of the saccharin CS was followed by self-administration of apomorphine, all rats were first trained to lever press to self-administer amphetamine and the first apomorphine access period was initiated with two priming injections (forced, passive injections). As such, not only did all rats receive preexposure to amphetamine self-administration but also to at least 2 non-contingent apomorphine administrations. While very effective to increase instrumental responding during operant training, a drug history of this nature could have influenced the magnitude of the suppressive effects of apomorphine in the Wise et al. report. US preexposure can either disrupt (Cappell & LeBlanc, 1977) or augment (for a discussion, see P. S. Grigson, Wheeler et al., 2001) drug-induced suppression of CS intake depending upon procedural manipulations. Indeed, Wise et al. (1976) reported clear evidence that apomorphine was reinforcing in only four of eleven animals. Experiment 1, then, used a more natural operant response (i.e., licking an empty spout) in an effort to eliminate potential CS and US preexposure effects and to facilitate the development of drug self-administration behavior. Experiment 2 compared the dose response function across a range of doses of cocaine (0.04, 0.08, 0.16, and 0.33 mg/infusion) when using a Fixed Ratio (FR) 10 lick contingency on the empty spout and then again when using an FR1 on a lever. Finally, Experiment 3 examined the responsiveness for the gustatory CS and drug (US) seeking (under extinction conditions) following at least one month of abstinence.
4.2 Experiment 1

4.2.1 Introduction

The acquisition of a conditioned response can be delayed not only by preexposure to the US (as discussed), but also by preexposure to the CS (Reilly, Harley, & Revusky, 1993). These preexposure effects are difficult to avoid when the experimental design requires the animal to voluntarily self-administer both the CS and the US, and, particularly, when the operant response is sufficiently foreign to require an initial training phase. Thus, in an effort to facilitate drug self-administration and to insure that each CS access period was followed by drug self-administration, licking an empty tube replaced the lever press as the operant response. Rats readily lick spouts. They lick at a rate of approximately 7 licks/sec (Corbit & Luschei, 1969) and quickly learn to complete a lick contingency on a filled or an empty spout for access to a preferred sucrose reward (Flaherty & Grigson, 1988; Reilly & Pritchard, 1997) or on an empty spout to self-administer a drug of abuse (Gallate & McGregor, 1999). In Experiment 1, then, rats were given 5 min access to 0.15% saccharin. This tube retracted and an empty tube advanced for 1 h during which time the rats could lick on an FR10 schedule of reinforcement for each iv infusion of cocaine (0.33 mg/infusion). There was one such taste-drug pairing a day for 13 days in succession. On the basis of the Wise et al. (1976) data, we predicted that the rats would avoid intake of the saccharin CS when paired with the opportunity to self-administer cocaine and, consistent
with the reward comparison hypothesis, that greater avoidance of the saccharin cue would be associated with greater drug self-administration.

4.2.2 Method

4.2.2.1 Subjects

The subjects were 35 naive, male, Sprague-Dawley rats (Charles River Laboratories) weighing between 300 - 400 g at the start of the experiment. They were housed individually in standard wire-mesh cages in a colony room with temperature, humidity, and ventilation automatically controlled. The rats were maintained on a 12/12h light/dark cycle with the lights on at 07:00 a.m. All experimental manipulations were conducted over an 8 h period starting 2 h into the light phase of the cycle. Except where noted otherwise, the rats were maintained on water and food (Teklad) ad libitum.

4.2.2.2 Self-administration catheter construction

The catheters are custom 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/tubing assembly is molded into a permanent dental
cement base using a custom-designed mold. A 2.5 x 2.5 cm mesh (Small Parts) is permanently fixed to the base via dental cement and functions as a backplate for the catheter assembly. A small silicon rubber ball 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 to the vein and muscle with super glue (Duro, Loctite Corp.). The entire catheter is flushed with and then soaked in 200 proof alcohol for 24 h prior to implantation.

### 4.2.2.3 Catheter implantation

The rat is anesthetized with the im administration of a Ketamine (70 mg/kg)/Xylazine (16 mg/kg). The hair is shaven on the rat in two places: 1) on the back of the rat between the shoulder blades and 2) directly on top of the jugular vein on the neck. One incision is made above the jugular vein at the neck (approximately 10 mm in length), at about a 30 degree angle away from midline. Another incision is made on the back of the rat (approximately 1" in length), horizontally positioned between the shoulder blades. The skin is separated from the muscle in both locations using hemostats. A cannula is 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 is inserted through the cannula, and then the cannula is removed. The rat is placed supine and the jugular vein is exposed by gently separating the muscle surrounding the vein using blunt microforceps. Once the jugular vein is located and cleared from surrounding
tissue, a stainless steel rod (3 mm diameter) is moistened with saline and gently placed under the jugular vein. Once the rod is in place it is 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) is then inserted into the vein through the incision. Verification that the catheter is in the jugular vein is 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. Following verification of proper placement, the catheter is secured into position by super gluing the silicon ball and catheter to surrounding muscle. Once anchored, the skin is sutured closed and Betadine antibiotic ointment (Baxter Co.) is applied over it. The rats are treated iv with 0.8 cc of 16.6% Tobramycin Sulfate and 0.2 cc of 400 mg Oxicillin Sodium once a day for 4 days following surgery. Patency is verified, when necessary, using 0.15 cc of 1% Brevital administered iv.

4.2.2.4 Coupling assembly

Prior to the start of each self-administration session, a coupling assembly is 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) is attached to the catheter assembly. The catheter tubing is attached to a counterbalanced swivel device (Instech, Inc.) that in turn is attached to a fluid injection assembly (syringe pump) in the experimental chambers. The fluid injection assembly enables iv
infusion of cocaine during self-administration sessions. In the animal's home cage, the catheter is sealed with a piece of Tygon tubing and a metal spacer is placed over the catheter assembly. General maintenance of catheter patency involves daily examination, cleaning of the coupling assembly, and flushing of the catheter with heparinized saline (0.2 ml of 13 IU/ml heparin).

4.2.2.5 Apparatus

The rats were trained in one of four identical modular operant chambers (MED Associates, Inc., St. Albans, VT) measuring 30.5 x 24.0 x 29.0 cm (length x width x height) and housed in a light and sound attenuated cubicle. All chambers have a clear Plexiglas top, front, and back wall. Side walls are made of aluminum. The grid floors consist of nineteen 4.8-mm stainless steel rods spaced 1.6-cm apart (center to center). Each chamber is equipped with two retractable sipper tubes that can enter the chamber through 1.3-cm diameter holes spaced 16.4-cm apart (center to center). A stimulus light is located 6 cm above each. In the extended position, the tip of the sipper tube is aligned in the center of the hole, flush with the right end wall. A lickometer circuit is used to monitor licking. Each chamber is also equipped with a house light (25 W), a tone (Solalert Time Generator, 2,900 Hz), and a speaker for white noise (75 dB). Cocaine reinforcement is controlled by an electronic circuit that operates a syringe pump (Razel Scientific Inst., Model A). Control of events in the chamber and collection
of the data are carried out on-line using a 33-MHz computer. Programs are written in the Medstate notation language (MED Associates, Inc.).

4.2.2.6 Procedure

The 35 subjects were run in 3 sets across different months. Approximately one week following surgery, all rats were placed on a water-deprivation regimen in which they received access to dH₂O for 5 min in the morning and for 1 h each afternoon. They were then habituated to the experimental chambers for 5 min a day for 3 days during which time they were given 5 min access to dH₂O in the chamber. The animals were then placed into one of two testing conditions: saccharin-saline iv or saccharin-cocaine iv. During testing, all rats were placed in the chambers with the house light and white noise on. The left tube was advanced and the rats were given 5 min access to 0.15% saccharin. This CS tube was then retracted and the empty US tube was advanced on the rats’ right. The stimulus light was illuminated above the US spout and the house light was off. The animals were placed on an FR10 schedule where completion of 10 licks lead to an iv infusion of 0.33 mg cocaine (n=18) or saline (n=17) over a 6 sec period. Drug or saline delivery was signaled by offset of the stimulus light, retraction of the spout, and onset of the tone and house light which remained on for a total of 20 sec. Further responding during this time was not reinforced. The total access period to the drug or saline lasted for 1 h. The patency of the catheter was evaluated after testing each day with Brevitol (0.2 ml of 10 mg/ml).
and the data were discarded when patency was not confirmed. 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 a total of 13 days. Finally, for the third set of rats only, blood samples (approximately 0.15 ml) were taken from the catheter 15 min after the final access period to water (24 h prior to the first conditioning trial) and then again 15 minutes after CS access on Trial 8. The blood was assayed for corticosterone using a standard radioimmunoassay kit (ICN). Unfortunately, the results from this assay yielded extreme scores. As a consequence, this aspect of the study currently is being replicated and will be presented in a separate report.

4.2.2.7 Data Analysis

All data were analyzed with SAS (SAS Institute Inc., Cary, NC) using a mixed factorial General Linear Model (GLM). Due to the loss of catheter patency, some animals failed to contribute data to all trials in all experiments. Both the statistical package, SAS, and the GLM are well suited to handle data with missing values of this sort. Post hoc tests were conducted where appropriate using Fisher’s Least Significant Difference (LSD) tests with α set at .05.
4.2.3 Results and Discussion

Due to catheter failure during testing, 16/35 rats (7 saline; 9 cocaine) contributed data to less than 13 trials. The available data from these 16 subjects were analyzed along with the data from the others using SAS and a 2 x 13 mixed factorial GLM varying US condition (saccharin or cocaine) and trials (1 - 13). Post hoc tests were conducted, where appropriate, using Fisher’s LSD tests with $\alpha$ set at .05.

4.2.3.1 CS Intake (licks/5min)

Intake of the saccharin CS was greatly suppressed when paired with the opportunity to self-administer cocaine, see Figure 4-1, left panel.
This conclusion was supported by post hoc tests of a highly significant US x Trials interaction, $F(12, 341) = 10.26, p < .0001$, which showed that rats in the
saccharin-cocaine group consumed less of the saccharin CS than did the saline treated controls on trials 2 - 13, ps < .05.

Although this was a robust finding, individual differences were evident in the cocaine-treated animals. As a consequence, these subjects were first divided into two groups on the basis of the median intake of saccharin on the final two days of conditioning. It then became evident, however, that 10 of the rats in the cocaine condition actually made fewer than 200 licks/5 min, while 8 of others in the cocaine condition made an average of approximately 1000 licks/5 min. We divided the rats accordingly. Thus, 10 rats in the saccharin-cocaine group were labeled as large suppressers and 8 were labeled as small suppressers. These data were then reanalyzed using a 3 x 13 mixed factorial GLM with group (saline, small suppressers, and large suppressers) and trials (1 - 13) as factors. The results of this analysis yielded a highly significant Group x Trials interaction, F (24,329) = 9.94, p < .0001, see Figure 4-1, right panel. Post hoc tests showed that while the large suppressers consumed less of the saccharin CS than the saline injected controls on trials 2 - 13 (ps < .05), the reduction in CS intake for the small suppressers attained significance only on trials 8 and 13 (ps < .05). The large suppressers also made fewer licks for the saccharin CS than did the small suppressers and this effect was statistically significant on trials 3 - 13, ps < .05. Taken together, the data demonstrate that rats will suppress intake of a saccharin CS when paired with the opportunity to self-administer cocaine. Moreover, the data show that there are very distinct individual differences, whereby approximately half of the Sprague-Dawley rats are more likely to avoid
the saccharin cue than are the other half. This finding is consistent with a similar dichotomy that occurs when the same saccharin CS is paired with the passive administration of either morphine and cocaine (Gomez, Leo et al., 2000; Gomez, Wheeler, & Grigson, 2000). Finally, it should be noted that these differences in intake cannot be attributed to differences in body weight as the results of a 3 x 13 mixed factorial GLM varying group (saline, cocaine small suppresser, cocaine large suppresser) and trials (1 - 13) found no significant effects, ps > .05.

4.2.3.2 Log 10 Latency (sec) to Lick the CS

As in the Flaherty and Grigson (1988) report, the latency data were logged and then analyzed with SAS using the GLM varying group (saline vs. cocaine) and trials (1-13). Once again, post hoc tests were conducted using LSD tests. The results of this analysis showed that the rats in the saccharin-cocaine group not only consumed less saccharin when it predicted the opportunity to self-administer cocaine (as shown in Figure 4-1), but that they also exhibited a longer latency to initiate licking the saccharin CS relative to the saline controls, see Figure 4-2, left panel.
This observation was confirmed by post hoc tests of a significant US x Trials interaction, $F(12, 341) = 2.62, p < .002$, showing that the rats in the saccharin-cocaine condition took longer to make the first lick of the saccharin CS than did the saline treated controls on trials 4, 5, and 8 - 13, $p < .05$.

Figure 4-2: **Left panel.** Mean (+/- SEM) log 10 latency (sec) to initiate licking the 0.15% saccharin solution following 13 pairings with the opportunity to self-administer either saline ($n=17$) or cocaine (0.33 mg/infusion, $n=18$) for 1 h using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small ($n=8$) and large suppressers ($n=10$) on the basis of the median split for saccharin intake. * = significantly different from saline controls.
Given the large individual differences obtained in the lick data, the latency data also were reanalyzed on this basis (i.e., on the basis of the group assignments determined for the bottle-1 saccharin intake) using the GLM with group (saline, small suppressers, and large suppressers) and trials (1-13) as factors. The results revealed a significant main effect of group, $F(2,32) = 16.15, p < .0001$, and Group x Trials interaction, $F(24,329) = 3.31, p < .0001$. Post hoc LSD tests of the main effect of group showed that the large suppressers took significantly longer to initiate licking the saccharin cue than did either the small suppressers or the saline treated controls overall, $p < .05$, which did not significantly differ from one another, $p > .05$. The results of the post hoc tests of the Group x Trials interaction were similar, showing that the large suppressers were slower to initiate licking the saccharin cue than were the saline controls on trials 4, 5, and 8 - 13, $ps < .05$, see Figure 4-2, right panel. Moreover, the large suppressers were slower to initiate licking the saccharin CS than the small suppressers on trials 5, 8 - 10, and 12 -13, $ps < .05$. This finding (i.e., a longer latency to initiate licking the saccharin CS when paired with cocaine self-administration) is interesting in that it parallels the data obtained in the anticipatory contrast paradigm where rats not only make fewer licks for the saccharin cue when it predicts the availability of the preferred sucrose reward, but are slower to initiate licking the saccharin cue as well (Flaherty & Grigson, 1988).
4.2.3.3 US Infusions/h

It should be noted that on no occasion were rats in the cocaine condition primed with cocaine (i.e., given a passive administration of the drug to initiate self-administration) and, more importantly, that all rats self-administered the drug during the very first conditioning trial using the empty tube operant. As such, both CS and US pre-exposure effects were successfully avoided, allowing for an evaluation of the development of the CS-US association over trials. In accordance with the prediction of the reward comparison hypothesis, rats in the cocaine group made more infusions than did the rats in the saline group, see Figure 4-3, left panel.
Support for this conclusion was provided by a significant US x Trials interaction, 
$F(12, 344) = 13.09, p < .0001$. Post hoc tests of this interaction showed that, 
although rats in the saline group made more infusions than did rats in the

Figure 4-3: **Left panel.** Mean (+/- SEM) number of infusions/h of either saline (n=17) or cocaine (0.33 mg/infusion, n = 18) following 13 saccharin-infusion pairings. The unconditioned stimulus (saline or cocaine) was infused using a fixed ratio 10 lick contingency on an empty spout. **Right panel.** A depiction of the same data, with the rats in the cocaine group divided into small (n=8) and large suppressers (n=10) on the basis of the median split for saccharin intake. * = significantly different from saline controls.
cocaine group on the first conditioning trial, this pattern was significantly reversed on trials 9 - 12 with the cocaine rats taking more infusions than the rats in the saline control group, ps < .05.

The self-administration behavior also was evaluated on the basis of individual differences obtained in the number of licks made for the saccharin CS in bottle 1. The results of this analysis were striking, revealing a highly significant main effect of group, F (2,32) = 11.16, p < .0002, and Group x Trials interaction, F (24,332) = 7.07, p < .0001, see Figure 4-3, right panel. Post hoc LSD tests of the 3 x 13 Group x Trials interaction were informative. As in the original analysis, the results showed that the saline rats made more infusions of saline on trial 1 than either the small or large suppressers made for cocaine, p < .05. Thereafter, the number of infusions decreased for the saline injected animals and increased for the large suppressers in the cocaine group. Post hoc tests indicated that the number of infusions made by the large suppressers was greater on trials 6 - 13 than was the number made by the saline treated subjects, ps < .05, and was greater on trials 2 - 13 than was the number made by the small suppressers, ps < .05. The number of infusions made by the small suppressers generally did not differ from those made by the saline controls, except on trials 1 and 4, where the small suppressers actually made fewer infusions of cocaine than the controls made of saline, ps < .05.

Taken together, these data suggest that the two groups of Sprague-Dawley rats not only differ in their consumption of the saccharin CS, but also in their propensity to self-administer the drug of abuse. In an effort to assess the
strength of this relationship, a correlational analysis was conducted with CS intake and US infusions as factors for all rats in the cocaine group (n = 18). Scores were used from the final two days of testing for each rat (i.e., when last patent). The results of this analysis revealed a strong negative correlation whereby low CS intake (i.e., avoidance of the saccharin cue) was associated with greater cocaine self-administration ($r = -.69, p < .001$), see Figure 4-4.
Thus, not only do approximately half of the rats from this outbred strain avoid the saccharin cue and "consume" more of the drug of abuse, but the degree of suppression of CS intake is strongly correlated with subsequent drug self-

Figure 4-4: A correlational analysis of each rat's terminal intake (i.e., licks/5 min on the final two days of testing) of the saccharin conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a strong negative relationship where low saccharin intake was highly correlated with high drug self-administration behavior.
administration behavior. This finding is consistent with our data and with those of others showing similar individual differences when the drug is passively administered (Farber et al., 1976; Gomez, Leo et al., 2000; Riley et al., 1978; Turenne et al., 1996). Thus, while it is possible that the differences in CS intake between the small and large suppressers in the present report were due to differences in the amount of drug self-administered, it is unlikely because in other experiments both large and small suppressers have emerged even when US exposure was held constant across all rats via passive drug administration (Gomez, Leo et al., 2000). Therefore, it is more likely that this enhanced suppression of saccharin intake reflects drug prone rats.

4.2.3.4 Log 10 Latency (sec) to First Infusion

Unlike the long latency with which the cocaine rats initiated licking the saccharin cue, the latency to obtain the first infusion of cocaine was short relative to the saline controls, see Figure 4-5, left panel.
Post hoc analysis of a significant US x Trials interaction, $F(12,322) = 2.93$, $p < .0007$, indicated that rats earned the first infusion of cocaine more quickly than the first infusion of saline on trials 8, 9, 10, 12, and 13, $ps < .05$. The pattern of data was similar when reanalyzed on the basis of the number of licks made on...
bottle 1 saccharin (i.e., saline, small, and large suppressers), see Figure 4-5, right panel. Post hoc LSD tests of a significant main effect of group, F (2,32) = 6.55, p < .004, showed that the large suppressers self-administered their first infusion more quickly than did either the small suppressers or the saline treated controls overall, p < .05. A similar finding was revealed by post hoc tests of the Group x Trials interaction, F (24,310) = 2.37, p < .0004. In this case, the data indicated that the large suppressers received their first infusion more quickly than the saline group on trials 8 - 10, 12, and 13, ps < .05. A similar reinforcement effect also was found in the small suppressers, but only on trial 12, p < .05, see Figure 4-5, right panel. Finally, the large suppressers were faster to administer their first infusion of cocaine each day than were the small suppressers, and this effect was significant on trials 4, 5, 7, and 8, ps < .05.

4.3 Experiment 2

4.3.1 Introduction

The results of Experiment 1 showed that (1) rats avoided intake of a saccharin cue when it predicted the opportunity to self-administer cocaine, (2) there were clear individual differences whereby approximately half of the rats were more likely to avoid the saccharin CS than were the others, and (3) those rats that avoided the saccharin CS also demonstrated a greater propensity to self-administer cocaine when given the opportunity. These findings are important
because they confirm and extend those of Wise et al. (1976) when using a less traditional empty spout as the operant. Use of this operant facilitated intake of both the saccharin CS and the cocaine US, and thereby averted the need for training and, consequently, CS and US preexposure. While it is the case that the empty tube operant is commonly used in animal learning studies (Reilly & Pritchard, 1997), it is somewhat new to the drug self-administration literature (Gallate & McGregor, 1999; Slawecki, Samson, & Chappell, 1997). Thus, Experiment 2 used the 15 rats from the third replication of Experiment 1 to examine the dose response function for decreasing doses of cocaine when using an FR10 on the empty tube and then again when using an FR1 on a lever. The saccharin CS was not presented in this experiment.

4.3.2 Method

4.3.2.1 Subjects

Fifteen rats from the third replication of Experiment 1 served as subjects. Seven of these rats were previously in the saccharin-saline group and 8 rats were in the saccharin-cocaine group (5 of these rats were identified as small suppressers and 3 as large suppressers). All rats were housed and maintained as described above.
4.3.2.2 Apparatus

The apparatus was the same as that used in Experiment 1, except that a lever, located on the opposite side of the testing chamber from the empty spout operant, was advanced into the chamber for the second phase (the lever phase) of the experiment.

4.3.2.3 Procedure

The rats continued on the water deprivation regimen described above. They were then given the opportunity to lick the empty spout on the FR10 schedule of reinforcement for the standard dose of cocaine (0.33 mg/infusion) used in Experiment 1. Once responding stabilized (5 days), a dose-response analysis was conducted where all rats were given the opportunity to respond for 1 h/day on the FR10 schedule for decreasing doses of cocaine (0.33, 0.16, 0.08, 0.04 mg/infusion) using a within subjects design. As described by Miczek and Mutschler (1996), responsiveness for each dose was evaluated across a 3 day period before switching to the next lowest dose. This dose-response function was then reevaluated in the same animals using an FR1 contingency on a lever, rather than the FR10 lick contingency on the spout.
4.3.3 Results and Discussion

The number of infusions made for each dose was averaged across the three day test session for each operant. The scores were analyzed using SAS and a 3 x 2 x 4 mixed factorial GLM varying history (i.e., identified as saline, small suppressers, or large suppressers in Experiment 1), operant (empty tube vs. lever) and dose (1 - 4). Once again, post hoc comparisons were made using Fisher LSD tests with $\alpha$ set at .05. The results of this analysis showed that the main effect of dose was significant, $F(3,32) = 21.19, p < .0001$, with the number of infusions/h decreasing significantly following the transition from 0.08 to 0.16 mg/infusion, $p < .05$, see Figure 4-6, left panel.
Although there was a tendency for the rats to make more infusions when using the lever than the spout (see Figure 4-6, middle panel), neither the main effect of operant, $F(1,5) = 1.16$, $p = .33$, nor the Operant x Dose
interaction, $F(3,13) = 1.88$, $p = .18$, was significant. The History (saline, small suppresser, large suppresser) x Dose interaction, $F(6,32) = 1.4$, $p = .24$, also failed to achieve statistical significance, in spite of the strong tendency for the rats identified as large suppressers in Experiment 1 to make more infusions than the rats identified as small suppressers, see Figure 4-6, right panel. Because of this strong tendency, we reanalyzed the dose data directly comparing the functions generated by the rats identified as small and large suppressers in Experiment 1. The results of this analysis revealed a highly significant History x Dose interaction, $F(3,15) = 5.22$, $p < .01$. Post hoc tests confirmed that the large suppressers made more infusions of the two lowest doses of cocaine than did the small suppressers, $ps < .05$. Finally, while the nature of the operant seemed to have little impact on the data, the three-way History (saline, small, and large suppressers) x Dose x Operant interaction was significant, $F(6,13) = 3.93$, $p < .018$ (data not shown). Post hoc tests of this interaction revealed that the large suppressers made significantly more infusions/h when using the spout to administer cocaine than did the saline rats or the small suppressers. This effect, however, was only significant with the lowest dose of cocaine, $ps < .05$. No other points were significantly different.

