NOVEL BEHAVIORAL FACTORS THAT INFLUENCE THE ACQUISITION OF
DRUG SEEKING AND DRUG TAKING IN RATS

A Dissertation in
Neuroscience
by
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ABSTRACT

Substance abuse and addiction persist as major health concerns in the United States. Not only does addiction impose direct financial and health threats to the addict, as well as society at large, but it also has a much more menacing effect: the devaluation of natural rewards (e.g., food, sex, work, money, caring for one’s offspring). Human studies have shown that addicts will seek and take drugs in lieu of all other activities, including going to work and caring for their children and themselves (Jones et al., 1995; Nair et al., 1997; Santolaria-Fernández et al., 1995). In addition, human addicts exhibit a decreased sensitivity to monetary rewards (Goldstein et al., 2007; Wilson et al., 2008). Consistent with the human data, animal studies have shown that rats suppress intake of a palatable solution (e.g., saccharin) when that solution serves as a cue predicting access to a drug of abuse (Cappell and LeBlanc, 