Three conclusions can be drawn from these data. First, individual differences in behavior are persistent. The rats identified as large suppressers (i.e., large drug-takers) in Experiment 1 continued to take more drug in the dose-response analysis in Experiment 2 than did those rats previously identified as small suppressers. This finding is interesting because it shows that, even with
repeated experience, the large suppressers continue to exhibit a sustained preference for cocaine, while the small suppressers appear to remain fairly resistant to the addictive properties of the drug. Second, as expected (Woods & Schuster, 1968), rats made fewer infusions for increasing doses of cocaine and this pattern was evident whether the operant response involved licking an empty spout or pressing a lever. The empty spout, then, is a valid and reliable operant. Finally, while the empty tube operant was highly effective in this paradigm, it is noteworthy that many of the rats were as likely to complete their "lick" contingency (i.e., to complete the circuit) by nose-poking rather than licking and nose-poking is an already well-established, facilitatory operant response (e.g., see De Vries, Schoffelmeer, Binnekade, & Vanderschuren, 1999).

4.4 Experiment 3

4.4.1 Introduction

It is now well known that classical conditioning or, more specifically, exposure to cues that previously have been associated with drug administration, can induce relapse following a period of extinction or abstinence in humans and animals (Ehrman et al., 1992; Weiss et al., 2000). Humans, of course, can relapse after months or even years of abstinence (Franken, de Haan, van der Meer, Haffmans, & Hendriks, 1999; Franken & Hendriks, 1999; Jaffe, 1990). The
animal data suggest that rats also can relapse following days, weeks, or even months of abstinence if exposed to stress (Stewart, 2000), primed with the drug of abuse (Chiamulera, Borgo, Falchetto, Valerio, & Tessari, 1996), or presented with cues or contextual stimuli previously associated with drug self-administration (Gracy, Dankiewicz, Weiss, & Koob, 2000; Grimm, Hope, Wise, & Shaham, 2001; McFarland & Ettenberg, 1997, 1998; Meil & See, 1997; Weiss et al., 2000). The present study was designed to test whether presentation of the taste cue, like other cues, would facilitate cocaine-seeking behavior following a period of abstinence. We reasoned that if the saccharin cue predicted the opportunity to self-administer the drug of abuse and the rats (particularly the large suppressers) retained this association, then they should avoid intake of the saccharin cue (as they had prior to the period of abstinence) and, when given the opportunity, they should exhibit strong drug-seeking behavior.

4.4.2 Method

4.4.2.1 Subjects

Twenty healthy rats (5 that served in Experiment 1 only and the 15 that served in both Experiments 1 and 2) served as subjects. They were maintained and housed as described above. Nine of these subjects were previously in the saccharin-saline treatment group, 11 were in the saccharin-cocaine group, 5 were identified as small suppressers, and 6 were identified as large suppressers.
4.4.2.2 Apparatus

Testing was conducted in the apparatus described in Experiment 1.

4.4.2.3 Procedure

The rats were tested for cue-induced drug seeking behavior after a period of at least one month of abstinence (16 rats were tested after 30 - 42 days of abstinence, 3 rats were tested after approximately 60 days of abstinence, and 1 rat was tested after 180 days of abstinence). All rats were returned to the water deprivation regimen in which they received 5 min access to water in the morning and 1 h in the afternoon. Once intake stabilized (5 days), all rats were placed in their test chamber. The test trial proceeded exactly as described in Experiment 1, with two exceptions. First, after having 5 min access to the saccharin CS, the empty tube operant was advanced, but neither saline nor cocaine was infused via the catheter following completion of the 10 lick contingency. This phase of the experiment, then, was essentially an extinction session during which time the spout and the associated cues were presented and drug-seeking behavior was evaluated. Second, the post-abstinence test session was conducted with the outer chamber door open to allow for videotaped analysis of the behavior. This procedure was conducted very quietly and did not appear to disrupt the behavior of the rats. These videotaped data, however, are preliminary and will not be presented here.
4.4.3 Results and Discussion

4.4.3.1 Post-abstinence CS intake

Intake of the saccharin CS was evaluated using a 1-way ANOVA with history (saline, small suppressers, and large suppressers) as a factor. Post hoc tests of a significant ANOVA, $F(2,17) = 11.38$, $p < .0007$, showed that, although the saline controls and the small suppressers consumed a relatively large amount of the saccharin CS, the large suppressers consumed virtually none of the CS solution, $p < .05$, see Figure 4-7, left panel.
Thus, the pattern of behavior obtained in the large suppressers before and then after at least one month of abstinence was relatively seamless. The saccharin CS was associated with the opportunity to self-administer the drug of abuse in Experiment 1 and the rats clearly remembered this association in

Figure 4-7: **Left panel:** Mean (+/- SEM) intake (licks/5 min) of the 0.15% saccharin solution for rats in the saline group (n=9), the small suppressers (n=5), and the large suppressers (n=6) in Experiment 1 when tested following a period of at least 30 days of abstinence. **Right panel:** Mean number of infusion attempts/h (i.e., completions of the 10 lick contingency) for the same subjects when given the opportunity to lick on the fixed ratio 10 lick contingency on the empty spout. This was essentially an extinction trial, however, during which time neither saline nor cocaine was delivered.
Experiment 3 as they continued to avoid intake of the natural saccharin reward even after a period of one to six months of abstinence from the drug of abuse.

4.4.3.2 Post-abstinence infusion attempts

Drug-seeking behavior (i.e., the number of attempts to infuse the drug) was evaluated using the same analysis as described for CS intake. Once again, the main effect of history, $F(2,17) = 4.34$, $p < .0003$, was highly significant. Post hoc tests of this analysis showed that the large suppressers made nearly three times as many completions of the 10 lick contingency in an attempt to infuse the US as did either the small suppressers or the saline controls, $ps < .05$, which did not differ from one another, $p > .05$, see Figure 4-7, right panel. The large suppressers, then, not only continued to avoid intake of the saccharin cue following a month of abstinence, but also continued to make greater attempts to self-administer the drug of abuse. Finally, although it may not have seemed ideal to test the animals after such varied periods of abstinence, it is noteworthy that the number of infusion attempts significantly increased with increasing periods of abstinence up to 60 days, $r = .86$, $p < .001$. This pattern of behavior is in perfect keeping with a recent report by Grimm et al. (2001) showing that drug-seeking behavior increased as the period of abstinence increased from 1 - 60 days. This “incubation effect”, however, may not remain linear, as the number of infusion attempts averaged 13.3 for the rats that were abstinent for 30 to 42 days, 32.3 for the rats that were abstinent approximately 60 days, but only 29 for the rat that
was tested at 6 months. Further study is needed to more fully elucidate the
effects of long-term abstinence on drug-seeking behavior.

4.4.3.3 Log latency (sec) to lick bottle-1 saccharin

The latency data were logged and analyzed using a one-way ANOVA. The
results showed that, although there was a slight tendency for the large
suppressers to take longer to initiate licking the saccharin CS than either the
small suppressers or the saline controls (mean = 76, 23, and 29 sec,
respectively) the main effect of history was not statistically significant, F < 1.

4.4.3.4 Log latency (sec) to obtain the first infusion

The results of a similar one-way ANOVA indicated that the latency to
complete the first mock infusion also did not differ significantly across three
groups, F < 1, in spite of a trend for both the small and the large suppressers to
obtain the first mock infusion more quickly than the saline controls. The absence
of a significant contrast effect in the latency measure on bottle 1 or a significant
reinforcement effect here likely relates to the sample size. Only 5 small and 5
large suppressers (the latency data from one large suppresser were lost)
contributed data to the latency measures. Even so, it is clear that rats (at least
the large suppressers) retain the taste-drug association over a 1 - 6 month
period, leading to continued avoidance of the saccharin cue and persistent drug-seeking behavior.

4.5 General Discussion

The results of Experiment 1 showed that rats initiated licking more slowly and made fewer licks for the saccharin cue when it was paired with the opportunity to self-administer cocaine. When this bottle was retracted and the empty tube operant was advanced, it was determined that low CS intake was correlated with greater, rather than fewer, infusions of cocaine. The intake data were then reevaluated on the basis of terminal intake of the saccharin CS and two distinct populations emerged: a group of small suppressers that made approximately 1000 licks of the saccharin CS in 5 min and then initiated approximately 4 infusions of cocaine per h and a group of large suppressers that made less than 200 licks of the saccharin CS in 5 min and then took approximately 10 - 12 infusions of cocaine per h. In Experiment 2, the usefulness of the empty bottle operant was confirmed when, as with the lever press, increases in the dose of cocaine led to predictable decrements in the number of infusions of cocaine per h. Finally, the results of Experiment 3 showed that the strength of the taste-drug association was long lived. The large suppressers continued to avoid intake of the saccharin cue and exhibited nearly three times as many attempts to infuse cocaine following a period of abstinence ranging from 1 to 6 months. Indeed, up to 60 days, drug-seeking behavior was positively
correlated with the length of the period of abstinence such that the rats made significantly more attempts to infuse the drug of abuse when tested following longer periods of abstinence.

In general, these findings confirm and extend those of Wise et al. (1976). First, it is clear that rats avoid intake of a saccharin cue when paired with passive (Cappell & Le Blanc, 1973; Cappell & LeBlanc, 1971; P. S. Grigson, Twining et al., 2000; Le Magnen, 1969) as well as active (i.e., self-) administration of a drug of abuse. Second, the rats suppress intake of a saccharin cue when paired with the opportunity to self-administer not only apomorphine (Wise et al., 1976), but also cocaine (present report). Third, the suppression of CS intake is robust even when the rats are drug naive at the start of testing (as in the present report). Fourth, anticipation of the availability of the drug of abuse during training not only suppresses intake of the saccharin CS, but also increases the latency to begin licking the saccharin CS. As stated, this finding (i.e., fewer licks and a longer latency to initiate licking during the acquisition phase) parallels that obtained when access to the same saccharin CS predicts the availability of a preferred sucrose reward in the anticipatory contrast paradigm (Flaherty & Grigson, 1988). Finally, the results of the present report also are consistent with the findings of Wise et al. (1976) in showing that, unlike a LiCl-induced CTA (White et al., 1977), avoidance of the saccharin CS was accompanied by an increase in responsiveness for the drug of abuse. Indeed, the correlational data revealed that the very same rats that exhibited the greatest avoidance of the saccharin
cue, demonstrated the greatest propensity to self-administer the drug of abuse when given the opportunity.

This apparent dissociation between reduced intake of the gustatory CS and increased instrumental responding for the drug US has been reported previously and was thought to reflect the paradox whereby rats would demonstrate aversion to a novel taste and then exhibit increased speed to get to the goal box to receive a passive drug injection or to self-administer drug (e.g., Switzman, Amit, White, & Fishman, 1978; Wise et al., 1976). As stated in the Introduction, however, our more recent data suggest that avoidance of the saccharin cue is highly correlated with 'intake' of the drug of abuse because the very same rewarding properties that increase drug self-administration also are responsible for the reduction in intake of the saccharin cue that predicts the drugs availability (P. S. Grigson, 1997). Moreover, evidence suggests that cocaine-induced suppression of CS intake is not due to conditioned anorexia because a similar reduction in CS intake occurs when using a drug of abuse such as morphine that is known to enhance appetite (Doyle, Berridge, & Gosnell, 1993b; Zhang & Kelley, 2000). Further, it also is not likely to be due to competing responses because, while not yet tested with drugs of abuse, it is known that competing responses do not account for sucrose-induced suppression of saccharin intake (Flaherty et al., 1996). Of course, it is unlikely that the suppressive effects of drugs of abuse and those of a rewarding sucrose US are one in the same. Even so, the data are most consistent with the conclusion that rats suppress intake of the saccharin CS in the present paradigm because they
are anticipating the opportunity to self-administer a more rewarding drug of abuse.

As such, the paradigm serves as a model for the study of two important phenomena. The first is cue-induced craving and relapse. That is, although there are several animal models for studying the role of cue-, context-, drug-, and stress-induced craving and relapse (Ehrman et al., 1992; Grimm et al., 2001; for a review see LeSage, Stafford, & Glowa, 1999; Neisewander et al., 2000; Stewart, 2000; Weiss et al., 2000), this is the first analysis of cue-induced craving and relapse using a gustatory cue. The data suggest that use of such a taste cue may be particularly effective in eliciting reinstatement of drug-seeking behavior following long periods of abstinence because in the present report, the animals exhibited strong drug-seeking behavior even after a period of 1 to 6 months of abstinence. In this case, re-exposure to the taste cue was associated with more than a two-fold increase in the number of attempts to infuse the drug of abuse relative to the saline controls. It is important to note, however, that highly persistent drug-seeking behavior occurred (as a group) only in the rats that were identified as the large suppressers in Experiment 1. This selective pattern emerged in spite of the fact that all rats (even those who served as the saccharin-saline controls in Experiment 1) had a great deal of experience with cocaine self-administration in Experiment 2, and all rats were tested after at least 30 days of abstinence. This finding suggests that the taste cue played an important role in the drug-seeking behavior because rats with a great deal of experience with cocaine, the context, and the discriminative stimuli (i.e., lights
and tones), but not the taste-drug association, failed to exhibit as many attempts to self-administer the drug of abuse under the same testing conditions.

Although one might suggest that greater drug-seeking behavior occurred in the large suppressors simply because they had more drug exposure than the small suppressors overall (see Figure 4-6, right panel), this argument seems unlikely. That is, the greatest number of infusion attempts in Experiment 3 (39 and 52) actually were generated by two large suppressers that did not serve in Experiment 2 (i.e., their catheters were no longer patent in Experiment 1 following 10 and 9 saccharin-cocaine pairings, respectively). Of course, this is not to suggest that the context and/or the discriminative stimuli, themselves, did not support drug-seeking behavior in Experiment 3. Drug-seeking behavior also was increased from approximately 4 infusions/h to 10 infusion attempts/h during the abstinence test in the saline rats and in the small suppressers. Moreover, two rats from the saline group in Experiment 1 that initiated the most infusions in the dose-response analysis of Experiment 2, also exhibited very strong drug-seeking behavior (19 and 29 attempts) in Experiment 3. Thus, we conclude that drug-preferring Sprague-Dawley rats are more likely to demonstrate persistent drug-seeking behavior when exposed to associated contextual or discriminative cues (Ehrman et al., 1992; Neisewander et al., 2000; Stewart, 2000; Weiss et al., 2000) and, as shown in the present report, when exposed to an associated gustatory stimulus as well. Future studies will directly examine the relative contribution of these cues by comparing drug-seeking behavior in the presence and in the absence of the gustatory CS.
In addition to serving as another useful model for the study of cue-induced craving and relapse, the present paradigm also is the first and only animal model for the systematic study of the mechanisms by which anticipation of the availability of a drug of abuse can come to devalue natural rewards. This phenomenon, ranging from a narrowing of interests to neglect of oneself (e.g., weight loss, ones job, or even one’s children) is common in the addicted human and is a pervasive problem for society and a cardinal feature of addiction according to the DSM IV (Jones et al., 1995; Nair et al., 1997; Santolaria-Fernandez et al., 1995). Even so, there is little known about the factors that influence, or the mechanisms that mediate, the phenomenon. Are the rewarding properties of drugs of abuse and natural stimuli mediated by the same (DiChiara, Acquas, Tanda, & Cadoni, 1993), or separate (Carelli, Ijames, & Crumling, 2000), circuits? Even if mediated by separate circuits, the present data demonstrate that the value of one reward type can pale in anticipation of another and, therefore, that these different rewards must be compared via some common substrate.

Although we know relatively little about this substrate, a few conclusions can be drawn from recently published data. First, the data suggest that the gustatory thalamus may be important for drug-induced devaluation of natural rewards because rats with bilateral lesions of the gustatory thalamus continue to lick the saccharin cue even when paired with sucrose or the passive administration of morphine or cocaine (P. S. Grigson, Lyuboslavsky et al., 2000; Reilly & Pritchard, 1996b; Reilly & Trifunovic, 1999). The same may be true for rats with bilateral lesions of the gustatory cortex (Mackey, Keller, & van der Kooy,
Second, drug-induced devaluation of natural rewards is greatest in selectively bred strains of rats (Lewis rats) and in individual outbred Sprague-Dawley rats that are known to be drug-preferring (Glowa et al., 1994, present report; P. S. Grigson & Freet, 2000). Finally, outbred Sprague-Dawley rats can become more sensitive to drug-induced devaluation of natural rewards following treatment with a highly potent dose of morphine (P. S. Grigson, Wheeler et al., 2001). This finding is consistent with other reports showing sensitization to the rewarding properties of a drug of abuse following chronic drug treatment (Horger, Shelton, & Schenk, 1990; Lett, 1989). In addition, the finding also may implicate a contributing role for cells in the dopaminergic pathway projecting from the ventral tegmental area to the nucleus accumbens because similar cellular/molecular characteristics have been reported in this pathway in reward-preferring Lewis rats and in Sprague-Dawley rats following a history of chronic drug treatment (for a review, see Nestler, 1995). In addition this pathway has recently been found to be sensitive to contrast effects with sweets and drugs (Genn et al., 2004; P. S. Grigson, Acharya, N.K., Hajnal, A., 2004; Roitman, Wheeler, & Carelli, 2005; Taha & Fields, 2005; Wheeler & Carelli, 2006).

In conclusion, rats suppress intake of a saccharin cue when paired with the opportunity to self-administer a drug of abuse and, consistent with the reward comparison hypothesis, greater avoidance of the saccharin cue is correlated with greater drug self-administration behavior. Indeed, as was found in Chapter 2, when reevaluated on the basis of individual differences in CS intake, two phenotypes emerged. In this case, however, the active administration of the drug
afforded the opportunity to evaluate these separate groups of rats on their relative motivation to self-administer cocaine. To that end, the group of small suppressers accepted the natural saccharin reward, but took little drug while the group of large suppressers avoided intake of the natural saccharin reward, and then initiated 3 times as many cocaine infusions. This suggests that those rats that are more sensitive to devaluation of the saccharin reward are also more sensitive to the reinforcing properties of cocaine and not to the aversive properties. Furthermore, these individual differences were persistent. Rats identified as the large suppressers (big drug takers) in Experiment 1 exhibited greater drug-taking behavior across several doses in Experiment 2 and greater avoidance of the saccharin cue and greater drug-seeking behavior when reexposed to the initial training complex following a period of 1 to 6 months of abstinence. Clearly, consideration must be given to the mechanisms that mediate these individual differences and to the factors/conditions that might “switch” the behavior of a small suppresser (i.e., a small drug-taker) into that of a large suppresser (i.e., a large drug-taker who also is more predisposed to drug-seeking behavior following a period of abstinence). Finally, it should be noted that, although the tendency to compare rewards can lead to the devaluation of a natural reward by a drug of abuse, evidence suggests that, under the right circumstances, reward comparison also can lead to the converse - the devaluation of a drug of abuse by a highly reinforcing natural reward. The treatment potential of this particular circumstance is evident as rats, primates, and humans are known to take less drug when provided with powerful alternative
reinforcers such as glucose, food, or vouchers for ski lift passes, fishing licenses, or continuing education (Carroll, Lac, & Nygaard, 1989; Higgins et al., 1993; M. A. Nader & Woolverton, 1991a, 1991b). Thus, the very model proposed for the study of cue-induced craving and drug-induced devaluation of natural rewards, also may prove useful in the study of treatment.
Chapter 5

Non-contingent cocaine enhances the devaluation of a saccharin cue and appears aversive in rats.

5.1 Introduction

In Chapters 2 and 3, the devaluation of a saccharin cue by drugs of abuse was investigated by presenting the saccharin cue in a bottle followed by an investigator administered “passive” injection. In Chapter 4 the model incorporated the self-administration technique and it was found that the greatest saccharin avoidance was exhibited by the rats that self-administered the greatest amount of cocaine. The switch to self-administered cocaine was an important step in the development of the model to determine whether primarily reinforcing or aversive properties were mediating the devaluation of the saccharin cue and to more closely model human drug addiction. However, it is unclear to what degree the ability to control the contingency of drug delivery (i.e., the exact temporal pattern and amount of the drug) contributes to either the suppressive effects of cocaine on saccharin intake or to the reinforcing efficacy of cocaine. In order to determine this, the current experiments will add to the model established in chapter 4 by employing pharmacological “yoked” controls that experience the same dose and pattern of cocaine administration as the actively administering rats but noncontingent upon their behavior. The addition of the yoked controls is another
important step in the development of the model as this control has never been evaluated in the reward comparison paradigm.

Traditionally, yoked administration is intended to serve as the perfect control to prevent self-administration behavior from confounding the interpretation of the biological effects of the drug and it is generally assumed that there is no real difference between these conditions. However, there is growing evidence that contingent and noncontingent cocaine administration have very different effects both on the brain and behavior. For example, self-administration of cocaine yields significantly higher DA turnover rates relative to yoked controls in the nucleus accumbens, ventral pallidum, and lateral hypothalamus while glutamate and GABA turnover rates are lower in the NAc and ventral pallidum (J. E. Smith, Koves, & Co, 2003). Additionally, turnover rates for acetylcholine (ACh) were differentially modulated in eleven different brain regions including relative increases in the ventral pallidum, hippocampus, medial and lateral hypothalamus, and a relative decrease in the VTA of self-administering rats (J. E. Smith, Vaughn, & Co, 2004). A strikingly large relative increase in the turnover of ACh was observed in the caudate putamen of the yoked rats (J. E. Smith et al., 2004). These data are consistent with microdialysis experiments that report increases of DA in the ventral pallidum and increases in both ACh and DA in the NAc of self-administering rats compared with yoked rats (G. P. Mark, Hajnal, Kinney, & Keys, 1999; Sizemore, Co, & Smith, 2000). Recently, a more specific analysis of DA concentrations in the NAc shell and core revealed that both active and yoked cocaine increase DA in both sub regions, and to a larger extent in the
shell, early in training (Lecca, Cacciapaqlia, Valentini, Acquas, & Di Chiara, 2007). However, with continued training DA release became sensitized in the shell for active rats and in the core for the yoked rats which resulted in opposite core/shell ratios. These yoked animals also exhibited greater stereotyped locomotor behaviors (e.g., head bobbing, sniffing down, gnawing) that were positively correlated with the core/shell dopamine ratio (Lecca et al., 2007). Taken together, these findings demonstrate that the contingency profoundly affects how the drug impacts neurobiological substrates including those responsible for natural and drug reward which may be directly responsible for differential effects on behavior.

Indeed, the behavioral effects of yoked administration of cocaine are quite different than those of self-administration and may augment the aversive effects of cocaine. For example, noncontingent cocaine is associated with more severe and sustained withdrawal symptoms than the contingent administration of the same dose (Mutschler & Miczek, 1998) and profoundly increases the lethality of cocaine in longer access sessions (Dworkin, Mirkis, & Smith, 1995). The increased mortality has been interpreted as a “failure of tolerance” which depends on interoceptive self-administration cues to initiate conditioned compensatory responses (CCRs) in anticipation of the drug effects (Siegel, 1978a; Siegel & Ramos, 2002). Interestingly, yoked administration also can alter the motivational properties of cocaine even when rats have a history of contingent cocaine access. For instance, rats that self-administer cocaine for 1 hr followed by 5 hrs of yoked administration exhibit reduced reinstatement to drug
seeking following a cocaine priming injection but not to the conditioned cues associated with drug delivery (Kippin, Fuchs, & See, 2006). Taken together, these data indicate that the motivational properties of cocaine are reduced by noncontingent administration of the drug and this effect may be mediated by an aversive mechanism.

As stated, it is not known how these different drug administration techniques affect the devaluation of the saccharin cue because a direct comparison has never been conducted in the reward comparison paradigm. Since a saccharin cue is avoided when it predicts cocaine self-administration it is likely that yoked administration of the same dose would yield similar results. However, if the non-contingent delivery of cocaine is also aversive, then greater avoidance in those rats relative to their actively administering counterparts would be predicted. Furthermore, if the mechanism is aversive then the yoked controls should exhibit reduced instrumental responding for the drug in subsequent tests of drug motivation.

Therefore, the purpose of this chapter is to characterize the effects of self-administered cocaine in rats in relation to that of the yoked cocaine controls within the reward comparison model. Thus, Experiment 1 compares the reduction in CS intake in rats that actively administer the drug of abuse vs. yoked controls that received the same drug infusions, but non-contingent upon their behavior. Experiment 2 tests whether the purported aversive effects of non-contingently administered cocaine are sufficient to affect motivation to self-administer cocaine under low fixed ratio and higher progressive ratio work
requirements. **Experiment 3** utilizes an alternating side choice procedure to test whether a history of yoked delivery shifts the relative preference for cocaine over water in thirsty rats.

### 5.2 Methods

#### 5.2.1 Subjects

The subjects were 111 naïve, male, Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing between 350-500 g at start of the experiment. They were housed individually in standard wire mesh cages, in a colony room with temperature, humidity, and ventilation automatically controlled. The rats were maintained on a 12-hr light-dark cycle with the lights on at 0700. All experimental manipulations were conducted over 10-hr starting 1.5 hrs into the light phase of the cycle. Except where noted otherwise, the rats were maintained on water and food (Teklad) ad libitum.

#### 5.2.2 Catheter Construction

The catheters were custom made in our laboratory using a modified procedure originally described by Koob and colleagues (1987) and described in detail previously (P. S. Grigson & Twining, 2002b, and Chapter 4).
5.2.3 Catheter Implantation and Maintenance

Again, the surgery was described in detail in Grigson and Twining (2002) and in Chapter 4.

5.2.4 Apparatus

The rats were trained and tested in one of 12 identical modular operant chambers (MED Associates, St. Albans, VT) that are identical to the ones described in Chapter 4.

5.2.5 Procedure

These 111 rats were run in two sets across different months. Approximately two-weeks after surgery, all rats were placed on a water deprivation regimen. *Water Deprivation*. Rats received access to distilled water (dH₂O) for 1 h each afternoon no sooner than 45 minutes after daily testing. If a rat failed to drink >5 ml of water during the rehydration period, they were given 10 ml overnight.

5.2.6 Habituation

All rats were habituated to the operant chambers for 20 minutes for 5 days prior to testing. During the first 5 min, a water spout was advanced on their left
followed by 15 minutes additional exposure to the chamber for 5 days.

**MATCHED GROUPS.** Rats were then matched on the basis of body weight and 5 min water intake and placed into one of 4 groups: **COC-ACTIVE (CA; n=32), COC-YOKED (CY; n=32); SAL-ACTIVE (SA; n=15), SAL-YOKED (SY; n=32).**

To control for different amounts of yoked saline delivery which is accompanied by cue light offset, tone onset and motorized spout retraction 8 of the saline yoked controls were yoked to the CA group and the rest to SA group.

### 5.2.7 Conditioning

#### 5.2.7.1 Experiment 1

During testing, all rats were placed in the operant chambers and given 5 min access to a 0.15% saccharin CS on the left side of the chamber. This CS tube was then retracted while the empty US tube (right) and inactive tube (center) were advanced. The house light remained on at all times and the stimulus light was illuminated above the US tube. For the next 1 h of testing, completion of 10 licks on the US tube (i.e., Fixed Ratio 10 schedule of reinforcement; FR10) by an actively administering rat lead to an iv infusion of either saline or 0.33 mg cocaine (0.2 ml) over a 6 sec period. Drug or saline delivery was signaled by retraction of the US tube, offset of the US-light, and initiation of a 20 sec time-out period signaled by the onset of a tone. Responding during the time-out period, or at anytime on the inactive spout, was not
reinforced. Simultaneously, the computer initiated the very same event sequence for both the saline-yoked and cocaine-yoked controls. Supplemental water was provided for 1 h, no sooner than 45 min after being returned to the home cage. CS-US pairings occurred once daily for a total of 18 pairings.

5.2.7.2 Experiment 2

All rats, regardless of their history in Experiment I, were given the opportunity to self-administer cocaine for 1 h/day on an FR10 schedule of reinforcement for 2 days (no saccharin was delivered). This was followed 24-72 h later by testing on a progressive ratio schedule of reinforcement where the lick contingency on the empty US tube was incremented progressively by 20 licks per reinforcement (PR10+20/inf). This PR session ended when the rat failed to receive a cocaine infusion in a 30 min time period. This last completed ratio is termed the “break point”.

5.2.7.3 Experiment 3

All rats were given 12 opportunities/h to choose water vs. cocaine in an alternating-side choice procedure by performing 10 licks on either the right or left empty-spout operant. At the beginning of each session both the right and left empty spout were advanced. The cue light was illuminated over the empty spout that lead to an i.v. infusion of cocaine (0.33 mg/inf) as it has been in Experiments
1 and 2, while no cue light was illuminated over the opposite empty spout which lead to a 20 sec access period to the center spout filled with dH2O (the 20 sec access period did not begin until the rats first contact with the water spout). The right/left location of the water and the cocaine operant was counterbalanced across trials and between rats. In addition, after successful completion of the FR10 lick requirement on the water or cocaine operant, both empty spouts were retracted and a 5 minute time-out was implemented. After the time-out period, when the right and left empty spout operants returned, the location of the rewards was alternated. The session was 1 h in length and therefore could lead to 12 choices maximally.

5.3 Results and Discussion

As stated above, the experiment was run in two iterations across different months. A mixed factorial analysis of variance (ANOVA) with repeated measures varying Group (CA, CY, SY) x Trials (1-18) x Replication (1, 2) indicated that the replication factor did not contribute meaningfully to any interactions whatsoever. There was, however, a significant main effect of replication, F(1, 94)=4.03, p<0.04, which indicated that rats in the first replication consumed more saccharin overall (Rep 1 Mean = 1,170.2; Rep 2 Mean = 1,040.7). The replication factor was dropped from further analysis since the relative differences between the treatment groups was the same for both replications. A preliminary statistical analyses was conducted on the saline
control groups (yoked to CA, yoked to SA, and SA), and found no significant
differences between them throughout any of the experiments and were therefore
collapsed into one group and will from this point forward be referred to as group
saline (SAL).

5.3.1 Experiment 1: CS Intake (Licks/5 min)

A mixed factorial ANOVA varying Group (CA, CY, SAL) x Trials (1-18)
indicated that rats that received non-contingent cocaine infusions exhibited
greater avoidance of the saccharin cue relative to their actively administering
counterparts, despite receiving the exact same dose of cocaine over the same
time course (see Figure 5-1).
Newman-Keuls post-hoc tests of a significant 2-way interaction, $F(34, 1836)=6.80, p<0.0001$, revealed that while both the CA and CY rats consumed significantly less of the saccharin cue than their saline controls on trials 2-18, the CY rats actually consumed significantly less than the CA rats on trials 3-5, and 7-18, $p<0.05$. Post-hoc tests of the significant main effect of Group, $F(2, 108)=25.58, p<.0001$, further supported this observation. Both the active and

Figure 5-1: Mean (+/- SEM) intake (licks/5 min) of 0.15% saccharin following 18 pairings with either active or yoked cocaine (0.33 mg/infusion) for 1 h. * = significantly different from saline controls, # = significantly different from active cocaine and saline controls.
yoked cocaine rats consumed less saccharin overall compared to the saline controls, ps<0.05, and the yoked rats consumed even less than their active counterparts, p<0.05.

5.3.2 Experiment 1: CS Intake and individual differences

Upon closer inspection of the data it became evident that two distinct response profiles exist within the actively administering group. The two groups consisted of rats that suppressed their intake of the saccharin CS to a large degree and rats that did not suppress their intake at all. These individual differences in intake are consistent with our previous report (see Figure 4-1), where CS intake was found to negatively correlate with cocaine self-administration (see (Figures 4-3 and 4-4). The use of yoked controls in the current report affords the opportunity to compare these subgroups across the contingencies. Therefore, a criterion was established whereby rats in the CA group that made < 50 licks of the saccharin CS by the last trial were considered “large suppressers” (CA_{Lg}) and their data were redrawn and reanalyzed along with the remaining rats referred to as the “small suppressers” (CA_{Sm}). Similarly, the data of the CY rats were redrawn and reanalyzed, based on the particular CA rat to whom they were yoked throughout the acquisition phase of the experiment and labeled accordingly (Cocaine Yoked: “large suppressers” = CY_{Lg}; “small suppressers” = CY_{Sm}).
The results of a mixed factorial ANOVA varying Group (CALg, CASm, CYLg, CYSm, SAL) x Trials (1-18) showed that the large suppressers exhibited a nearly identical pattern of robust saccharin avoidance irrespective of the cocaine contingency (see Figure 5-2, right panel).

Figure 5-2: A depiction of the same data as in figure 5-1 but divided into small (left panel) and large (right panel) suppressers. Mean (±SEM) intake (licks/5 min) of 0.15% saccharin following 18 pairings with either saline or active vs yoked cocaine (0.33 mg/infusion) for 1 h. * = significantly different from saline controls, # = significantly different from active cocaine and saline controls.
This observation was supported by Newman-Keuls post hoc tests of a significant 2-way interaction, $F(68, 1802)=10.53, p<0.0001$, which revealed that both the active large suppressers and their yoked controls significantly avoided saccharin consumption relative to the saline group beginning on trial 2 ($p<.05$). In contrast to the large suppressers, the saccharin intake of the small suppressers was significantly more suppressed in the yoked controls than their actively administering counterparts (see Figure 5-2, left panel). Post-hoc tests of the same 2-way interaction confirmed this finding. First, although the actively administering small suppressers ($\text{CAsm}$) appeared to exhibit mild avoidance of the saccharin CS relative to the saline controls, this numerical difference did not attain statistical significance on any trial ($p>.05$). However, the yoked small suppressers ($\text{CYSm}$) exhibited significant avoidance relative to the saline controls on trials 3-18, $p<.05$. Moreover, group $\text{CYSm}$ consumed significantly less saccharin than the $\text{CAsm}$ on trials 3-5, 7, 9-12, 14-18 ($p<.05$). Importantly, when collapsing across trials, post hoc tests of a highly significant main effect of Group, $F(4, \text{106})=59.65, p<0.0001$, revealed an identical pattern across conditions (data not shown).

### 5.3.3 Experiment 1: Cocaine Intake and individual differences

As stated above and in Chapter 4, it was found that two distinct response profiles existed with respect to saccharin intake and, further, that this saccharin intake was negatively correlated with cocaine self-administration. In the section
above, this dichotomy in saccharin intake also was apparent in this new set of rats. This finding was extended to include the yoked controls. Of course, the active and yoked cocaine groups received identical amounts of cocaine at the discretion of the particular executive rat controlling the cocaine infusions (Figure 5-3). The following analysis on cocaine infusions was therefore conducted only on the actively administering animals (as the results would be identical if conducted on the yoked rats).

**Figure 5-3:** Mean (+/- SEM) number of infusions/h of either saline or active (left panel) vs. yoked cocaine (right panel) following 18 saccharin-cocaine (0.33 mg/inf) pairings. The data are depicted with the rats in the cocaine groups divided into small and large suppressers on the basis of the saccharin intake of the active cocaine rats. * = significantly different from saline controls and large suppressers; # = large suppressers significantly > saline and small suppressers.
The results of a mixed factorial ANOVA varying Group (CASm, CAfg, SAL) x Trials (1-18) indicated that the active large suppressers self-administered significantly more cocaine than did the active small suppressers. Newman-Keuls post hoc tests of this significant 2-way interaction, F(34, 731)=4.64, p<.0001, indicated that the large suppressers self-administered significantly more cocaine on each and every trial than the small suppressers (p<.05). Furthermore, the small suppressers, while averaging between 2-6 infusions per trial never obtained significantly more infusions of cocaine than the saline controls obtained of saline on any trial day (p>.05). These results were mirrored by the post hoc tests of the highly significant main effect of Group, F(2, 43)=70.21, p<.0001 (data not shown), whereby the large suppressers self-administered significantly more cocaine infusions overall than the small suppressers and this latter group did not differ from saline controls.

Taken together the data indicate that these rats differ not only in their consumption of saccharin but also in the propensity to self-administer cocaine. However, while the yoked controls received the same pattern of cocaine infusions as the active rats, they consumed less saccharin overall. Furthermore, this greater suppression of saccharin intake can be attributed to the rats yoked to the active small suppressers. Therefore, separate correlational analyses were conducted to assess the strength of the relationship between the saccharin intake and cocaine intake in both the CA (n=32) and CY (n=32) groups across trials. The results of the analyses revealed a strong negative correlation for both
of the cocaine contingencies whereby low saccharin intake (i.e., avoidance of the saccharin cue) was associated with high drug intake either by self \((r=-0.83; p<0.0001; r^2=0.69)\) or yoked \((r=-0.76; p<0.0001; r^2=0.58)\) drug administration (Figure 5-4).

Figure 5-4: A correlational analysis of each rat’s average intake (i.e., licks/5 min) across trials 2-18 of the saccharin conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a strong negative relationship where low saccharin intake was highly correlated with high drug self-administration behavior (left panel) or high administration of yoked cocaine (right panel).
Despite the similar significant negative correlation between saccharin intake and cocaine infusions, group CY exhibited greater avoidance of the saccharin cue than did group CA and this greater avoidance seems to be carried by the CY_{sm} sub group. Therefore, further correlations were conducted on the small suppressers of each contingency to assess the relative strength of the relationship between saccharin intake and this relatively modest (relative to the large suppressers) cocaine intake. Interestingly, even at this very modest level of cocaine intake there was a significant negative correlation in both the active, r = -0.46; p<0.02; r^2=0.22, and yoked, r = -0.48; p<0.02; r^2=0.24, small suppressers across trials 2-18, whereby lesser saccharin intake predicted greater cocaine intake whether active or yoked (data not shown). However, by the final 2 trials of the experiment, this predictive relationship disappeared for the active small suppressers, r = -0.30; p=0.15; r^2=0.09, but not for their yoked controls, r = -0.56; p<0.005; r^2=0.30, by the final 2 trials of the experiment (Figure 5-5).
Figure 5-5: A correlational analysis of each small suppresser's terminal intake (i.e., licks/5 min on the final two days of testing) of the saccharin conditioned stimulus as a function of the number of infusions of cocaine administered/h on these same trials. The results revealed a negative relationship where lower saccharin intake was correlated with higher levels of cocaine administration in both the active (left panel) and yoked (right panel) small suppressers. However, this relationship attained statistical significance only when the drug was delivered non-contingently.
5.3.4 Experiment 2: Fixed and Progressive Ratio Responding

5.3.4.1 Introduction

As stated above, it is rarely considered that contingent and noncontingent drug administration would have any distinct behavioral consequences. However, in Experiment 1, it was established that avoidance of a saccharin cue that reliably predicted i.v. cocaine infusions was greater in rats when the cocaine was non-contingently delivered. It is unclear why the very same dose of the drug, delivered over the same time course, resulted in a greater behavioral avoidance of the paired sweet reward. Therefore, the primary purpose of Experiment 2 was to compare the lasting impact that a history with either self-administered or yoked cocaine would have on the subsequent self-administration of cocaine. The use of water deprivation and a permissive operant where rats can lick, nose poke, or otherwise come into contact with an empty spout operant any way they choose, was essential to the success of this experiment because it facilitates persistent investigation of spouts by even the yoked controls. To take further advantage of their deprivation state, and to eliminate different levels of thirst as a potential interpretive confound, the saccharin cue was not presented before drug trials in this experiment. Therefore, all rats (i.e., CA, CY, SAL) had access to cocaine infusions for two days on a non-taxing, low work requirement, fixed ratio 10 schedule for two days. Following the FR 10, motivation to work for cocaine was probed by use of a progressive ratio test. This schedule of reinforcement involves systematically increasing the work requirement for each subsequent cocaine
infusion until the rat reaches some breaking point and quits responding (see methods for details). Obtaining a higher number of cocaine infusions or a higher break point (i.e., the last successfully completed reinforcement requirement) indicates a ‘greater motivation to work for cocaine’.

5.3.4.2 Results and Discussion

As mentioned in the methods, the delivery of saline infusions in an active or yoked manner had no differential impact whatsoever on any data points throughout the entire experiment. This fact was abundantly clear in Experiment 2. Prior establishment of a contingency between the operant behavior and the delivery of saline and the cue complex, or not (as in the yoked saline controls), did not differentially effect subsequent responding on either the fixed or progressive ratio schedules when self-administering cocaine for the first time in Experiment 2. This was surprising because it was assumed that this lack of establishing a contingency in the yoked saline controls during Experiment 1 would have retarded acquisition of cocaine self-administration and necessitated a prolonged training period on the FR 10, however, it did not. In fact, cocaine self-administration was quite similar for both groups on both days on the FR schedule (history of saline active = 16.7 ± 3.76, and 18.45 ± 3.48; vs. history of saline yoked = 18.04 ± 4.41 and 16.55 ± 4.96).

Furthermore, even for the cocaine treated rats, this previous contingency (yoked vs. active) during Experiment 1 also had no differential effect on the
subsequent self-administration of cocaine during the 2 days on the FR 10 schedule of reinforcement. This observation was supported by the null results of a mixed factorial ANOVA varying Group (CA, CY) x Day (1-2) which indicated nearly identical response rates between the groups across both days. Neither the main effects of Group, $F(1, 59)=0.0004; p>0.98$, and Day, $F(1, 59)=0.38; p>0.53$, nor the 2-way interaction, $F(1, 59)=0.0617; p>0.80$, were even close to significant (see Figure 5-6, left panel, left of the dashed line).
Figure 5-6: Left Panel: Mean (± SEM) active cocaine intake (0.33 mg/inf) on a fixed (left of dashed line) and progressive (right of dashed line) schedule of reinforcement for rats with a history of either active or yoked cocaine in Experiment 1. Right Panel: Mean (± SEM) number of total contacts with the active empty spout on the progressive ratio day for the rats with a history of either active or yoked cocaine in Experiment 1.
However, when performing on a progressive ratio schedule, where the work requirement to obtain a cocaine infusion increases after each earned infusion, the previously yoked rats self-administered significantly less cocaine than their active counterparts $t(1, 58)=6.28; p<0.015$ (see Figure 5-6, left panel). This difference does not reflect a lack of responding on the part of the previously yoked controls as they averaged a respectable $726.1 \pm 323.2$ responses on the operant (Figure 5-6, right panel).

5.3.5 Experiment 2: Fixed and Progressive Ratio Responding and individual differences

The previous section demonstrated that yoked exposure to i.v. cocaine does not significantly retard acquisition of cocaine self-administration when it is relatively easy to obtain (i.e., on an FR10) under the particular permissive parameters of the test. In contrast, the history of yoked cocaine reduced motivation to work for cocaine when the work requirement was progressively increased per infusion. This is an interesting result on its own as prior exposure to cocaine is typically thought to sensitize rats to the reinforcing properties of the drug (Horger et al., 1990), not hinder it. The discovery of the individual differences in Experiment 1 affords a deeper level of analysis on the subsequent effects of this history with either self-administration of cocaine or yoked cocaine on both fixed and progressive ratio performance in Experiment 2.

In experiment 1, it was evident that two populations of rats emerged in the active group. One group greatly avoided the saccharin cue and self-administered
a large amount of cocaine while the other did not avoid the saccharin cue and infused relatively small amounts of cocaine. The rats that were yoked to the actively administering rats were separated on that basis and there was evidence of enhanced avoidance of the saccharin cue in the yoked controls. The augmented avoidance was carried by the rats that received a relatively small amount of cocaine since the saccharin avoidance in both active and yoked large suppresser groups was maximal and nearly identical. This floor effect was likely due to the relatively large number of cocaine infusions experienced. Nevertheless, since a history of yoked cocaine reduced subsequent motivation to self-administer cocaine relative to a history of the identical but self-administered dose, this effect should be larger when comparing the large suppressers because they had a significantly greater number of active or yoked infusions respectively. To investigate this possibility, the data from Experiment 2 were reanalyzed with rats separated into their subgroups based on their saccharin intake in Experiment 1.

5.3.5.1 Experiment 2: Fixed Ratio Days

The results of a mixed factorial ANOVA conducted on only the fixed ratio days varying Group (CA, CY) x I.D. (Large, Small) x Day (1-2) revealed that only the large suppressers exhibited differing effects of the prior cocaine contingency (Figure 5-7, both panels, left of dashed line).
This effect was supported by post hoc tests of a significant Group x I.D. interaction, $F(1, 57)=8.36; p<0.005$, which indicated that CY$_{Lg}$ rats that were yoked to CA$_{Lg}$, and thus received comparatively more yoked cocaine than the CY$_{Sm}$ rats yoked to CA$_{Sm}$, self-administered significantly less cocaine even on the FR10 schedule. Furthermore, this reduction was not evident in the CY$_{Sm}$ rats.
relative to the $CA_{Sm}$ rats. In fact, if anything post-hoc tests indicated a tendency for this relatively small amount of prior yoked exposure to augment responding for cocaine relative to $CA_{Sm}$ rats, $p=0.15$.

5.3.5.2 Experiment 2: Progressive Ratio Challenge

The results of a factorial ANOVA varying Group (CA, CY) x I.D. (Large, Small) revealed that the relative responding for cocaine on the progressive ratio challenge resembled that of the fixed ratio days when accounting for drug history in Experiment 1 (Figure 5-7, both panels, right of the dashed line). Post hoc tests of this significant 2-way interaction, $F(1, 57) = 5.21; p<.026$, revealed that the $CY_{Lg}$ rats obtained significantly fewer cocaine infusions than the $CA_{Lg}$ rats on the progressive ratio test day despite having received the same dose of the drug delivered over the same time course throughout the 18 trials of Experiment 1. There was no such difference on the progressive ratio test in the small suppresser groups that had a relatively small amount of cocaine during Experiment 1 regardless of the contingency. In fact, these two groups averaged to practically identical amounts of cocaine ($CA_{Sm} = 8$ vs. $CY_{Sm} = 7.9$ infusions).

5.3.6 Experiment 3: Alternating-Side Choice Test

In Experiment 2, it is clear that the prior exposure to yoked cocaine reduced subsequent motivation to work for cocaine on a progressive ratio test.
This effect was exhibited by the yoked large suppressers. Although unlikely, due to their stable rates of responding on both fixed and progressive ratios, one potential concern is that these rats simply did not adequately learn the contingency between their behavior and the drug consequence. Furthermore, given adequate learning it is still unclear as to why the yoked exposure reduced subsequent operant responding for cocaine. The following experiment was therefore designed to evaluate whether these rats are exhibiting reduced motivation for operant responding in general, for cocaine specifically, or whether cocaine has been rendered aversive by the yoked administration.

An important addition to this experiment are the saline controls. As mentioned above, they participated in each of the experiments and it did not matter whether they received saline in a yoked or active manner. However, for the purposes of Experiment 3, it is important to note that their unique unpaired experience with saccharin in Experiment 1 and cocaine in Experiment 2 renders them important controls. In Experiment 1, they had access to the saccharin cue on the left side of the chamber followed by a saline consequence. Saccharin therefore was never directly paired with and, thus, not devalued by cocaine in this group. In Experiment 2, no rats had access to saccharin and instead all rats had access to cocaine on the right side of the chamber.

First and foremost, a one-way ANOVA conducted between the SAL, CA, and CY groups revealed that there were no differences between any groups whatsoever in the number of total choices, $F(2, 92)=1.13, p=.33$. This null finding demonstrates that none of the groups had impaired operant performance. In
addition, a mixed factorial ANOVA on Reward (water, cocaine) x Group (SAL, CA, CY) revealed no preferences for any of the groups as evidenced by the null 2-way interaction, F(2, 90)=1.66, p=.20. Instead, the groups varied significantly in their preference to perform on either the left (i.e., side previously paired with saccharin) or the right (side previously paired with cocaine-self administration and the associated compound cues) side of the operant chamber. This was true in spite of the fact that the rewards would alternate sides, with the cue light tracking where cocaine was available.

A mixed factorial ANOVA varying Group (CA, CY, SAL) x Side (Left, Right) revealed that their respective histories in Exp 1 with either saline, or active vs. yoked cocaine and their subsequent active cocaine history in Exp 2 substantially influenced the side of the chamber on which they chose to respond during Experiment 3 (Figure 5-8).
Newman-Keuls post hoc tests of a significant 2-way interaction, $F(2, 91)=5.26$; $p<0.007$, revealed that the SAL group chose to respond on the right side of the chamber. This SAL group had access to saccharin in Exp 1 on the left side of the chamber and then, as with all groups, the saccharin cue was removed and all groups had access only to cocaine self-administration in Exp 2 on the right side.

Figure 5-8: Mean (±SEM) choices on the left or right empty spout operant to gain access to either water for 20 sec or a cocaine infusion (0.33 mg/inf) in the 1 hour choice tests for the rats separated by their history of saline or active vs. yoked cocaine in Exp 1.
of the chamber. Not altogether surprising, the side previously paired with self-administered cocaine in Exp 2 was preferred over the side previously paired with access to saccharin. The CA group, on the other hand, exhibited no preference for either the left side or the right side of the chamber, because of the opposing preferences of the large and small suppressers (see analysis below). Finally, the CY group was the only group that, as a whole, preferred to perform on the left side of the chamber. This last finding was most likely due to avoidance of the side paired with the yoked administration of cocaine and its associated compound cues (i.e., bottle retraction, light offset, tone).

5.3.7 Experiment 3: Alternating-Side Choice Test and Individual Differences

Upon closer inspection of the data, it was clear that the responses to the choice test were complex and varied within each of the groups. Therefore, as was done for Exp 1 and Exp 2, the rats were divided into large and small suppressers as identified in Exp 1 and the data were reanalyzed. The reanalysis provided clear data and further clarified the differential effect of a history with active vs. yoked cocaine. Specifically, the results of a mixed factorial ANOVA varying group (CA_Lg, CA_Sm, CY_Lg, CY_Sm, SAL) x Side (Left, Right) shed further light on the conditions that influenced side preferences during the choice test (Figure 5-9).
Post-hoc tests of the significant 2-way interaction, $F(4,89)=7.78; p<0.0001,$
revealed that the $\text{CA}_{Lg}$ rats, like the $\text{SAL}$ rats preferred the side that was
previously associated with cocaine-self-administration. In fact, the $\text{CA}_{Lg}$ rats
exhibited an even stronger preference for the right side than the saline rats,

Figure 5-9: A depiction of the same data as in Figure 5-8 but divided into large
and small suppressers on the basis of saccharin intake in Exp 1. Mean (±SEM)
choices on the left or right empty spout operant to gain access to either water for
20 sec or a cocaine infusion (0.33 mg/inf) in the 1 hour choice tests for the rats
separated by their history of saline or active vs. yoked cocaine in Exp 1.
p<0.01. This effect was most likely due to the avoidance of the saccharin paired side by devaluation of the saccharin cue in Exp 1 added to a preference for the cocaine paired side. In contrast, for the saline group, the saccharin cue was never devalued by cocaine so the saccharin paired side still retains value. Also for this group, the cocaine paired side was experienced only for 3 days during Exp 2 so it may not have evoked as strong a preference for the right side. The CA_{Sm} rats, on the other hand, significantly preferred the side previously associated with the saccharin cue. This observation confirms that these rats not only prefer the natural reward over cocaine-self administration (as in Exp 1), but prefer to perform operant behaviors in the place that was previously associated with the saccharin cue. Taken together, it explains why the CA group average “washed out” as the rats that suppressed intake of saccharin and subsequently self-administered a large amount of cocaine preferred to perform on the right side of the chamber while the rats that preferred saccharin over self-administered cocaine preferred the opposite.

In stark contrast to the groups with a history of active cocaine, the CY_{Lg} and CY_{Sm} rats exhibited a relatively uniform left-side preference. However, this observation was not perfect. Of the 8 CY_{Lg} rats, 1 heavily preferred the right side while all others preferred the left side. Omission of this one rat resulted in significant left side preferences for both of the yoked subgroups, ps<.05. While this near uniform left-side preference of the CY_{Lg} rats was almost exactly opposite that of the CA_{Lg} rats which uniformly preferred the right side of the chamber, the omission of the outlier is an important caveat. It appears that yoked
administration of cocaine in large amounts is aversive and reduces the
motivational properties of cocaine. However, this effect will not effect all
individuals the same way. Therefore, the inescapable, unpredictable nature of
yoked-cocaine seems to dose dependently augment the drugs aversive qualities,
delay acquisition of self-administration on fixed and progressive ratios, and
increase place aversion in most rats. Nevertheless, some rats may still develop a
preference for cocaine and cocaine associated environmental stimuli – it is, after
all, euphorigenic.

5.4 General Discussion

To summarize, when a saccharin CS predicts access to cocaine self-
administration it is devalued and subsequently avoided. In fact, intake of the
saccharin CS is negatively correlated with cocaine self-administration primarily
because two very distinct groups emerge. Large suppressers consume very little,
if any, saccharin and self-administer a large amount of cocaine while small
suppressers drink a lot of saccharin and self-administer a comparatively small
amount of cocaine. The rats that were yoked to these subgroups had identical
amounts of cocaine delivered noncontingently and this resulted in enhanced
avoidance of the saccharin cue compared with their active counterparts. This
enhanced effect was entirely carried by the CY_{Sm} subgroup which experienced a
relatively small amount of yoked cocaine (~2-6 infusions/trial on average) and
was not exhibited by the large suppressers perhaps because of a floor effect.
Furthermore, the saccharin intake of the active and yoked small suppresser subgroups was found to reliably predict even this small number of cocaine infusions across trials 2-18. However, when considering only the final 2 trials this predictive relationship was lost for the CASm subgroup but remained for the CYSm subgroup.

These augmented effects in the CYSm subgroup were most likely due to a failure of the development of ‘normal’ Pavlovian associations that ordinarily facilitate the expression of drug tolerance (MacRae, Scoles, & Siegel, 1987; Poulos, Hinson, & Siegel, 1981; Ramos, Siegel, & Bueno, 2002; Siegel, 1975, 1978b; Siegel, Baptista, Kim, McDonald, & Weise-Kelly, 2000; Siegel, Hinson, Krank, & McCully, 1982). The only cue that was different between the contingencies was that of the internal decision to initiate and engage in seeking or approach behaviors to self-administer cocaine (i.e., “self-administration cue”). Since the self-administering rat is always sure of when and if an infusion of cocaine is forthcoming it is “prepared”. In contrast, a yoked rat is never sure of when or if an infusion of cocaine is forthcoming and, thus, is not “prepared” because each of the location specific, drug associated, compound cues are simultaneous with onset of the drug infusion. Therefore, the CYSm rats do not exhibit the same degree of tolerance to the drug because the compensatory conditioned responses (CCRs) that precede and counteract the unconditioned properties of the drug are either absent or sufficiently reduced in the yoked condition (Weise-Kelly & Siegel, 2001). At higher doses and longer access periods than in the present experiments, yoked administration kills rats even
when their actively administering counterparts readily survive (Dworkin et al., 1995). Furthermore, the elimination of drug associated contextual cues have been implicated in the real cause of apparent heroin overdose in humans. Specifically, it was found that heroin addicts that either died or were hospitalized due to an apparent “overdose” in fact merely took a previously tolerated drug dose in a different setting. In these cases, a more accurate description of the condition is a “…failure of [learned] tolerance…” brought about by a failure of the environmental cues to evoke the appropriate antecedent compensatory responses that counteract the effects of the drug (Siegel, 2001). While this interpretation explains the enhanced avoidance of saccharin in the CY_{Sm} subgroup, there was no such effect in the large suppressers. The uncertainty about the mechanism of saccharin avoidance in the large suppressers underscores the need to evaluate subsequent drug motivation in both groups.

One of the most important arguments of this dissertation is that avoidance of the saccharin CS observed in the reward comparison model cannot be mechanistically understood when the US is passively delivered and has complex effects. This is not true, however, if it is evaluated within the context of instrumental behavior aimed at establishing drug motivation. This was the purpose of both Experiments 2 and 3. Experiment 2 established that yoked administration of cocaine results in reduced motivation to work for cocaine on a progressive ratio. Moreover, when evaluated based on saccharin intake in Exp 1, it was found that the CY_{Lg} subgroup, which received a comparatively large amount of yoked cocaine, exhibited reduced cocaine self-administration on both
a fixed and progressive ratio schedule. For the small suppressers, however, the drug contingency in Exp 1 did not differentially affect subsequent motivation to self-administer cocaine on an FR or PR schedule. Therefore, the reduced tolerance that led to enhanced saccharin avoidance in the CYSm subgroup in Exp 1 did not have any lasting significant impact on the motivational properties of cocaine in Exp 2. For the large suppressers, however, the effects of the contingency were not observable in Exp 1 yet carried over to Exp 2 and, for the CYLg subgroup, significantly reduced the ability of cocaine to motivate instrumental performance even when work requirements were low.

The goal of Experiment 3 was to establish the underlying cause of the reduced motivational properties of cocaine exhibited by the CYLg subgroup. There were three possible explanations of the effects of yoked cocaine: 1) yoked cocaine retards instrumental learning; 2) yoked cocaine is not as rewarding as active cocaine but is not aversive; and 3) yoked cocaine is aversive and therefore reduces the motivational properties of cocaine. The results of Experiment 3 support the third explanation. The alternating-side choice procedure was originally intended to pit brief access to water against a cocaine infusion. This particular choice, however, was not distinguished by any of the rats. All of the rats, regardless of prior contingencies, learned equally well to perform on the empty spouts that brought fourth either of these rewards. There was a surprising lack of preference for the type of reward and none of the groups differed in total choices made. This eliminates the first explanation for the effects of the yoked contingency because these rats clearly have no difficulty working on the
operants. The fact that these rats seemed to exhibit a near-perfect lack of preference for reward type that was alternating across right and left locations per choice was suspicious (especially given the results of the prior experiments). This encouraged a deeper analysis. As it turned out, an extremely strong side preference not only interfered with the development of a preference for reward type but also fully accounted for the differential effects of prior contingency on the responding in Experiment 3. The yoked rats preferred to perform operant behaviors on the left side of the chamber which effectively avoids the place and cues that are associated with the inescapable, unpredictable delivery of cocaine in Exp 1. In contrast, all other groups, which never experienced yoked cocaine, strongly preferred to perform operant behaviors on the right side of the chamber where they practiced actively seeking/approaching the places and cues that are associated with cocaine infusions.

In conclusion, noncontingent cocaine infusions are a terrible control procedure for self-administration and are superficially similar at best. Elimination of self-administration cues or other contextual cues that predict drug administration causes more severe withdrawal from cocaine (Mutschler & Miczek, 1998), reduces tolerance to morphine’s analgesic and hypothermic effects (Siegel, 1978b, 1982; Siegel, Hinson, & Krank, 1978), and renders cocaine (Dworkin et al., 1995), pentobarbital (Vila, 1989), alcohol (Melchior, 1990) and heroin (Siegel et al., 1982) more lethal. Furthermore, yoked cocaine leads to markedly different turn over rates of virtually every neurotransmitter across several disparate brain regions including those that mediate the rewarding
effects of the drug (J. E. Smith et al., 2003; J. E. Smith et al., 2004). In fact, yoked cocaine reverses core/shell ratios of NAc DA release, augments the emergence of sensitized stereotypical behaviors (Lecca et al., 2007), and reduces the cocaine primed reinstatement to drug seeking behavior (Kippin et al., 2006). The present report adds to this body of literature by providing the first evidence that yoked cocaine enhances the suppressive effects of the drug on saccharin intake, and reduces subsequent drug motivation by an aversive mechanism.

Finally, the implications of these findings point toward an unusual and previously unexplored adjuvant to drug treatment for cocaine addiction. Cocaine, if delivered in a random, unpredictable, uncontrollable, and inescapable manner reduces the incentive properties of cocaine and, in most cases, renders it aversive. Therefore, cocaine itself may serve as an effective means to reduce compulsive drug seeking in humans.
Chapter 6

Reward History, rather than current reward value, affects the motivation to acquire cocaine self-administration

6.1 Introduction

As has been discussed and demonstrated in previous chapters, animals readily compare rewards over time. In fact, reward comparison effects occur within the same modality, as when comparing lettuce with a more palatable banana or two different levels of sucrose concentration, and across different modalities such as saccharin vs. cocaine. So far, this dissertation has focused on this cross modal contrast effect in only one direction (i.e., How the administration of more powerful drugs of abuse come to devalue less powerful natural rewards). We have not yet explored the converse: Whether this brief daily experience with natural sweet rewards alters responding for drugs of abuse and, in particular, reduces the motivation to acquire drug self-administration.

Evidence suggests that the phenomenon of devaluation in cross modal reward comparison is indeed bidirectional where both the drug and the natural reward reduce the value of the other. In rats, the opportunity to self-administer cocaine reduces wheel running, and the opportunity to run in a running wheel, in turn, reduces cocaine self-administration behavior (Cosgrove, Hunter, & Carroll, 2002). Likewise, access to a non-drug reinforcer, like a glucose and saccharin mixture, reduces cocaine self-administration and drug-induced relapse (Carroll et
al., 1989; Liu & Grigson, 2005). When food and cocaine are concurrently available, monkeys also make fewer choices for cocaine as the value of the food alternative is increased or the response requirement for cocaine is increased (Czoty, McCabe, & Nader, 2005; M. A. Nader & Woolverton, 1991b). Finally, as described in Chapter 1, drug addicted humans weigh less, are more often absent from work, and more often have their children removed from the home due to neglect (Jones et al., 1995; Nair et al., 1997; Santolaria-Fernandez et al., 1995). Even so, the availability of natural rewards such as money (Donny, Bigelow, & Walsh, 2003, 2004) or vouchers for community based activities (e.g., ski lift passes, course credits) can serve to greatly reduce cocaine self-administration in addicted humans (Higgins et al., 1994). Taken together, these data clearly demonstrate that the availability of multiple rewards in the immediate environment forces comparisons and choices to be made that ultimately reduce the responding for both rewards when compared with responding to either reward in isolation.

While the acute availability of multiple reinforcers reduces responding for each, there is also evidence that the valence of environmental circumstances or the availability of alternative reinforcers, on a more chronic time scale, can affect the motivation for drug self-administration. For example, individually housed rats exhibit enhanced acquisition of drug self-administration (Bozarth, Murray, & Wise, 1989) and socially isolated monkeys choose a cocaine reward over a food reward more frequently when compared with socially housed control animals (Czoty et al., 2005; for review see, M. A. Nader & Czoty, 2005). Moreover, under
social housing conditions, only the dominant monkeys were shown to have upregulated D2 receptors in the basal ganglia and to exhibit decreased sensitivity to cocaine as a reinforcer (Morgan et al., 2002). This may indicate that access to the benefits of social dominance such as increased grooming by subordinates, and unmolested ‘first dibs’ on food and mates alter the reward substrate to selectively enhance responding to social or natural rewards and, in turn, reduce the reinforcing efficacy of cocaine (Kuehn, 2005; Morgan et al., 2002). Finally, according to the Office of Applied Studies in the Substance Abuse and Mental Health Services Administration the National Survey on Drug Use there is a strong negative predictive relationship between family income and the prevalence of drug use at an early age which may suggest that a history with a high level of reward imparts some protection to acquiring compulsive drug use and that a lack of this history enhances the motivation to seek drugs (found at the official website of the National Institute on Drug Abuse: http://www.nida.nih.gov/DrugPages/Stats.html).

Since an impoverished or isolated environment increases drug use while an enriched environment tends to exert the opposite effect, one wonders whether shifting an animal’s access from the expected high or low reward magnitude to the opposite would also influence the motivation to acquire drug self-administration. Interestingly, rats that exhibit reduced motivation for cocaine when a high level of sweet reward is concurrently available reportedly show an immediate 4-fold increase in cocaine self-administration if that sweet is unexpectedly removed (Carroll et al., 1989). Additionally, in Chapter 5,
Experiment 2, the removal of the saccharin cue prior to cocaine access (which had been presented reliably for 18 trials in Exp 1) apparently elevated cocaine intake on a progressive ratio schedule, but only for rats that were relatively cocaine naïve and not for rats with a history of saccharin intake that predicted active cocaine access (# of cocaine infusions of PR test: cocaine naïve = 25.98 ± 5.02 vs. cocaine experienced = 10.16 ± 1.52; \( t_{(1, 68)} = 7.85; p<.007 \)). While these reports may suggest that the loss of expected reward enhances cocaine seeking, neither report was explicitly designed to test that hypothesis and, therefore, neither had adequate control groups to eliminate alternative explanations. For instance, neither report had a cocaine control group that was maintained on the same level of sweet reward and had a similar amount of prior experience with cocaine.

Therefore, the present investigation will use the successive negative and positive contrast paradigms to investigate whether a history of access to high and low sucrose reward and/or the unexpected gain or loss of that reward influences the motivation to self-administer cocaine on a progressive ratio schedule during acquisition. These well known models of contrast (see Chapter 1) are uniquely suited to this investigation because they already contain adequate controls for caloric intake on the day of the shift. That is, rats that experience an unexpected gain or loss of reward just prior to cocaine access will be compared to rats that have had a consistently high or low level of reward and remain unshifted just prior to cocaine access. This controls for the effect of relieving or increasing hunger immediately before cocaine access.
6.2 Methods

6.2.1 Subjects

The subjects were 34 male Sprague-Dawley rats (Charles River) housed individually in stainless steel hanging cages and maintained with free access to dry Harlan Teklad rodent diet and water except where otherwise noted. All rats were implanted with chronic indwelling iv jugular catheters.

6.2.2 Apparatus

The rats were trained in one of twelve identical modular operant chambers housed in sound attenuated cubicles (MED Associates, Inc., St. Albans, VT). Each chamber was equipped with retractable sipper tubes and a lickometer circuit was used to monitor licking. All tastant solutions were delivered through the left sipper tube while the cocaine operant consisted of a similar, but empty, sipper tube that advanced through a hole on the right of the chamber. Cocaine was delivered by a syringe pump.

6.2.3 Prior Experimental History

All rats had prior experience with 5-min access to a 0.15% saccharin solution for 14 days (data not shown). This was followed by cocaine self-administration (0.33 mg/inf) in the operant chambers. For the first two-days,
cocaine was available for 1 h during which the rats would lick an empty spout operant 10 times per infusion (Fixed Ratio; FR10). On the third day, the lick requirement was increased progressively per infusion (Progressive Ratio; PR10(+20/inf)) (data not shown).

6.2.4 Procedure

Rats were food deprived to 82% of their free-feeding body weight for the duration of testing. **Matched Groups.** Rats were then matched on the basis of their prior history of cocaine intake and placed into one of four conditioning groups. **Sucrose Conditioning.** The unexpected gain of reward was evaluated using a Successive Positive Contrast design consisting of an UNSHIFTED-HIGH (n=8) and a SHIFT-UP (n=9) group. The UNSHIFTED-HIGH group had 5-min access to 1.0 M sucrose daily for 11 days. The SHIFT-UP group had 5-min access to 0.1 M sucrose for 10 days and then, on the 11th day, was “shifted up” to the 1.0 M sucrose. Similarly, the unexpected loss of reward was evaluated using a Successive Negative Contrast design consisting of an UNSHIFTED-LOW (n=8) and a SHIFT-DOWN (n=9) group. The UNSHIFTED-LOW group had 5-min access to 0.1 M sucrose for 11 days. The SHIFT-DOWN group had 5-min access to 1.0 M sucrose for 10 days and then, on the 11th day, was “shifted down” to the 0.1 M sucrose. **Cocaine-SA.** Immediately following the unexpected loss or gain of sucrose reward on day 11, an empty spout advanced on which rats self-
administered iv cocaine (0.33 mg/inf) on an a progressive ratio schedule (PR10+5 x # inf for the first 10 infusions and then +50/inf thereafter).

6.3 Results

6.3.1 Sucrose Intake

The results indicated that for all 10 preshift trials rats ingest more of the 1.0 M sucrose than 0.1 M sucrose (concentration effect; see Figure 6-1). Furthermore, on the shift day, a significant concentration effect was observed for the rats that were shifted up to the 1.0 M sucrose but this effect was not ‘elated’ above the unshifted high group (i.e., positive contrast was not observed, see left panel). However, the rats that were shifted down to the 0.1 M sucrose solution drank significantly less than their unshifted low controls (i.e., negative contrast was observed, see right panel). These observations were supported by the results of a mixed factorial ANOVA varying Preshift Solution (Hi vs Low) x Post-Shift Solution (Shift Up vs Shift Down) x Trials (1-11). The three-way interaction was significant, F(10, 290)=3.37, p<.0004, and all differences were verified by Newman-Keuls tests, ps<.05.
6.3.2 Cocaine Self-Administration on the Post Shift Day

The number of cocaine infusions obtained on the progressive ratio test were dependent on the pre-shift solution. However, the unexpected increase or decrease in sucrose concentration had little to no effect on the motivation to work.

Figure 6-1: Mean (+/- SEM) intake (licks/5 min) of 0.1 M or 1.0 M sucrose across preshift trials 1-10, followed by the post shift trial (11).
for cocaine (see Figure 6-2). These observations were supported by the results of a factorial ANOVA varying Pre-Shift Solution (High vs. Low) x Post Shift Solution (Shift Down vs. Shift Up). Neither the two-way interaction nor the main effect of Post Shift Solution were significant, $F_s < 1$.

Figure 6-2: Mean cocaine (0.33 mg/inf) intake on a progressive ratio test on trial 11 (shift day). **Left Panel:** Rats that had access to a consistently high (1.0 M) or low (0.1 M) concentration of sucrose for all 11 trials. **Right Panel:** Rats that had access to the high or low concentration of sucrose for 10 trials and were shifted to the opposite concentration for trial 11 (shift day) just prior to cocaine access. Red box indicates sucrose history over the 10 pre-shift trials.
However, there was a significant main effect of Pre-Shift Solution, $F(1, 27)=8.23$, $p<.008$, indicating that rats with brief daily access to the high 1.0 M sucrose reward exhibited a reduced motivation to work for cocaine on a PR test relative to rats with brief daily access to the low 0.1 M sucrose reward (see Figure 6-3). Unexpectedly shifting half of the rats to the opposite condition for one day, just prior to the PR test, did not alter the differential effects of this high vs. low sucrose history on cocaine self-administration.
Figure 6-3: Brief (5 min) daily access to a high concentration of sucrose (1.0 M) for 10 (or 11 days for the Unshifted-High controls) reduced cocaine self-administration on a progressive ratio schedule relative to control rats that had similar access to a low concentration (0.1 M). The relative loss or gain of reward on the sift day (trial 11) did not override this effect. * = significance at the p<.01 level.
6.4 General Discussion

This investigation revealed that even brief (5 min) daily access to a high, but not a low, concentration of sucrose significantly reduces the drive to work for cocaine on a progressive ratio test in food deprived rats. This finding is consistent with published data demonstrating that access to an alternative reinforcer reduces the motivation to self-administer cocaine (Carroll et al., 1989). The current data set extends these findings by demonstrating that the sucrose access need not be simultaneously available, can be brief, but must be consistent (i.e., daily) to effectively reduce the drive for cocaine because a one-time increase in sucrose reward did not provide protection. Likewise, a transient decrease in sucrose reward does not offset the protective effects of a history with high sucrose reward.

Importantly, while food deprivation is known to augment the rewarding effects of cocaine, amphetamine, and the opiates (Cabeza de Vaca & Carr, 1998; Cabeza de Vaca, Krahne, & Carr, 2004; Carr, 2002; Carr, Kim, & Cabeza de Vaca, 2000), the suppressed cocaine drive in the rats with a history of high sucrose access cannot be attributed to reduced hunger, per se. This is because the rats that were shifted up to the high concentration of sucrose just prior to cocaine access, but were accustomed to a low concentration of sucrose for trials 1-10, exhibited no evidence of reduced cocaine drive (Fig 6-2, right panel). In fact, their cocaine intake was virtually identical to that of the rats with a consistently low level of sucrose reward for all 11 trials (Fig 6-2, left panel).
Surprisingly, the transient unexpected gain or loss of sucrose reward did not alter this overarching effect of sucrose history on the motivation to work for cocaine. This was particularly surprising for the negative contrast group because negative contrast effects are known to increase anxiogenic stress (Flaherty et al., 1985; Mitchell & Flaherty, 1998) and stress has been shown to increase cocaine self-administration both during acquisition and maintenance, induce reinstatement to drug seeking after extinction, and sensitize animals to cocaine’s rewarding and locomotor effects (Ahmed & Koob, 1997; Covington & Miczek, 2005; Erb, Shaham, & Stewart, 1996; Haney, Maccari, Le Moal, Simon, & Piazza, 1995; Tidey & Miczek, 1997). Since the current experiment was designed to evaluate the effect of varied sucrose histories on the propensity to acquire cocaine self-administration all animals were tested with relatively little cocaine experience. Therefore, while the relative gain or loss of sucrose reward does not affect cocaine drive during acquisition it may do so late in the maintenance phase or precipitate relapse to drug seeking following extinction. Furthermore, these data do not rule out the possibility that the gain or loss of reward would affect the propensity to acquire self-administration of other drugs of abuse such as heroin and alcohol. Finally, the stress associated with a negative contrast effect simply may not be sufficient to enhance the motivation for cocaine, particularly under circumstances where the relative loss of sucrose is offset by the subsequent opportunity to self-administer cocaine.

Nevertheless, the fact that a history of brief access to a high sucrose reward reduces the motivation to work for cocaine in spite of the unexpected loss
of that reward may have far reaching implications for human populations. These data imply that living in environments enriched with natural rewards may help prevent humans and rats from developing compulsive drug taking habits even when those rewards are temporarily threatened. Moreover, these data underscore the need for addiction treatment programs to incorporate more consistent naturally rewarding alternatives and to address the fact that addicts from impoverished environments may require extra attention. One enriched day is not enough to offset a lifetime of poverty.
Chapter 7
Lesions of the ventral tegmental disrupt drug-induced appetite stimulating effects but spare reward comparison.

7.1 INTRODUCTION

In the previous chapter it was established that rats will not obtain as many cocaine infusions on a PR schedule if they are given relatively brief but repeated access to a highly concentrated sucrose solution across several days. This effect was not apparent when a low concentration of sucrose was used instead. Drugs of abuse, then, can devalue natural rewards and the availability of a natural reward can reduce drug self-administration behavior. As mentioned in Chapter 1, animals are motivated to consume goals in serial fashion. Therefore, when rewarding stimuli are consumed they must first be compared at a fundamental level with other rewarding options. All of these incentive stimuli can be distilled to the common dimension of reinforcement value. If they are compared at this level of reinforcement then the brain substrate that mediates reinforcement is likely involved, in some way, in the comparison between rewards.

Despite the pervasive and fundamental nature of this bi-directional phenomenon, relatively little is known about the underlying neural substrates. One potential player is accumbens dopamine. As discussed in Chapter 1, dopamine is increased in the nucleus accumbens (NAC) following consumption
of a natural reward such as saccharin or sucrose (Colantuoni et al., 2002; Hajnal & Norgren, 2001, 2002; Hajnal et al., 2004), food (Mirenowicz & Schultz, 1996; N. R. Richardson & Gratton, 1996; G. P. Smith & Schneider, 1988), sex (Everitt, 1990; Meisel, Camp, & Robinson, 1993; Pfaus et al., 1990), and following ingestion of salt when salt hungry (Roitman, Patterson, Sakai, Bernstein, & Figlewicz, 1999). It also is increased following the administration of drugs of abuse including morphine, cocaine, and ethanol (Di Chiara, 2002; Di Chiara et al., 1999; Ito, Dalley, Howes, Robbins, & Everitt, 2000). Finally, accumbens dopamine is increased in response to cues that predict cocaine administration (Ito et al., 2000; Ito, Dalley, Robbins, & Everitt, 2002; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Schultz, 1998) and its release can elicit approach in cocaine experienced rats (Phillips et al., 2003).

While accumbens dopamine plays an important role in responding to the absolute rewarding properties of reinforcing stimuli evidence suggests that it is not essential for the comparison of different levels of a given reward over time (i.e., relative rewarding properties). Accumbens dopamine appears to track the relative value of rewarding sucrose stimuli in the successive negative contrast paradigm (Genn et al., 2004). Specifically, the rats downshifted from 32% to 4% exhibited a blunted accumbens dopamine peak to the 4% sucrose solution, relative to unshifted rats that only experienced the 4% sucrose reward. Even so, other data suggest that dopamine is not necessary for comparing two levels of the same reward over time. For example, successive negative contrast effects are not altered by pretreatment with dopamine antagonists such as
chlorpromazine or haloperidol (Flaherty, Becker, Checke, Rowan, & Grigson, 1992). Likewise, successive negative contrast effects in consummatory behavior also are not affected by extensive bilateral 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (Leszczuk & Flaherty, 2000). Finally, this same lesion also fails to prevent the development of anticipatory contrast effects when saccharin predicts access to a preferred sucrose solution (Flaherty & Checke, 1982b; Leszczuk & Flaherty, 2000).

Although not essential for the comparison of two different levels of a natural reward, dopamine may be required for the comparison of rewards from different modalities and especially for comparing a natural reward with a drug of abuse. As with the successive negative contrast paradigm, the results from a microdialysis study show that dopamine tracks this cross-modal reward comparison process over time. In particular, the dopamine peak that typically is associated with the consumption of saccharin was fully blunted when the saccharin cue came to predict morphine following a single saccharin-morphine pairing (P. S. Grigson, Acharya, & Hajnal, 2004). Moreover, in that same experiment, access to the saccharin solution blunted the DA peak to morphine relative to explicitly unpaired controls. The data gleaned from neurotoxic lesions, on the other hand, are more mixed. Neurotoxic lesions induced by the administration of 6-OHDA into the lateral ventricles eliminated drug-induced suppression of CS intake (Wagner, Foltin, Seiden, & Schuster, 1981), while more selective 6-OHDA lesions of the nucleus accumbens did not (van der Kooy, Swerdlow, & Koob, 1983). The following experiments will revisit this important
issue in an effort to determine whether accumbens dopamine simply tracks this cross-modal reward comparison process (as described) or whether it is, in fact, essential for it. Since rats with dopamine lesions of the VTA do not self-administer cocaine (Roberts & Koob, 1982), the role of accumbens dopamine in the current report will be examined with experimenter delivered, rather than self-administered, cocaine. The resulting data will be considered in light of the relative effectiveness of the same lesion on appetite stimulating effects induced by chlordiazepoxide and by morphine.

7.2 Experiment 1a (Saccharin-Morphine)

As alluded to above, methylamphetamine-induced suppression of CS intake was prevented by the administration of 6-OHDA into the lateral ventricles (Wagner et al., 1981) and morphine and ethanol-induced suppression was prevented by the ip administration of alpha-methyl-para-tyrosine (Sklar & Amit, 1977). Apomorphine-induced suppression of CS intake, however, was not disrupted by the more selective administration of 6-OHDA into the nucleus accumbens (van der Kooy et al., 1983). Experiment 1, then, was designed to revisit this issue by testing whether morphine-induced suppression of saccharin intake is disrupted by bilateral 6-OHDA lesions of the ventral tegmental area (VTA). The VTA is the source of dopaminergic innervation for the nucleus accumbens and for the prefrontal cortex (Dallvechia-Adams, Kuhar, & Smith, 2002; Dallvechia-Adams, Smith, & Kuhar, 2001).
7.2.1 Methods

7.2.1.1 Subjects

All experimental procedures have been reviewed and approved by the Institutional Animal Care and Use Committee at the Penn State College of Medicine. The subjects were 34 naïve, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 275 and 300 g at the start of testing. All rats were individually housed in stainless steel cages in a colony room where temperature (21°C), humidity, and lighting (12L:12D) were controlled automatically. All experimental manipulations began 3 h into the light phase of the cycle. Food and water were available *ad libitum*, except where noted otherwise. In Experiment 1, 20 rats received bilateral, stereotaxically-guided 6-OHDA lesions of the VTA (Group VTAx) and 14 rats served as control subjects (Group SHAM): Six of these SHAM rats received vehicle infusions of 1% ascorbic acid into the VTA (SC), while 8 served as non-surgical controls (NSC).

7.2.1.2 Surgery

Twenty minutes prior to anesthesia, the rats were injected intraperitoneally (ip) with atropine sulfate (0.25 mg/rat) and Gentamicin (6 mg/rat). They were then anesthetized with sodium pentobarbital (50 mg/kg, ip) and supplemented as necessary throughout surgery. To protect norepinephrine containing neurons, the norepinephrine reuptake inhibitor, protriptyline (15
mg/kg), was administered (ip) 30 ± 10 min before the 6-OHDA infusion. The rat’s head was then mounted in a stereotaxic instrument, using non-traumatic earbars, with the skull level between bregma and lambda. The skin over the skull was opened with a mid-line incision. Using a 4 mm diameter trephine, a hole was drilled in the skull on either side of the midline, ~4.0 mm posterior to bregma. The dura mater was left intact and kept moist throughout the surgery with physiological saline. The coordinates for placement of the Hamilton (10µl) syringe into the VTA were -4.8 to -5.0 mm posterior to bregma; ± 0.8 to ± 1.0 mm lateral to the midsagittal suture; -8.7 mm below the skull surface. Three minutes after the syringe was lowered in place, 3 µl of 6-OHDA was infused over 10 min followed by a 10 min diffusion period before removing the syringe. The same procedure was followed for the opposite side. The surgical control rats were treated identically, except that 3 µl of 1% ascorbic acid was infused instead of 6-OHDA. After removal of the Hamilton syringe, the hole in the skull was filled with Gelfoam and the wound closed with wound clips.

7.2.1.3 Recovery

In general, the animals recovered from the initial surgery over about 2 days and body weight returned to presurgical levels within a week. After 7 days, however, 6 of 72 rats treated with 6-OHDA did not eat enough regular chow to maintain body weight. These rats were given high calorie sweetened condensed milk mixed with a powdered chow to offset their weight loss. Four of these rats
continued to lose weight and were then tube fed sweetened condensed milk (5 – 8 ml) twice daily by an oral gavage until they recovered. Tube feeding lasted no more than 7 days and, although some rats were lost (2/36 in Experiment 1 and 3/36 in Experiment 2), the surviving rats were healthy and eating normally by the 3rd week post surgery.

7.2.1.4 Apparatus

Experiment 1a and 1b were conducted using inverted Nalgene-graduated cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Fluid intake was recorded to the nearest 0.5 ml.

7.2.1.5 Solutions

Sodium saccharin and L-alanine were obtained from Sigma Chemical Co., St. Louis, MO, and sucrose (saccharose) was obtained from Fisher Chemical, Pittsburgh, PA. All solutions were prepared at least 24 h in advance and presented at room temperature. Morphine sulfate and cocaine hydrochloride were generously provided by NIDA. Both were mixed in saline immediately before testing. Cocaine was injected in a stock solution (1.5 mg/ml), adjusted for body weight to avoid necrosis (Durazzo, Gauvin, Goulden, Briscoe, & Holloway, 1994). The 6-hydroxydopamine (6-OHDA; 2 µg/µl in 1% ascorbic acid vehicle) was prepared fresh each day and maintained on dry ice between injections.
Chlordiazepoxide (CDP) was obtained from the Hershey Medical Center Pharmacy and was mixed with 0.9% saline 1-2 hrs before injection.

7.2.1.6 Procedure

**Deprivation State.** Once recovered, all rats were weighed and handled daily and placed on a water deprivation schedule in which they received access to distilled water (dH2O) for 5 min in the morning and for 1 h in the afternoon. Once morning intake stabilized (5-10 days), rats were matched into groups on the basis of morning intake over the final 2 days of baseline and assigned to one of two US conditions: saline or 10 mg/kg morphine, ip, (SHAM: n=7/cell; VTAx: n=10/cell). **Conditioning.** When testing began, all rats were weighed and given 5 min access to a 0.15% saccharin solution. After a 5-min interstimulus interval they were injected i.p. with saline or 10 mg/kg morphine. One such CS-US pairing occurred every other day for a total of eight trials. In addition to their daily 1 h afternoon rehydration period, all rats were given 5 min access to water on mornings between conditioning trials.

7.2.1.7 Analysis

All statistical analyses were conducted using 3-way, mixed factor analyses of variance (ANOVAs) varying the between factors (drug and lesion) by a single within factor (trials). When appropriate, all post hoc tests were conducted using
Newman-Keuls with alpha set at .05. Preliminary statistical analyses were conducted between the surgical control and the non-surgical control groups. No significant differences were obtained. As a consequence, these groups were collapsed and will, hereafter, be referred to as group SHAM.

7.2.1.8 HPLC Analysis

**Brain Dissection.** The rats were sacrificed by decapitation and their brains were rapidly removed and placed on the dorsal surface (~ 3 min). The brains were dissected on a cold microtome stage with a glass surface (~ 3 min). The initial coronal slice was taken approximately 2.0 mm anterior to the hypothalamus. The next slice was taken directly anterior to the hypothalamus. The striatum was then removed from the caudal surface of this slice of brain, based on its distinct morphological appearance. The caudate putamen included tissue dorsal to the anterior commissure, ventral to the corpus callosum, and medial to the external capsule. The medial prefrontal cortex also was dissected. The samples were immediately put in separate cold microcentrifuge tubes and weighed. **Homogenization.** 500 µl of 0.1 M ice-cold PCA containing 0.01% cysteine, and DHBA was added to the samples and then homogenized for 2 min, and centrifuged at 12,000 x g for 20 min at 4°C. Supernatants (300 µl) were transferred onto a 0.2 µm-pore filtering tube and frozen at -72°C. For experiment 2, tissue was sonified in 0.1M NaOAc (pH 4.0) at 10 µL/mg. The homogenate was centrifuged at 14,000 x g, 4°C, for 10 min. Supernatant was filtered through
0.45 μm microspin filter tubes (Alltech Associates, Deerfield, IL) by centrifuging at 14,000 x g, 4°C, for 2 minutes, and a 20 μL aliquot was analyzed.

**Biochemical Determinations.** Concentrations of dopamine (DA), norepinephrine (NE), serotonin (5-HT), and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA), and 5-Hydroxyindoleacetic acid (5-HIAA) were analyzed by reverse-phase HPLC with coulometric detection. Samples (15 µl) were injected with an autosampler (ESA 540, Chelmsford, MA) to a 15-cm column with 3-mm bore and 3-μm C-18 packing (ESA MD-150). The mobile phase contained 60 mM sodium phosphate, 100 μM EDTA, 1.24 mM heptanesulfonic acid (Sigma), and 6% (vol/vol) methanol at pH 3.6. Once separated, the compounds were measured with a Coulochem II system (ESA; analytic cell: model 5014B, electrode 1 -175 mV, electrode 2 +175 mV; guard cell: model 5020, + 300 mV). The system detection limit for DA is ~2.0 fmol/15µl standard sample. In brain microdialysates, DOPAC levels typically are >100-fold higher than DA, so detection limits are not an issue. For Experiment 2, samples were analyzed by HPLC equipped with a C18, MD-150 column (150 mm length x 3 mm interior diameter, ESA, Inc. Chelmsford, MA) and 4 coulometric electrochemical detectors. Electrochemical sensor potentials were set at 150, 250, 350 and 500 mV. Mobile phase consisted of 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid sodium salt, 25 μM EDTA and 8% acetonitrile (pH 2.9) at a flow rate of 0.6 ml/min. Compounds were identified and quantified by comparing retention time, sensor ratio measures and peak height to known standards.
7.2.2 Results and Discussion

7.2.2.1 HPLC Analysis

*Neurochemical levels in the VTAx rats*: Relative to SHAM controls, the infusions of 6-OHDA into the VTA led to an 80% depletion of DA in the nucleus accumbens and an approximate 35% depletion of NE and 5-HT. This depletion profile was similar in the dorsal striatum. In the medial prefrontal cortex, however, the VTA lesion led to an approximate 15% depletion of DA and NE and a 25% depletion of 5-HT (see Table 7-1).

<p>| Table 7-1: Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines relative to SHAM Controls for the Rats in Experiment 1. |</p>
<table>
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<th>mPFC</th>
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<td><strong>AVG % ±SEM</strong></td>
<td><strong>AVG % ±SEM</strong></td>
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<tr>
<td>DOPAC</td>
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<tr>
<td>5HIAA</td>
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<td>68.1 ± 7.2</td>
</tr>
</tbody>
</table>

Data are expressed as a percent of the SHAM group’s average tissue concentration (± 4.9-10% S.E.M.) for the respective monoamine and metabolite. The concentrations were collapsed for both right and left hemispheres. NAC, nucleus accumbens; dSTR, dorsal striatum; mPFC, medial prefrontal cortex; ND, not detectable.
7.2.2.2 Saccharin-CS Intake

All rats (SHAM and VTAx) suppressed intake of the saccharin cue following pairings with the 10 mg/kg dose of morphine, see Figure 7-1, left and right panels.

Figure 7-1: Mean (± S.E.M) intake (ml/5 min) of 0.15% saccharin in SHAM and VTA-lesioned (VTAx) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings.
This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial analysis of variance (ANOVA) varying lesion (VTAx or SHAM), drug (morphine or saline) and trials (1-8). The results showed that the Drug x Trials interaction was significant, F (7, 210) = 13.41, p<.0001. Post hoc tests of this two-way interaction showed that all morphine treated rats, SHAM and VTAx, consumed less saccharin than their saline injected controls on trials 2-8, ps < .05. Neither the Lesion x Drug, F<1, nor the Lesion x Drug x Trials interaction, F (7, 210) = 1.64, p<.12, however, attained statistical significance, indicating that the lesion of the VTA had no impact on morphine-induced suppression of CS intake. The main effect of lesion, on the other hand, was significant, F (1, 30) = 5.27, p<.03, showing that the VTAx rats consumed less saccharin than the SHAM rats overall.

7.3 Experiment 1b (Alanine-Cocaine)

It is clear that the dopamine lesion (~80% depletion) did not affect morphine induced suppression of CS intake in the present report. Although morphine administration causes a substantial increase in dopamine in the nucleus accumbens (Di Chiara et al., 1999), µ opioid receptors are widely distributed outside of the VTA, most notably in the NAC and lateral hypothalamus (MacDonald, Billington, & Levine, 2004). Thus, it is plausible that many of the effects of morphine, including the rewarding effects, are conserved in rats with a selective depletion of accumbens dopamine. Therefore the following experiment used the SHAM and the VTAx rats from Experiment 1a in a crossover design to
test whether the lesion would disrupt the suppressive effects of cocaine. Cocaine is an indirect DA agonist and its rewarding effects are generally thought to be mediated by inhibition of the DA transporter on presynaptic terminals of VTA neurons in the NAC (Giros, Jaber, Jones, Wightman, & Caron, 1996). Direct administration of the neurotoxin into the VTA, then, would be expected to damage these terminals and, in so doing, may more selectively disrupt cocaine-induced suppression of CS intake.

7.3.1 Methods

7.3.1.1 Subjects

The subjects were the same as those described in Experiment 1a.

7.3.1.2 Procedure

Experiment 1b began one week after completion of Experiment 1a. Water deprivation was maintained and a complete crossover design was employed whereby the rats that had received the saccharin CS paired with morphine in Experiment 1a were now presented with a novel sweet tasting amino acid, 0.3 M alanine. This CS was paired with saline (VTAx: n=10; SHAM: n=7). Rats that previously served in the saccharin-saline condition in Experiment 1a, on the other hand, also received access to the alanine CS, but this CS was now paired with cocaine (VTAx: n=10; SHAM: n=7). Testing. During testing, all rats were
weighed and given 5 min access to the 0.3 M alanine solution. After a 5 min interstimulus interval they were injected subcutaneously with saline or a 10 mg/kg dose of cocaine. One such CS-US pairing occurred every other day for a total of eight trials. To maintain proper hydration, all rats received 1 h access to water each afternoon and 5 min access each morning between conditioning trials.

7.3.2 Results and Discussion

7.3.2.1 Alanine-CS Intake

Unlike the SHAM rats, the VTAx rats did not suppress intake of the alanine CS following 8 pairings with the 10 mg/kg dose of cocaine (Figure 7-2).
This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (cocaine or saline), and trials (1-8). The results showed that the Lesion x Drug x Trials interaction was significant, F (7,210) = 3.27, p < .003. Post hoc tests of this 3-way interaction revealed that the

Figure 7-2: Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTA-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings.
cocaine treated SHAM rats exhibited a significant reduction in intake of the alanine CS on trials 5, 7, and 8 relative to their saline treated controls, ps < .05. The cocaine treated VTAsx rats, in comparison, actually consumed significantly more of the alanine CS than their saline treated controls on trials 1 and 4, ps < .05. The Drug x Trials interaction also was significant, F (7, 210) = 8.33, p<.0001. Post hoc tests of this 2-way interaction indicated that all of the rats receiving saline (VTAsx and SHAM) consumed significantly less of the alanine CS than their cocaine injected controls on trials 1 and 2. This finding probably was due to carryover effects (see General Discussion) for the rats previously serving in the saccharin-saline condition in Experiment 1a. While there was a significant main effect of Lesion, F (1,30) = 6.01, p < .02, indicating that the VTAsx rats consumed significantly less alanine overall, neither the main effect of Drug, F<1, nor the Lesion x Drug interaction, F (1,30) = 2.14, p=.15, attained statistical significance. Thus far, these results suggest that bilateral lesions of the VTA may disrupt cocaine- but not morphine-induced suppression of CS intake. Experiment 2a will directly test the validity of this conclusion.

7.4 Experiment 1c (CDP-Induced Appetite)

Although it is possible that the VTA lesion selectively disrupts the suppressive effects of cocaine, but not morphine, it also is possible that the lesion was simply too small to adequately disrupt both phenomena. The overall reduction in saccharin and alanine intake by the VTAsx rats in both Experiment 1a
and 1b may be indicative of a good VTA lesion (e.g., a motivational deficit) or it may, on the other hand, reflect damage to the neighboring substantia nigra (i.e., a motor deficit). Thus, it is crucial to the current investigation to implement a behavioral test that is sensitive to the motivational deficit induced by DA depletion of the VTA-accumbens pathway, while at the same time ruling out a potential motor deficit. The present experiment, then, will employ a task known to be disrupted by a similar VTA lesion. Specifically, pretreatment with a benzodiazepine induces appetite stimulating effects when measured in an intake test and increases appetitive taste reactivity (Berridge & Treit, 1986; Shimura, Kamada, & Yamamoto, 2002). Dopaminergic lesions of the VTA have been shown to block midazolam-induced increases in intake of a 0.1 M sucrose solution using a 24 h two-bottle intake test (Shimura et al., 2002). This earlier report, however, did not control for a potential motor deficit, did not verify DA levels in either the dorsal or ventral striatum, and did not employ stimulus parameters that are relevant to the current investigation. For the current experiment, we modified the parameters to match those employed in Experiments 1a & 1b, controlled for a potentially confounding motor deficit, and measured extracellular DA concentrations in both the dorsal and ventral striatum. Specifically, we used a 0.1 M sucrose solution and a 5 min one-bottle test in non-deprived rats to determine whether the present VTA lesion, which appears to have disrupted cocaine-induced suppression of CS intake, is sufficient to prevent chlordiazepoxide-induced appetite stimulating effects.
7.4.1 Methods

7.4.1.1 Subjects

The subjects were the same as those described in Experiment 1a and 1b. In this experiment, however, all rats were given access to food and water *ad libitum*.

7.4.1.2 Apparatus

Testing was conducted in one of four modular operant chambers (MED Associates, St. Albans, VT) measuring 30.5 x 24.0 x 29.0 cm, housed in a light- and sound-attenuating cubicle equipped with a ventilation fan. All chambers had a clear Plexiglas top, front, back, and one side wall (the side with the sipper tubes). The remaining side wall was made of aluminum. The grid floors consisted of nineteen 4.8-mm stainless steel rods spaced 1.6 cm apart (center to center). Each chamber was equipped with a retractable sipper tube that could enter the chamber through 1.3-cm diameter holes. A stimulus light was located 6 cm above the tube. In the extended position, the tip of the sipper tube was aligned in the center of the hole, flush with the wall. A lickometer circuit (0.3 uA) was used to monitor licking. A shaded houselight reflected light off the ceiling. Each chamber was also equipped with a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN) and a speaker for white noise (75 dB) on the wall opposite the sipper tubes. Events in the chamber and collection of the data
were controlled on-line with a Pentium computer. Programs were written in Medstate notation language from Med-PC® for windows (WMPC™).

7.4.1.3 Procedure

For trials 1-10, each SHAM or VTAx rat was taken from his home cage, weighed and placed in the operant chamber. When the trial was initiated, the house light was illuminated and a bottle containing a palatable 0.1 M sucrose solution was advanced for 5 minutes. After the 5 min access period, the bottle retracted, the house light was turned off and the subject was removed from the operant chamber and placed back in his home cage. Baseline responding for 0.1 M sucrose was determined across trials 1 – 5. On trials 6 – 7, the rats were habituated to an i.p. injection of saline. Approximately, 35 min (± 5 min) later, they were given 5 min access to 0.1 M sucrose. Test trials were conducted over trials 8 – 10. Using a standard ABA design, 5 min intake of the 0.1 M sucrose solution was assessed following an ip injection of saline (trial 8), a 10 mg/kg dose of chlordiazepoxide (trial 9), and saline (trial 10).

7.4.2 Results and Discussion

Bilateral 6-OHDA lesions of the VTA were uniformly successful in disrupting the appetite stimulating effects of chlordiazepoxide. Thus, the SHAM
rats, but not the VTA lesioned rats, significantly increased intake of the 0.1 M sucrose solution following the injection of CDP (Figure 7-3).

Figure 7-3: Mean (± S.E.M) intake (licks/5 min) of 0.1 M sucrose in SHAM and VTA-lesioned (VTAx) rats. Baseline intake was assessed on trials 1-5. Intake on the remaining trials was assessed 35 min after an intraperitoneal injection of either saline (trials 6-8 &10) or a 10 mg/kg dose of chlordiazepoxide (trial 9).

This conclusion was supported by the results of a 2 x 10 mixed factorial ANOVA varying lesion (SHAM or VTAx) and trials (1-10). The Lesion x Trials
interaction was highly significant, $F(9,288) = 6.82, p < .0001$. Post-hoc tests of this 2-way interaction revealed that the SHAM rats consumed significantly more 0.1 M sucrose after the CDP injection on trial 9 than they did after saline injections either before (trial 8) or after (trial 10) the test trial, $p < .05$. This effect was completely abolished in the VTaX rats. In addition, there was a tendency for the SHAM rats to drink more sucrose than the VTaX rats overall, as indicated by a significant main effect of lesion, $F(1,32) = 4.14, p < .05$. However, post-hoc tests of the two-way Lesion x Trials interaction described above revealed that the SHAM rats drank significantly more 0.1 M sucrose only on trial 9, after CDP was injected. We conclude, therefore, that the significant main effect is carried by the increase in intake exhibited by the SHAM rats following the injection of CDP on trial 9. Further, it is important to note that the failure of the VTaX rats to increase sucrose consumption after the CDP injection cannot be attributed to a motor deficit or to a ceiling effect. In Experiments 1a and 1b, the same VTaX rats demonstrated much higher lick rates during equivalent 5 min sessions when tested under water-deprived conditions. Specifically, given that rats take approximately 5 µl/lick (Corbit & Luschei, 1969), the VTA lesioned rats made between 1000 – 2000 licks/5 min when tested in the water-deprived state in Experiments 1a and 1b. These rats, then, could have made more licks/5 min in the present experiment, but they did not. Thus, while the lesion exerted an apparent mixed disruptive effect on drug-induced suppression of CS intake, it was sufficient to fully eliminate the appetite stimulating effect induced by the ip administration of CDP.
7.5 Experiment 2a (Alanine-Cocaine)

The results of Experiment 1 suggest that bilateral lesions of the VTA disrupt avoidance of a palatable taste cue when paired with cocaine, but not when paired with morphine. If true, this would indicate that the suppressive effect of cocaine, but not morphine, relies on intact dopaminergic transmission within the striatum. While a seemingly parsimonious conclusion, confidence is diminished by the relatively small size of the suppressive effect of cocaine in the SHAM subjects. In our hands, this dose of cocaine generally supports a substantial reduction in CS intake in naïve rats (P. S. Grigson, 1997; P. S. Grigson, Cornelius et al., 2001; P. S. Grigson, Wheeler et al., 2001). Thus, it appears that prior experience with the saccharin-saline condition in Experiment 1a may have disrupted cocaine-induced suppression of alanine intake in both the intact and the lesioned subjects in Experiment 1b. The purpose of the present experiment was to eliminate this interpretive confound by replicating cocaine-induced suppression of alanine intake in a set of naïve VTA lesioned rats. If an intact VTA is essential for the development of cocaine-induced suppression of alanine intake, then the effect should be disrupted even when both the CS and the US are novel. If, on the other hand, the disruptive effect of the lesion found in Experiment 1b was due to carry-over effects from Experiment 1a, then cocaine-induced suppression of CS intake should be robust in both the SHAM and the VTA lesioned rats.
7.5.1 Methods

7.5.1.1 Subjects

The subjects were 33 naïve, male Sprague-Dawley rats obtained and maintained as described. They weighed between 275-300 g at the start of testing. Fourteen rats received bilateral, stereotaxically-guided 6-OHDA lesions of the VTA and 19 rats served in group SHAM: Twelve received vehicle infusions of 1% ascorbic acid into the VTA (SC) and 7 served as non-surgical controls (NSC).

7.5.1.2 Apparatus

Testing was conducted in the home cage as described in Experiment 1a and 1b.

7.5.1.3 Procedure

Deprivation State. The rats (SHAM: n=9/cell; VTAX: n=7/cell) were tested as described in Experiment 1b.
7.5.2 Results and Discussion

7.5.2.1 HPLC Analysis

*Neurochemical levels in the VTAx rats:* The HPLC results for the NAC are similar to those reported in Experiment 1. Bilateral 6-OHDA lesions of the VTA led to an approximate 80% depletion of DA in the accumbens and a 25%-30% depletion of NE and 5-HT. The VTA lesion also led to an 80% depletion of DA in the dorsal striatum and a 73% and a 44% depletion of NE and 5-HT, respectively. Finally, the VTA lesion led to a 37% depletion of DA and a 31% depletion of NE in the prefrontal cortex (Table 7-2).

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Table 7-2: *Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines Relative to SHAM Controls for the Rats in Experiment 2.*

Table 2. Data are expressed as a percent of the SHAM group’s average tissue concentration (± 4.9-10% S.E.M.) for the respective monoamine and metabolite. The concentrations were collapsed for both right and left hemispheres. NAC, nucleus accumbens; dSTR, dorsal striatum; mPFC, medial prefrontal cortex; ND, not detectable.
7.5.2.2 Alanine-CS Intake (Experiment 2a)

Similar to the results of Experiment 1a with morphine, all rats (SHAM and VTAx) suppressed intake of the alanine cue following pairings with the 10 mg/kg dose of cocaine, see Figure 7-4.

Figure 7-4: Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTAx-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings.
This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (cocaine or saline), and trials (1-8). The results showed that the Drug x Trials interaction was significant, F (7,203) = 11.90, p < .0001. Post hoc tests of this two-way interaction showed that all cocaine treated rats, SHAM and VTAx, consumed less alanine than their saline injected controls on trials 3-8, p<.05. Neither the Lesion x Drug, F<1, nor the Lesion x Drug x Trials interactions, F<1, however, attained statistical significance indicating that the lesion of the VTA had no impact on cocaine-induced suppression of CS intake. The main effect of Lesion, F (1,29) = 9.64, p<.004, on the other hand, was significant, showing that the VTAx rats consumed less alanine than the SHAM rats overall. When taken with the results of Experiment 1, these data demonstrate that depletion of accumbens dopamine via 6-OHDA lesions of the VTA is not sufficient to disrupt either morphine- or cocaine-induced suppression of CS intake in naïve rats.

7.6 Experiment 2b (Saccharin-Morphine)

In Experiment 1, the suppressive effects of morphine were intact for all of the rats while those of cocaine were eliminated in the rats with bilateral 6OHDA lesions of the VTA. The results of Experiment 2a suggest that this dissociation had more to do with the order of testing than with the nature of either the conditioned or unconditioned stimuli employed. Experiment 2b was designed to test the validity of this conclusion.
7.6.1 Methods

7.6.1.1 Subjects

The subjects were the same as those described in Experiment 2a.

7.6.1.2 Procedure

Experiment 2b began one week after completion of Experiment 2a. Water deprivation was maintained and a complete crossover design was employed whereby the rats that had received the alanine CS paired with cocaine in Experiment 2a were now presented with a novel palatable CS, 0.15% saccharin. This CS was paired with saline (VTAx: n=10; SHAM: n=7). Rats that previously served in the alanine-saline condition in Experiment 2a, on the other hand, also received access to the saccharin CS, but this CS was now paired with morphine (VTAx: n=10; SHAM: n=7). **Conditioning.** During testing, all rats were weighed and given 5 min access to the 0.15% saccharin solution. After a 5 min interstimulus interval they were injected i.p. with saline or a 10 mg/kg dose of morphine. One such CS-US pairing occurred every other day for a total of eight trials. To maintain proper hydration, all rats received 1 h access to water each afternoon and 5 min access each morning between conditioning trials.
7.6.2 Results and Discussion

As predicted, the SHAM, but not the VT Ax rats, suppressed intake of the saccharin CS following pairings with a 10 mg/kg dose of morphine (see Figure 7-5).

Figure 7-5: Mean (± S.E.M) intake (ml/5 min) of 0.15% saccharin in SHAM and VT A-lesioned (VT Ax) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings.
This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (morphine or saline), and trials (1-8). The results showed that there was a highly significant Lesion x Drug x Trials interaction, $F(7, 189) = 3.84, p < .0006$. Post hoc tests of this 3-way interaction revealed that the VTAx rats in the morphine condition actually consumed more saccharin than their saline controls on trial 1. This effect was also exhibited by the SHAM rats and persisted until trial 2. Thereafter, only the SHAM rats injected with morphine demonstrated significant avoidance to the saccharin CS relative to their saline injected controls (e.g., on trials 5-8). Neither the main effect of drug, $F < 1$, nor the main effect of trials, $F < 1$, was significant. The main effect of lesion approached statistical significance, $F(1, 27) = 4.09, p < .053$, consistent with a tendency for the VTAx rats to drink less saccharin than the SHAM rats overall. Taken together, the results demonstrate that naïve rats, with or without VTA lesions, will learn to avoid a palatable taste cue paired with either morphine or cocaine. However, conditioned avoidance of a palatable taste paired with either morphine or cocaine is retarded by prior non-reinforced experience with a different sweet-tasting CS.

7.7 Experiment 2c (Morphine Induced Appetite)

The results thus far show that, while an intact VTA is not essential for morphine- or cocaine-induced suppression of saccharin intake, it is required for the expression of a CDP-induced appetite stimulating effect. This pattern of data
suggests that the dopaminergic pathway is not essential for responding to the devaluation that occurs following saccharin-drug or saccharin-sucrose (Leszczuk & Flaherty, 2000) pairings, but is essential for responding to the increase in palatability that occurs following the administration of a benzodiazepine. The final experiment examined this dichotomy more directly using morphine to induce the appetite. Like CDP, morphine increases food intake, and the associated appetitive taste reactivity behaviors, and these effects have been shown to depend upon opiate receptors in the nucleus accumbens (Doyle, Berridge, & Gosnell, 1993a; Soderpalm & Berridge, 2000). Shimura et al. (2002) showed that the appetite stimulating effect of morphine also was disrupted by VTA lesions, but again, controls for motor deficits were not included and accumbens and striatal dopamine were not measured. If an intact VTA is required for the expression of appetite stimulating effects in general, then our rats with VTA lesions also should fail to exhibit a morphine-induced appetite stimulating effect. Such a finding would be striking, given that the same drug (morphine) readily suppressed CS intake in naïve rats with similar VTA lesions in Experiment 1a. In the present experiment, powdered chow intake was measured in non-deprived rats over a 1 h test period. This approach allowed ample time for the VTAX rats to demonstrate an increase in food consumption on the day of the morphine injection. Finally, a manipulation was conducted at the end of testing to verify that any disruption in intake was not simply due to a motor deficit. This control is particularly important because our VTA lesion also led to an 80% depletion of DA
in the dorsal striatum and a general reduction in 5-minute intake was obtained in all of the conditioning experiments.

7.7.1 Methods

7.7.1.1 Subjects

Two surgical control rats that served in the first two phases of Experiment 2 were lost due to infection. The remaining thirty-one rats were healthy and served in this final experiment. The subjects had access to both food and water ad libitum, except where noted otherwise.

7.7.1.2 Apparatus

All testing was conducted in the home cage as described.

7.7.1.3 Procedure

For trials 1-5, each rat was taken from his home cage, weighed, and returned. While the rats were being weighed, their food pellets were removed and a food dish containing powdered chow was weighed and placed in the center of the home cage for 1 hour. After this 1h-access period to powdered chow, both the food dish and the rat were weighed again. Spillage was minimal, but when it occurred, was brushed into the dish before the final weight measurement was
taken. Again, a standard ABA design was used to evaluate the hyperphagic
effects of a 4 mg/kg dose of morphine administered ip. Thirty minutes prior to the
start of the 1 h test session, all rats were injected with saline on trial 3, morphine
on trial 4, and saline on trial 5. In order to test for potential motor deficits, all rats
were food-deprived for 24 hrs on trial 6 and then were given 1 h access to
powdered food.

7.7.2 Results and Discussion

Once again, the bilateral 6-OHDA lesions of the VTA were successful.
Only the SHAM rats significantly increased intake of powered food following the
injection of morphine (Figure 7-6).
This conclusion was supported by the results of a 2 x 5 mixed factorial ANOVA varying lesion (SHAM or VTAx) and trials (1-5). The Lesion x Trials interaction was highly significant, $F(4,116) = 8.76, p < .0001$. Post-hoc tests of

Figure 7-6: Mean (± S.E.M) intake (g/h) of powdered chow in SHAM and VTA-lesioned (VTAx) rats. Baseline feeding was assessed on trials 1 & 2. Food intake on the remaining trials was assessed 30 minutes after an intraperitoneal injection of either saline (trials 3 & 5), a 4 mg/kg dose of morphine (trial 4), or after 24 h food deprivation (trial 7).
this 2-way interaction revealed that the SHAM rats consumed significantly more food after the morphine injection on trial 4 than they did after saline injections on trial 3 or trial 5, ps < .05. This effect was completely absent in the VTAx rats. Powdered chow intake did not differ between the VTAx rats and the SHAM lesioned rats on any trial, except after the morphine injection on trial 4. The main effect of lesion was not significant, F<1, indicating that the SHAM and the VTAx rats consumed roughly equivalent amounts of the powdered chow when collapsed across trials. The failure of the VTAx rats to increase food consumption after the injection of morphine cannot be attributed to a motor deficit or to a ceiling effect as they, and their SHAM counterparts, exhibited much higher consumption of powdered chow on trial 7 following 24 h of food deprivation than on any other day. A t-test confirmed that intake by the SHAM and the VTA lesioned rats did not differ following 24 h food deprivation, p > 05. Taken together, the results show that dopaminergic transmission in the ventral striatum is essential for the expression of appetite stimulating effects, whether elicited by CDP or by morphine and whether measuring consumption of a sucrose solution or powdered chow.

7.8 General Discussion

Bilateral 6-OHDA lesions of the VTA led to an 80% reduction in dopamine in the NAC and to a 15 – 37% depletion of dopamine in the prefrontal cortex in rats. This lesion was sufficient to fully eliminate the appetite stimulating effects
induced by both a 10 mg/kg dose of CDP and a 4 mg/kg dose of morphine. The lesion also served to augment the disruptive effects of prior non-reinforced exposure to a sweet-tasting CS (i.e., prior experience with a sweet paired with saline). Even so, use of a counterbalanced design confirmed that this effect was not dependent upon either the nature of the CS or the nature of the US. Finally, while the lesion was sufficient to eliminate the appetite stimulating effects of both CDP and morphine and to augment the disruptive influence of prior experience, the same lesion had no impact whatsoever on either morphine- or cocaine-induced suppression of CS intake in naïve rats. We conclude that the dopamine projection from the VTA to the NAC need not be intact to compare rewards over time. It is, however, required for the increase in sustained intake that occurs following the administration of a benzodiazepine or morphine and for new learning following a period of non-reinforced exposure to a similar CS.

The VTA-NAC pathway clearly tracks both absolute and relative reward properties. It is not, however, an obligate relay. As discussed, neurochemical and electrophysiological data show that NAC dopamine tracks stimuli of import (rewarding, aversive, or when neutral stimuli become associated) and the neural response to these stimuli is altered by experience (Carelli & Deadwyler, 1997; Datla, Ahier, Young, Gray, & Joseph, 2002; Di Chiara et al., 1999; Hajnal et al., 2004; Kiyatkin & Stein, 1996; Kiyatkin, Wise, & Gratton, 1993; G. P. Mark et al., 1994; Roitman et al., 2005; Tobler, Fiorillo, & Schultz, 2005; Young, 2004; Young, Ahier, Upton, Joseph, & Gray, 1998). The accumbens also tracks relative reward properties. The activity of some single cells in the NAC is reduced for a
low concentration of sucrose when alternated with the availability of a preferred, higher concentration (simultaneous negative contrast, Taha & Fields, 2005; Wheeler, Roitman, Grigson, & Carelli, 2005) and it is increased for a high concentration of sucrose when alternated with a less preferred, low concentration (simultaneous positive contrast, Wheeler et al., 2005). Similarly, Tobler et al. (2005) recently showed in the Macaque monkey that the activity of VTA dopamine neurons for a given reward magnitude depends upon the relative magnitude of the alternative reward that is predicted. Finally, as described, the peak in accumbens dopamine following the ingestion of saccharin or sucrose is blunted for the same solution if the rats are expecting, on the basis of prior experience, to have access to a more palatable, more concentrated sucrose solution or a drug of abuse (Genn et al., 2004; P. S. Grigson et al., 2004).

In contrast to a critical role identified for the gustatory thalamus and cortex (Geddes, Han, Baldwin, & Grigson, 2004; P. S. Grigson, Lyuboslavsky et al., 2000; Mackey et al., 1986; Reilly & Pritchard, 1996a; Schroy et al., 2004), however, the VTA-NAC pathway need not be intact for the expression of such reward processing in behavior. Indeed, in many instances extensive lesions of the dopaminergic VTA-NAC pathway do not disrupt responding to either absolute or relative reward properties. Regarding absolute reward properties, rats with accumbens DA depletions (Berridge & Robinson, 1998a), or no forebrain at all for that matter (Grill & Norgren, 1978a), exhibit normal positive hedonic orofacial movements that increase lawfully with the concentration of sucrose infused into the oral cavity (Grill & Norgren, 1978b). Further, drug-induced increases in
appetitive taste reactivity measures (rather than intake) can be augmented by the administration of either morphine or benzodiazepines (Berridge & Treit, 1986; Doyle et al., 1993a), even when rats are depleted of almost 99% of DA in the NAC (Berridge & Robinson, 1998a). Similar lesions of the VTA only minimally reduce intake of increasing concentrations of rewarding and aversive taste stimuli in a 24 h 2-bottle intake test (Shimura et al., 2002). Finally, regarding relative reward properties, bilateral 6-OHDA lesions of the NAC fail to disrupt successive negative contrast effects in consummatory behavior (Leszczuk & Flaherty, 2000) and similar lesions of this pathway fail to disrupt avoidance of a saccharin cue when paired with a rewarding sucrose solution (Leszczuk & Flaherty, 2000). The results from the present report are in keeping. Large bilateral lesions of the VTA failed to prevent avoidance of a palatable taste cue following pairings with either cocaine or morphine. The dopaminergic projection from the VTA to the NAC, then, need not be intact for drug-induced suppression of CS intake.

Although large lesions do not disrupt responding to either absolute or relative properties of reward, several other behaviors are disrupted, and as such, provide insight into the obligate function of the VTA-NAC pathway in behavior. Of most relevance to the current report, Leszczuk and Flaherty (2000) showed that the same NAC lesion that failed to disrupt consummatory successive negative contrast effects following the unexpected downshift from 32% to 4% sucrose, completely prevented the successive negative contrast effect in instrumental behavior in a runway. An intact NAC, then, is not required to detect the gustatory
stimuli, accurately identify their relative value, remember the value of the previously received reward, compare the available 4% sucrose reward with the memory of the preferred 32% sucrose reward, or to suppress consummatory behavior accordingly (steps also required for the comparison of a saccharin cue with a drug of abuse). The lesion-induced deficit occurs when the loss of reward should impact upon instrumental performance. In this case, the lesioned rats decreased running speed in the runway, but only to the level of the unshifted controls. Interestingly, this finding parallels that obtained with bilateral excitotoxic lesions of the hippocampus which also selectively disrupt successive negative contrast effects in instrumental (runway), but not consummatory, behavior (Flaherty, Coppotelli, Hsu, & Otto, 1998). The hippocampus is known to be critical for context learning (Honey & Good, 1993) and regulates “up” and “down” states of neurons in the NAC (Goto & O'Donnell, 2002). Taken together, these observations may suggest that the failure to demonstrate contrast in instrumental performance following a lesion that disrupts the VTA-NAC pathway relates to the context dependent nature of the instrumental behavior.

The most obvious lesion-induced deficit in our hands was the complete elimination of the appetite stimulating effects of chlordiazepoxide and morphine. These effects could not be attributed to a general motor deficit because the same lesioned rats exhibited a higher level of food and water intake when food- or water-deprived. In the same light, the data cannot be attributed to a general motivation deficit, as the VTA lesioned rats clearly were motivated to consume the stimuli when hungry or thirsty. According to Kelley (2004) and Berridge and
Robinson (1998a), opiates and benzodiazepines serve to increase the perceived palatability of sweets, leading to an increase in the associated appetitive orofacial responses for the stimuli when infused directly into the oral cavity. These drugs also are thought to lead to an increase in motivation as reflected by an increase in the animal’s willingness to work for access to the sweet on a progressive ratio schedule of reinforcement (Zhang et al., 2003). As briefly mentioned above, rats with extensive lesions of the NAC continue to demonstrate a benzodiazepine-induced appetite stimulating effect in taste reactivity behavior following intraoral delivery (Berridge & Robinson, 1998a). The lesioned rats, then, are appropriately sensitive to the drug-induced palatability shift, if the stimuli are infused directly into the oral cavity. They fail, apparently, when having to seek access to the fluid. Thus, even though changes in perceived palatability are likely intact, the present data show that the VTA lesioned rats fail to increase consumption of the rewarding stimulus when having to approach the location of the reward.

As stated, the VTA lesioned rats had no difficulty approaching the same rewarding stimuli when motivated by internal cues such as food or water deprivation. Hanlon, Baldo, Sadeghian, and Kelley (2004), however, have shown that the motivation underlying these drug-induced appetite stimulating effects differs from that induced by food deprivation. Of course, the VTAx rats did approach and sample the stimuli in the present report. Indeed, with the drug on board, they consumed a volume that did not differ from that consumed on non-drug trials. This exposure to this presumably more palatable stimulus, however, was not sufficient to facilitate a drug-induced increase in intake. One possible
explanation relates to “work”. Salamone has published a number of papers clearly showing that accumbens dopamine is required to appropriately respond to increasing work requirements (Salamone et al., 2001). It would seem unlikely, however, that licking a spout on a continuous reinforcement schedule such as this would constitute “work”. Indeed, Salamone, et al. (2001) showed that a similar lesion of the NAC disrupted lever pressing for food pellets when using a Fixed Ratio (FR) 20 or greater, but not when using a FR5 schedule of reinforcement. A second possibility relates to Pavlovian-to-instrumental transfer. Specifically, selective lesions of the nucleus accumbens have been found to disrupt Pavlovian to instrumental transfer, where responding for a reward is increased in the presence of a reward-associated cue (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001). Were this interpretation to provide an explanation, we would have to conclude that the context plays a critical role in drug-induced appetite stimulating effects and that the lesioned rats are either unable or unmotivated to use this information about the context to direct their behavior following the administration of the drug. Some support for this conclusion is provided by the finding that contextual cues support conditioned feeding following repeated intra-accumbens injections of morphine (Kelley et al., 2000). Whether the phenomenon is, or is not, dependent upon contextual cues, it would appear that the drug-treated VTA lesioned rats perceive the food as more palatable when it comes into contact with the oral cavity, but that this experience is not sufficient to either maintain the ingestive behavior or to facilitate approach to the stimulus during an ‘interburst’ or ‘intermeal’ interval. Interestingly, Higgs and
Cooper (2000) have shown that benzodiazepines increase intake by increasing burst length and that this effect is dopamine mediated. Thus, accumbens dopamine may be required to sustain burst length during voluntary consumption, even though the palatability of the stimulus is appropriately augmented by the drug pretreatment. Perhaps accumbens dopamine is required to sustain consumption, even on a continuous reinforcement schedule, when the licking behavior is motivated in a given context (perhaps via incentive salience) by exteroceptive, rather than interoceptive, stimuli (i.e., by the incentive associated with the perceived value of the reward rather than by the drive induced by hunger or thirst).

Finally, the VTA lesion also exerted disruptive effects on drug-induced suppression of CS intake by augmenting carry-over effects from Experiments 1a and 2a. The retarded development of a CS-US association as a function of prior non-reinforced experience is referred to as latent inhibition (Lubow, 1973, 1989; Lubow & Moore, 1959). Although the present experiments did not include a specific CS preexposure group, the present report is consistent with literature showing augmented latent inhibition in rats with targeted disruption of dopaminergic signaling in the NAC via accumbens 6-OHDA lesions or intra-NAC infusions of the DA antagonist haloperidol or chronic interferon alpha (Bethus, Stinus, & Goodall, 2003; Gray et al., 1997; Joseph et al., 2000; Weiner & Feldon, 1997). Of course, this would suggest that stimulus generalization occurred between the two CSs (saccharin and alanine). Even so, it is unlikely that disrupted performance by the lesioned rats is due solely to an increase in
stimulus generalization. Were the VTA lesioned rats to exhibit greater CS
generalization, then they should have exhibited greater carry-over effects (i.e.,
greater suppression of CS intake) on the first trial following the switch from the
“drug-associated” to the “safe” CS than the SHAM controls. This, however, was
not the case in either Experiment 1b or 2b. Rather, the lesion-induced deficit
became evident over the latter trials during reversal learning. This finding is
consistent with the view posed by Joseph et al. (2000) that dopamine signaling is
required during conditioning to “learn that the to-be-conditioned stimulus is
familiar” (page 929). From a Schultz perspective (Tobler et al., 2005), dopamine
signaling may be required during conditioning to recognize that presentation of
the CS led to a consequence that differed from that which was expected.
Dopamine’s role in the tracking of rewards and in the development of
expectancies, then, may be critical when behavior needs to change in some
exteroceptive/context-dependent manner. Interestingly, latent inhibition is context
dependent (Honey & Good, 1993) and is similarly augmented by lesions of the
hippocampus (Reilly et al., 1993). Since neural activity in the NAC is modulated
by input from both the hippocampus (Goto & O’Donnell, 2002) and VTA, and
producing lesions of either structure augments latent inhibition, the NAC may be
the final common pathway for the proper expression of reversal learning. Indeed,
the accumbens is a more likely focal point of the two major dopaminergic
projections from the VTA because evidence suggests that the PFC is not involved
in cases where prior non-reinforced experience disrupts subsequent learning
(Broersen, Heinsbroek, de Bruin, & Olivier, 1996; Ellenbroek, Budde, & Cools, 1996; Lacroix, Broersen, Weiner, & Feldon, 1998; Weiner & Feldon, 1997).

In sum, we have found that an 80% depletion of accumbens dopamine was sufficient to fully eliminate the appetite stimulating effects induced by a benzodiazepine and morphine. Internal data showed that this finding could not be explained by either a general motor impairment or a general motivational deficit. The lesion also augmented the disruptive influence of a latent inhibition-like effect, where prior non-reinforced exposure to one sweet-tasting CS retarded the development of drug-induced suppression of intake of another sweet-tasting CS. Use of the cross-over design demonstrated that the disruptive effect of the lesion in this paradigm was not due to a general inability to detect or respond to either the gustatory CS or the drug US. Finally, while the lesion was sufficient to eliminate the appetite stimulating effects of both CDP and morphine and to augment the disruptive influence of a latent inhibition-like effect, it had no impact on either morphine- or cocaine-induced suppression of CS intake in naïve rats. This expands upon earlier findings (van der Kooy et al., 1983) by demonstrating that the suppressive effects of morphine and cocaine are intact following extensive lesions of the VTA-NAC pathway. Similar lesions of the NAC also failed to alter both successive and anticipatory contrast effects in consummatory behavior (Leszczuk & Flaherty, 2000). Thus, while dopamine neurons in the VTA-NAC pathway track this drug-induced devaluation of the natural reward, they need not be intact to respond to this information, at least not in consummatory behavior. Indeed, the essential nature of the substrate appears to
become evident in contrast, in classical conditioning, and in ingestion when a shift in reward parameters calls for a shift in behavior in the presence of reward-associated exteroceptive/contextual cues.

7.8.1 Acknowledgements

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Chapter 8
Cue-induced negative affect: A behavioral and neural mechanism of cocaine seeking

8.1 Introduction

In chapter 7, it was determined that depleting the NAc of dopamine has very little effect on the devaluation of a saccharin cue by either cocaine or morphine. Nevertheless, as previously outlined, accumbens dopamine tracks both absolute and relative rewarding properties of natural and drug reinforcers. Moreover, single neurons in the NAc have been shown to track the valence of both natural reinforcers and drugs of abuse. Subpopulations of these neurons exhibit phasic responses to lever presses for either water and food, cocaine (Carelli et al., 2000) or to the intraoral delivery of either sucrose or quinine (Roitman et al., 2005). Interestingly, the phasic responses to intraoral sucrose were predominately inhibitory while the responses to quinine were predominately excitatory. Furthermore, accumbens units also track the relative value of sucrose solutions in either positive or negative simultaneous contrast (Taha & Fields, 2005; Wheeler et al., 2005). The current chapter, then, sought to characterize the response of NAc neurons in the reward comparison paradigm. In addition, due to necessary parametric alterations to the paradigm, a previously undiscovered behavioral effect was unmasked which led to novel insights into the relationship
between the devaluation of a saccharin cue and the role of conditioned negative affect in the motivation to self-administer cocaine.

The devastating emotional impact of chronic cocaine use includes the neglect of employment, social, and recreational activities, a major symptom for the diagnosis of substance abuse disorder (DSM-IVr). Further, the negative affect experienced by cocaine addicts predicts the drug’s euphoric effects (Newton, Kalechstein, Tervo, & Ling, 2003). It has been proposed that the motivation to consume cocaine results from the negative affective state associated with acute withdrawal (Ahmed, Kenny, Koob, & Markou, 2002). Addiction, however, is a chronic disorder that resists therapeutic intervention, not because of the motivational properties of drug need per se, but because learned associations can repeatedly precipitate this motivation over time (Hyman, Malenka, & Nestler, 2006). The current animal models of addiction (Ahmed et al., 2002; Deroche-Gamonet et al., 2004; Robinson & Berridge, 1993a; Vanderschuren & Everitt, 2004), however, do not assess the emotional impact of drug taking on natural reward processing, nor have they examined the impact of cue-induced negative affect on drug seeking behavior. This analysis is critical to accurately model the persistent nature of human cocaine addiction and to understand its neural foundation. Here we demonstrate that a drug-associated taste cue elicits a conditioned aversive state that is both behaviorally and neurally quantifiable and is proportional to the motivation to consume cocaine.

In this report, affective responses to a saccharin taste cue were assessed in a novel rodent model of cocaine seeking. Rats exhibit stereotyped oromotor
responses to palatable and unpalatable taste stimuli when infused directly into the oral cavity that correspond to the hedonic valence of the stimulus (Berridge, 2000) and are detectable by examining the electromyographic (EMG) activity of the anterior digastric muscle, a muscle coupled to licking (Kaplan, Roitman, & Grill, 1995; Roitman et al., 2005). These responses, termed taste reactivity, reflect not only innate taste preferences but conditioned changes in affect (Berridge, 2000; Grill & Norgren, 1978a). Taste preferences may be assessed neurophysiologically as well. It has recently been shown that electrophysiological patterns of activity in the NAc, an area of the brain essential for the expression of motivated behavior (Berridge & Robinson, 2003; Wise, 2002a), reflect the hedonic valence of palatable and unpalatable taste stimuli (Roitman et al., 2005). Here it was hypothesized that the sweet taste cue would be devalued by its association with cocaine, elicit a quantifiable change in affective state (as determined by both behavior and neurophysiology), and facilitate subsequent cocaine-seeking behavior.

Mildly water deprived rats (n=9) learned that an orange or grape flavored saccharin solution predicted the opportunity to self-administer cocaine (Paired Conditioned Stimulus, CS+), while the other flavor predicted the opportunity to self-administer saline (Unpaired Conditioned Stimulus, CS-). After the training period (8-15 taste-cocaine pairings), rats were given a test session in which each was intraorally infused with the CS-, then the CS+, while electrophysiological and EMG recordings were conducted. Commensurate with intraoral delivery, rats consumed both the paired and unpaired solutions as indicated by a review of
videotaped ingestive behavior (for further procedural details see section 8.2.4 below).

8.2 Detailed Methods

8.2.1 Subjects

Twelve male Sprague-Dawley rats (300-350g) were individually housed with ad libitum food and water with a 12/12 hr light/dark cycle (lights on at 7:00 a.m.). All experiments were conducted in the light phase between 10:00 a.m. and 6:00 p.m.

8.2.2 Surgery and Histology

Rats were anesthetized with a ketamine (100mg/kg)-xylazine (20mg/kg) mixture. For EMG recordings, the uninsulated tips of 2, 7-strand stainless steel wires (A-M Systems, Carlsborg, WA) were implanted into the anterior digastric muscle and the other ends were led subcutaneously out an incision in the top of the head where they mated with an omnetics connector, adapted from Kaplan et al., (1995). Another wire wrapped around a skull screw served as ground for EMG recordings. For electrophysiological recordings, 8-wire microelectrode arrays (N-B Labs, Dennison, TX) were implanted bilaterally in the NAc. The coordinates used, in accordance with the atlas of Paxinos and Watson, were AP: +1.7mm, ML: ±0.8 (shell) to ±1.3mm (core), DV: -6.3mm from surface. For each
array, another wire was wrapped around a skull screw and implanted in brain to serve as ground. Finally, rats were bilaterally implanted with intraoral cannulae. Each cannula consisted of an approximately 6cm length of PE-100 tubing which was phalanged at one end with a Teflon washer. The cannula was inserted just lateral to the first maxillary molar with the Teflon washer flush against the molar. The other end was exteriorized out the incision at the top of the head and held in place along with the EMG connector and arrays with dental acrylic. Rats were permitted at least 1 week to recover from surgery.

Following experiments, rats were deeply anesthetized with ketamine/xylazine and electrode tips were marked by passing current (10µA, 5s) through the electrodes. Rats were then transcardially perfused with saline, then formalin, brains were removed and, after post-fixing and freezing, sliced into 40µm sections through the forebrain. Sections were then mounted on slides and stained with potassium ferocyanide and counterstained with thionin to visualize electrode tips using well-established procedures (Roitman et al., 2005).

8.2.3 Electromyographic and Electrophysiological Recordings

Electrophysiological procedures have been described in detail previously (Roitman et al., 2005). Briefly, before the start of the behavioral session, the rat was placed into a plexiglas chamber within a sound attenuating box. Rats were connected to a flexible recording cable (Plexon Inc., Dallas, TX) attached to a commutator (Crist Electronics, Hagerstown, MD) that permitted virtually
unrestrained movement within the chamber. The headstage contained 16 miniature unity-gain field effect transistors. NAc activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (multichannel acquisition processor (MAP) system; Plexon, Inc., Dallas, TX). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium IV computer. Another computer controlled behavioral events of the experiment (Med Associates, Inc.) and sent digital outputs corresponding to each event to the MAP box to be time-stamped along with the neural data. The neurophysiological system has the capability of recording up to four neurons per microwire using real-time discrimination of neuronal action potentials. However, in the present study, typically one or two neurons were recorded per microwire, as in previous reports (Roitman et al., 2005). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Roitman et al., 2005). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template and principle component analysis procedures provided by the neurophysiological software system (MAP system). The template analysis procedure involves taking a
sample of the waveform and building a template of that extracellular waveform. Subsequent neurons that match this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment was over using additional principle components analysis in Offline Sorter (Plexon Inc., Dallas, TX). After sorting, firing rates of individual neurons were aligned to solenoid opening onset for the intraoral infusion. Perievent histograms were constructed using commercially available software (NeuroExplorer, Plexon Inc., Dallas, TX). Firing rates were calculated for each neuron across 1s intervals from -1 to +4s relative to fluid delivery in 100ms bins. Data were then imported into Excel.

For EMG recordings, rats were attached to a second flexible cable (Plexon Inc., Dallas, TX) and EMG potentials were recorded differentially. Briefly, wires were led to an amplifier (Plexon Inc., Dallas, TX) and signals were amplified (1000X). Processed analog signals were then led through a national instruments board to the same computer that collected electrophysiological data. The same program (Sort Client, Plexon Inc. Dallas, TX) that collected electrophysiological data also collected EMG data. To analyze EMG signals, a horizontal threshold was positioned higher than at least 6σ of the noise. Threshold crossings were time stamped and examined relative to solenoid opening using NeuroExplorer.

8.2.4 Experimental Design

Mildly water deprived rats were trained in daily sessions to press a lever for water. Upon acquisition of this behavior, surgery was conducted as described
above. After recovery, rats were mildly water deprived and tested for retention of lever pressing for water. Rats were then given daily drug conditioning sessions. In each session rats were intraorally infused with a grape or orange kool aid™ flavored 0.15% saccharin solution, the CS+, (0.2 ml delivered over 3.5 sec/trial for 30 trials) that predicted cocaine access (2 h access to 0.33 mg/inf). The following day these rats were similarly infused with the other flavored 0.15% saccharin solution, the CS-, that predicted saline access. Rats received 8-15 taste-drug pairings, until behavioral oromotor evidence of the association was observed. On the test day, rats were placed in the taste reactivity chamber and were connected to EMG and electrophysiological cables as well as infusion lines to the intraoral cannulae. After neurons were sorted on line, the experimental session began. Rats were infused, in the same session, with the CS- and then the CS+ (0.2 ml delivered over 3.5 sec/trial for 30 trials each) while the hedonic impact was simultaneously evaluated using electromyographic (EMG) recordings of the anterior digastric muscle and verified by video analysis using the taste reactivity test. Three additional mildly water deprived rats were tested in the same way for naïve responses to the flavored saccharin solutions.
8.2.5 Data analysis

8.2.5.1 Behavior

Taste reactivity was analyzed in a frame-by-frame analysis using videotape recorded on the test day. Appetitive and aversive taste reactivity was counted using the technique of Grill and Norgren (1978b). Briefly, mouth movements that matched the ‘triangle’ shape shown in fig 8-1B for a duration exceeding 90 ms were counted as aversive. These criteria excluded all neutral and ingestive mouth movements. Instances in which the tongue protruded and crossed the midline (as in Fig 8-1A) were counted as appetitive. Counts for each animal were analyzed with a paired t-test for both appetitive taste reactivity and aversive taste reactivity. Intake of the CS- and CS+ during a 2 bottle post-test also was analyzed with a paired t-test. Taste reactivity scores were generated by 2 raters with an inter-rater reliability of 91%.

8.2.5.2 Determination of Phasic Responses

Consistent with other reports from our lab (Roitman et al., 2005), NAc neurons were classified as exhibiting a phasic excitation or inhibition in firing rate during saccharin delivery. The transient effect of gustatory stimulation is initiated approximately 1 second after solenoid opening and typically is resolved within 5 seconds (see Fig. 8-1C and D). Therefore analyses were confined to each cell’s firing rate across a 5 second period over the 30 trial test session. To this end,
neural activity was characterized via the activity of each neuron 1s prior to saccharin delivery (baseline period) and 4, 1s bins following delivery (effect period). Specifically, NAc neurons were classified as phasic (excitatory or inhibitory) to tastant delivery if their averaged activity across all 30 trials/session during any of the 4, 1s effect period bins (4s post infusion) differed significantly from the baseline period. A repeated measures ANOVA was run on each neuron’s activity in these 5 time bins with planned contrasts between the 4 effect periods and the baseline.

8.2.5.3 Neural representation of devaluation

After determination of phasic responses to infusions of the CS- and CS+, groups of cells were averaged based on similar response profiles. These groups (e.g. inhibitory for CS- and excitatory for CS+) were averaged and analyzed as above to determine significant differences in responsiveness for the stimuli in the subpopulations. It was hypothesized that neural representations of conditioned aversive gustatory stimuli would resemble intrinsically aversive gustatory stimuli. That is, rewarding taste stimuli produce a majority of inhibitory responses and aversive stimuli produce predominantly excitatory responses. Therefore ‘inhibitory to excitatory’ cell counts were calculated as percentages of the total number of phasic cells for each rat across stimuli (as illustrated in Fig 2). These percentages were analyzed with a X-Square test to determine alterations in phasic responses in the population. Statistical analyses of all electrophysiological
recordings were performed using commercially available software (SPSS, Tulsa, OK).

8.3 Results and Discussion (Behavior)

As stated above, the rats consumed both of the intraorally delivered flavored saccharin solutions. However, oromotor behavior and associated EMG activity were different for the CS- and the CS+. The unpaired CS- (Figure 8-1A, C) elicited mouth movements that were more rapid and of lower amplitude than were those elicited by the paired CS+ (Fig. 8-1B, D). Detailed video analysis of the test session revealed that the rapid, low amplitude responses were due to neutral ingestive responses and appetitive taste reactivity (i.e., lateral tongue protrusions, Fig. 8-1E, t(8)=4.33, p<.05). Conversely, aversive taste reactivity (i.e., gaping) was exhibited for the cocaine-paired saccharin solution, the CS+ (Fig. 8-1F, t(8)=4.08, p<.05). Following the test session, rats preferred the CS- to the CS+ in a 2 bottle test (CS- 10.3 ± 1.5 mean/SEM, CS+ 2.8 ± 0.5 ml/30 min, p<.05). The strength of the aversion was indexed using a preference ratio (CS+ intake/CS- + CS+ intake) and results showed that the degree of aversive taste reactivity exhibited to the CS+ during the intraoral test was significantly correlated with the strength of the aversion demonstrated in the 2-bottle intake test (r^2=.50, p<.05). These data demonstrate a severe hedonic devaluation, a reversal in the affective response, to the saccharin cue that predicted cocaine availability.
The degree of hedonic devaluation of the drug-associated taste cue predicted the motivation to take cocaine (multiple R=.94). Specifically, aversive taste reactivity scores at test correlated significantly with the acquisition of stable drug self-administration, $r^2=.73$, $p<.05$, (Fig. 8-1G). Aversive taste reactivity also correlated with drug loading (i.e., the number of lever presses for cocaine in the first 10 minutes that occur at ½ of the inter-press-interval for the entire session prior to test), $r^2=.72$, $p<.05$ (Fig 8-1H). The relationship between aversive taste reactivity and motivation negate the possibility that a classic conditioned taste aversion developed to the cocaine-paired saccharin solution (CS+). Partial correlation of these and other factors revealed that drug loading behavior accounted for the greatest proportion of variance in aversive taste reactivity at test (Drug loading $r^2=.38$, Latency to acquire $r^2=.15$). Other indices of learning, which are poor indices of motivation, did not contribute to the relationship between taste reactivity and drug taking (e.g., Day 1 cocaine presses $r^2=.08$, Final session cocaine presses $r^2=.01$). Together these data demonstrate a robust reversal of palatability (from rewarding to aversive) for a taste cue that predicts cocaine availability. In turn, the magnitude of this affective response is proportional to the motivation to self-administer cocaine.
Figure 8-1: Behavioral responses to saccharin that predicts cocaine access. Rats exhibited predominantly appetitive orofacial reactions to infusion of the CS- (A) but aversive reactions to the CS+ (B). These responses were reflected in the patterns of EMG activity (C and D). An analysis of oromotor behavior during the session revealed appetitive taste reactivity expressed for the CS- (E) and aversive taste reactivity for the CS+ (F). Asterisks denote significant differences, p<.05. Aversive taste reactivity scores were highly correlated with the acquisition of stable cocaine self-administration (G) and Drug loading in the final training day before testing (H).
8.4 Results and Discussion (Neural)

The hedonic devaluation of the CS+ also was evident in the electrophysiological activity of NAc neurons. The brief infusion of orange and grape saccharin solutions in naïve rats (n=3) elicited transient inhibitory responses, in most responsive NAc neurons (77% of responsive neurons; see section 8.2.5.2 for details). The number and magnitude of inhibitory responses were identical for orange and grape flavored saccharin solutions, F<1 (Figure 8-2A, B). Further, intake of these solutions in a 2 bottle test administered the following day did not differ (orange 9.2 ± 0.4 mean/SEM, grape 8.7 ± 1.2 ml/30 min, p>.05). These data are consistent with the previous finding that palatable gustatory stimuli elicit predominantly inhibitory responses in NAc neurons (Nicola, Yun, Wakabayashi, & Fields, 2004; Roitman et al., 2005). Similarly, infusions of the CS- in the 9 cocaine-trained rats also elicited a predominantly inhibitory profile (Fig. 8-2C). In contrast, significantly more excitatory responses were elicited by infusion of the taste stimulus paired with impending cocaine availability, the CS+, $X^2=23.5$, p<.01, (Fig. 8-2D). This pattern of activity is consistent with the predominant response to the intraoral delivery of an aversive tastant (Roitman et al., 2005).

The switch in the activity of NAc neurons resulted from two populations. The first group (26% of responsive neurons), responded with inhibitions for the
CS- and excitations for the CS+, F(4,8)=6.78, p<.05, (Fig. 8-2E and F). The second group (15% of responsive neurons), exhibited no response for the CS-, but transiently increased firing rates for the CS+, F(4,8)=7.63, p<.05 (Fig. 8-2G and H). This pattern of neural activity cannot be attributed simply to goal directed behavior (Carelli & Deadwyler, 1994a; Nicola et al., 2004; Taha & Fields, 2006) because the tastant was directly administered into the oral cavity. Instead, the neural responses appear to be linked to the affective aspects of the taste stimulus itself. The data could in part be attributed to Pavlovian conditioning, because neutral cues (i.e., tones and lights) for cocaine also elicit excitatory responses (Carelli & Wightman, 2004), as in Fig. 8-2 G and H. However, the reduction obtained in inhibitory responses for the CS+, shown in Fig 8-2 E and F, is best accounted for by the reversal of palatability for the CS+ (from rewarding to aversive). In support, this excitatory response profile matches the pattern of neural activity elicited following the infusion of a bitter tastant into the oral cavity (Roitman et al., 2005). As such, the present data provide the first evidence for a learned shift in the NAc signal commensurate with a shift in affect.
Figure 8-2: The activity of NAc neurons reflects hedonic devaluation. Infusions of orange (A) and grape (B) flavored saccharin solutions in naïve rats primarily elicited inhibitory responses. Infusions of the CS- at test primarily elicited inhibitory responses (C) while infusions of the CS+ primarily elicited excitations (D). Two populations of neurons were responsible for the altered response profile. Twelve neurons exhibited inhibitory responses for the CS- (E) and excitatory responses for the CS+ (F). Seven neurons exhibited no response to the CS- (G) but increased firing rate to the CS+ (H). Horizontal lines indicate the infusion period.
8.5 General Discussion

Importantly, this conditioned shift in affect (as reflected by a quantifiable alteration in behavior and in the neural code) is predictive of drug taking. Given sufficient drug access, chronic cocaine self-administration engages homeostatic mechanisms that disrupt reward processing and result in anhedonia (i.e., reduced sensitivity to rewards) (Ahmed & Koob, 1998). The end result of this process is negative affect and tolerance to the hedonic effects of cocaine (Ahmed et al., 2002; Ahmed & Koob, 1998; Koob et al., 2004). This negative state is thought to be alleviated by drug loading (Ahmed et al., 2002), a phenomenon observed in our rats that show pronounced aversion to the CS+. However, learning is vital to the expression of the consequences of chronic drug use; if a cue predictive of drug delivery is omitted, tolerance ‘fails’ and can result in death by apparent overdose in human addicts (Siegel & Ramos, 2002). Similarly, learning is essential to the development of this transient aversive state as it was selectively precipitated only after the formation of a learned association between the tastant (CS+) and cocaine. Once elicited, this precipitated state was proportionally corrected by drug loading.

The behavioral and electrophysiological data presented here provide a new framework to consider the impact of cocaine experience on responding for natural rewards. Drugs devalue natural reward cues and greater devaluation is associated with greater drug taking (P. S. Grigson, 1997; P. S. Grigson & Twining, 2002a). Here, however, we observe severe devaluation of a previously
palatable tastant due to its predictive association with cocaine availability. This
devaluation is manifest by a reversal of affective behavioral responses and a shift in NAc neuronal activity. The emergence of aversive taste reactivity to an appetitive stimulus that predicts cocaine provides compelling behavioral data that a conditioned negative affective state is rapidly inducible and pronounced. The magnitude of this conditioned aversive state is significant because it is highly correlated with the motivation to rapidly take cocaine. The dynamic nature of the finding also is significant because it provides an example of how conditioned cues can transiently alter behavior and neurophysiology to precipitate drug-seeking behavior in time, a key aspect of cocaine addiction in humans.
Chapter 9

General Discussion

9.1 Summary of Findings

These experiments, detailed in Chapters 2-8, were conceived with the intention to develop a rodent model of drug-induced devaluation of natural rewards because there are no animal models that focus on this key aspect of substance abuse disorder in humans. The first part of the development process was to establish a reasonable degree of construct validity. This was achieved not only by the experiments described herein but also from other published studies from the Grigson laboratory that distinguish between LiCl induced conditioned taste aversions and drug of abuse mediated reward comparison. To that end, the first experiment was designed to establish generality to all drugs of abuse. That is, if the rewarding properties of drugs of abuse are responsible for the devaluation of an otherwise palatable saccharin cue, then all reinforcing drugs should support avoidance of the tastant. Almost every drug of abuse was reported to support saccharin avoidance except heroin (Cappell & Le Blanc, 1977; Cappell & LeBlanc, 1971; Etkind et al., 1998; Goudie et al., 1978; Le Magnen, 1969; Riley & Freeman, 2004; Shoaib & Stolerman, 1995; Vogel & Nathan, 1975). There was a published account that indicated morphine, but not heroin, supported avoidance of saccharin (Switzman et al., 1981). Since heroin is
even more reinforcing than morphine this posed a problem for the reward comparison hypothesis but was convincingly resolved by using superior parameters (e.g., use of both non-deprived and water-deprived rats, multiple pairings instead of one, less restrictive water deprivation). Indeed, heroin supported a robust avoidance of the saccharin cue. Moreover, it was found that water-deprivation dichotomized the response. One group of heroin treated rats avoided the saccharin cue to a large degree (the large suppressers) and the other group did not significantly avoid the saccharin cue (the small suppressers) until after several CS-US pairings.

This discovery of individual differences led to two different lines of investigation within the current set of experiments. The first, Chapter 3, was to evaluate the devaluation of the saccharin cue across a range of doses of morphine, cocaine, and the aversive agent LiCl in water-deprived, food-deprived, and non-deprived rats. This comprehensive but not exhaustive study provided important basic parametric data that simply did not exist in the literature despite having been a phenomenon of interest for almost 40 years. Importantly, these dose response functions were conducted under nearly identical laboratory conditions which facilitates a comparison between the compounds. The principle finding, then, was that the suppressive effects of drugs of abuse on saccharin intake differ from those of LiCl across the dose response function (see Table 3-1). In fact, depriving rats of either water or food rendered the suppressive effects of morphine and cocaine either impotent or transient (at best) while having far less impact on the suppressive effects of LiCl. This finding is consistent with
several findings from other laboratories as well as internal published data (Bell et al., 1998; Flaherty et al., 1991; Gomez & Grigson, 1999; P. S. Grigson et al., 1999; Parker, 1991, 1993, 1995). Taken together, these internal data suggest that the reinforcing properties of drugs of abuse mediate the devaluation of the saccharin cue. However, while the data in Chapter 3, by themselves, are an important contribution to the field, they do not rule out the possibility that the alleged aversive properties of drugs of abuse are contributing to saccharin avoidance, but are merely less salient. This interpretive confound stems from the use of experimenter delivered drug injections without any measure of drug motivation to compare with the saccharin avoidance. Therefore the model was adapted to incorporate the drug self-administration technique.

This second line of investigation precipitated also by the discovery of individual differences, Chapter 4, was designed to establish the mechanism of cocaine-induced devaluation of saccharin by examining saccharin intake in relation to self-administration behavior. Indeed, the results demonstrate that the rats that consume the least of the saccharin cue (large suppressers) self-administer more cocaine, not less, than the rats that drink the most of the saccharin cue (small suppressers). The relationship between saccharin intake and the motivation to seek cocaine in the large suppressers remained strong for up to 6 months with no further exposure to cocaine. Rats, and humans, self-administer cocaine because it is rewarding and reinforces the behavioral contingencies that result in its delivery. The large suppressers are more sensitive to these reinforcing properties and, therefore, more readily avoid an otherwise
palatable saccharin solution that signals the imminent availability of cocaine. Taken together, Chapters 2-4 establish that the devaluation of a saccharin cue when paired with any drug of abuse depends upon the reinforcing properties of the drug US as it does when paired with a sucrose US in anticipatory contrast. Moreover, these findings underscore the need to evaluate saccharin avoidance in the context of instrumental behaviors that are reliable indicators of drug motivation.

There is no better example for this need than in Chapter 5. The switch to self-administered cocaine was integral to advance the model; however, it was unclear to what degree the ability to control the contingency of drug delivery contributes to either the suppressive effects of cocaine on saccharin intake or to the reinforcing efficacy of cocaine. Therefore, yoked controls were added to the reward comparison paradigm and subsequently compared to their active counterparts on several measures of drug motivation. It was found that the yoked controls (particularly when they are yoked to large drug takers) consumed less of the saccharin cue in Exp 1, were less motivated to self-administer cocaine on both fixed and progressive ratios in Exp 2, and avoided the side of the chamber associated with the delivery of the cocaine infusion in Exp 3. Together, these effects show that yoked cocaine is not only a bad control for self-administration but, in fact, may protect against the acquisition of subsequent cocaine self-administration. This finding is consistent with a recent publication which demonstrates that rats with an extended history of cocaine self-administration were less prone to drug-induced relapse if they were also “treated” with yoked
exposure (Kippin et al., 2006). These results suggest that agonist replacement therapies may benefit from altering the method of drug delivery.

Chapter 6 was uniquely conceived for three reasons. The first was to test the ability of a natural reward to reduce the motivation to self-administer cocaine. The second was to test the potential cause for what appeared to be an augmented motivation to self-administer cocaine by the removal of the expected saccharin cue just prior to cocaine self-administration in a previous experiment (See Chapter 5, Experiment 2). Since the experiment in Chapter 5 was not designed to test this potential cause it did not have controls that would render a viable answer. However, this posed an additional intriguing question. If it were true that the unexpected loss of reward could augment the motivation to self-administer cocaine, then would the unexpected gain of reward have the opposite effect? Therefore, rats were given access to cocaine self-administration following an unexpected shift in reward. The results of this study revealed that the shift in reward magnitude, in either direction, had no effect on the subsequent motivation to self-administer cocaine. Instead, brief daily access to a consistently high level sucrose reward greatly reduced the motivation to self-administer cocaine relative to rats that had daily access to a low one.

The final two chapters dealt with the neural systems of reward and their role in modulating reward comparison. Dopamine lesions of the VTA had no effect on the devaluation of a taste paired with passive injections of morphine or cocaine. However, single neurons in the NAc differentially tracked the valence of intraorally delivered saccharin cues as they predicted access to either saline
infusions (CS-) or cocaine infusions (CS+). For example, a subpopulation of the responsive NAc neurons exhibited excitatory responses to the devalued saccharin CS+ but inhibitory responses to the still-palatable saccharin CS-.

Interestingly, the fact that NAc neurons respond predominately with excitations to an intraoral infusion of aversive quinine solution and predominately inhibitions to that of a palatable sucrose solution implies that the devalued saccharin CS+ is actually aversive. Moreover, the behavioral analysis confirmed that aversive gaping reactions were emitted to the CS+ and appetitive responses to the CS-.

However, the most interesting result of this study was the relationship between this aversive taste reactivity and the subsequent motivation to self-administer cocaine. The more averisive taste reactivity exhibited by the rats, the faster those rats consumed cocaine in the first 10 minutes when access was granted. This finding is a clear demonstration that the severity of the shift in hedonic response is indicative of the motivation to seek and consume cocaine.

### 9.2 Conclusions

Whenever one reward alternative is compared to another, a complex set of calculations must be made for an animal to make a choice. There are many factors that dictate what type of reward is preferred, and how much of a reward is consumed at any given time or place. Animals need to gauge internal states, be aware of the relative availability of different rewards at different times, and integrate this information with their experience with the available rewards.
Despite this complexity, animals do this extremely well - perhaps because this calculation is reduced to the basic element of reward value. Comparison along this dimension has the potential to put all reward alternatives on the same playing field and to facilitate rapid decision making that is integral to survival. Contrast effects aid these decisions because they increase behavioral flexibility. However, this complex and contrasting reward system is exquisitely tuned to sort between ‘natural’ rewards that have been in the environment for thousands of years. Evolution did not entrain rats or humans to perform these calculations with drugs of abuse in the equation. As a result, our modern environment is laced with artificial pleasure so powerful that, when it is experienced, it devalues less powerful rewards and causes many of those exposed to develop compulsive drug seeking habits. How does this happen?

The interpretation of behavioral findings detailed in Chapter 8, within the context of contrast effects and learning, unite two opposing theories of the causes of drug addiction. It was long thought that the reason addicts consume copious amounts of drug over time was because they were escaping the aversive effects of drug withdrawal (i.e., negative reinforcement). However, this theory lost favor with a majority of addiction scientists because it could not explain why addicts would relapse to drug addiction long after withdrawal symptoms had passed (Baker, Piper et al., 2004). The opposing view held that addicts take drug because the incentive motivational properties of the drug and its associations are becoming sensitized. That is, through associative learning and positive reinforcement, neutral stimuli that are associated with drug
administration become reinforcing by themselves (i.e., they acquire “incentive salience”) and can drive drug seeking behavior even if they are encountered many years later. Interestingly, addicts report experiencing tolerance to the subjective rewarding properties (i.e., “liking”) of the drug despite increased drive to seek (i.e., “wanting”) the drug.

The pendulum may be swinging in the other direction again as recent data suggest a critical role for negative reinforcement in the drive to seek drug (Ahmed & Koob, 1998, 2005; Hyman et al., 2006; Koob et al., 2004). For example, rats dependent on heroin will exhibit severe aversive withdrawal symptoms if treated with the opiate antagonist naloxone (Kenny et al., 2006). Critically, these dependent rats also will exhibit enhanced drive for heroin consumption and increased ICSS thresholds, which is indicative of an anhedonic state, when they are treated with naloxone or if they are exposed to stimuli that had been repeatedly paired with naloxone administration (Kenny et al., 2006). Moreover, a similar anhedonic state powerfully predicts a return to cigarette smoking in humans (Baker, Brandon, & Chassin, 2004; Baker, Piper et al., 2004; Brandon, Tiffany, Obremski, & Baker, 1990; Zelman, Brandon, Jorenby, & Baker, 1992). Taken together, these data demonstrate that a negative affective state akin to withdrawal is precipitated by conditioned stimuli that have been paired with withdrawal and that this state drives the consumption of heroin or nicotine.

Ultimately, it is the contention of this author that these opposing views are not mutually exclusive. Instead, there is clear evidence that both positive and negative reinforcement processes are continually operating to produce the
symptoms of an addicted state. In fact, associative learning and contrast mechanisms appear to work interdependently. The missing link between them is beautifully demonstrated by the results of Chapter 8 but requires an understanding how conditioned compensatory responses are formed. This concept was first fully described and elaborated by Siegel and colleagues (1976; 1977; 1978a; 1978b; 1982; 1987; 1988a; 1988b; 1991; 2001; 1979; 1981; 1982; 2002) and was discussed in Chapter 5 to explain the enhanced effects of cocaine in the yoked rats. In that chapter, the lack of self-administration cues in the yoked rats reduced the predictability of drug delivery and thus failed to elicit necessary conditioned compensatory responses. These conditioned compensatory responses are, as the name implies, a product of learning, oppose the effects of the drug, are aversive and similar to withdrawal, and enhance tolerance. They occur because the effects of the drug are strong and, as the organism attempts to restore homeostasis, it begins to do so, after repeated pairings, in an anticipatory (preventative) fashion following the presentation of cues that reliably predict drug consumption. The model of reward comparison fits this associative structure perfectly.

However, evidence of a negative affective state brought on by the saccharin CS was difficult to demonstrate until the design changes implemented in Chapter 8. This is because the mere avoidance of a taste cue that predicts drug provides little information about the nature of that avoidance. This lack of information is why many have referred to drug induced devaluation of an otherwise palatable taste as a paradox for 35 years. A significant reversal of that
lack of information arrived in the form of aversive taste reactivity. The short (≈3.5 s) intraoral infusion of the flavored saccharin solution every minute for 30 min prior to access to cocaine resulted in hedonic devaluation of the taste severe enough that rats emitted gapes to its infusion. Gaping has never been observed in this paradigm despite several attempts to look for it (Parker, 1988, 1991, 1993, 1995; Parker, Maier, Rennie, & Crebolder, 1992). At first glance, it might seem appropriate to conclude that a classic conditioned taste aversion occurred. However, the paradox still held true to form. The greater the devaluation (i.e., greater # of gapes) the faster the rats acquired cocaine self-administration and the faster they consumed cocaine in the first 10 minutes of access on the test day.

In fact, the data of Chapter 8 go well beyond the implications of a naloxone paired cue precipitating enhanced heroin self-administration. The CS+ was never paired with a drug precipitated withdrawal of any kind. All the CS+ ever did was reliably predict future access to cocaine. This implies that exposure to any cue that reliably predicts the self-administration of a drug of abuse has the potential to precipitate relapse. However, this is not merely because they have acquired incentive salience through positive reinforcement but, perhaps, because this exposure precipitates withdrawal-like responses and selectively motivates drug seeking and craving through negative reinforcement. Therefore, it is the conclusion of this investigator, at this time, that the saccharin CS+ elicits conditioned compensatory responses that manifest as negative affect. This negative affect is measurable by the degree of hedonic devaluation (i.e.,
operationalized by the # of gapes emitted to the saccharin CS+) and it is reliably stronger in those rats that are more driven to take cocaine. This relationship between negative affect and drug consumption should be the primary target for therapeutic intervention. A rodent model of drug induced devaluation of natural rewards and cue induced craving has finally arrived.

9.3 Future Directions

There are always competing interpretations that run counter to one’s conclusions. Such is the case with this new model; however, these counter possibilities are also exciting new research directions. For instance, it is unclear to what degree the intraoral infusion of a devalued taste contributes to the development of negative affect and to the subsequent motivation to self-administer cocaine. Could this infusion cause the increase in load up behavior? To test this, one could present a neutral cue that reliably predicts access to cocaine and then evaluate intraoral infusions of a saccharin solution that was familiar but was never predictive of cocaine access. If negative affect alone is responsible for the gaping then it should still be present and predictive of load up behavior. If negative affect is not the sole reason for the emergence of aversive taste reactivity and it, instead, relies on the explicit association of the saccharin CS+ with the anticipation of cocaine self-administration, then another exciting research direction takes shape. It is possible that the degree of palatability of an intraorally infused taste during this anticipatory period would influence the
motivation to self-administer cocaine (much like access to sucrose did in Chapter 6). The prediction would be that aversive tastes would increase drug motivation while palatable tastes would decrease it. Finally, these data suggest a role for the mesolimbic dopamine system in the predictive relationship between the negative affective state and the drive to self-administer cocaine. Briefly, there is evidence that kappa antagonists attenuate the anhedonic state associated with drug withdrawal (Carlezon et al., 2006; Carlezon et al., 2005). The endogenous kappa agonist, dynorphin, is known to be released as negative feedback onto VTA dopamine neurons and shuts down dopamine release (Nestler & Carlezon, 2006). Therefore, dynorphin mediated kappa activation is a good candidate mechanism for the emergence of conditioned compensatory responses in anticipation of cocaine self-administration. The prediction would be that kappa antagonists, delivered systemically or directly into the VTA, would block gaping in this model.

9.4 A Final Word

In closing, I would like to add a few words on the potential role that the devaluation of natural rewards by drugs of abuse plays in the development of addiction: This dysfunctional devaluation of natural rewards leaves ‘the addicted’ bereft of any compelling reasons to work for a better tomorrow. We all know that our happiness does not arrive without considerable effort. And the joys we do experience from day to day are the reason we live, and die… if but only for the
freedom to pursue them. How would any of us, drug addict or not, react to these pleasures losing their motivational properties? What if they were stolen ever so gradually, without our knowledge, and we awoke one day and realized there was no thing worth obtaining, no one worth protecting, no meal worth making? What would we turn to, how would we cope in a world devoid of varied natural motivations and filled only with the regular provocation of an insatiable and intense need that never tires, never heeds to the demands of other drives, and grows stronger with each ritualistic attempt to escape it? Why live if but to exist merely out of spite? In this state, how wonderful, how beautiful, how pleasing, how absolutely necessary, that next “hit” must seem. Once all else has lost its luster and pales by comparison, it is an easy choice for the addicted. This is because, by contrast, there is nothing like it.
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Period: August 1, 2003 - July 31, 2006
Award Total: $78,933.00

2003  Selected to attend the Cold Spring Harbor Laboratory course: The Cellular Biology of Addiction
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1996  Robert S. Davidson Award for Research in Psychology
1996  Departmental Honors, Experimental Psychology

MANUSCRIPTS


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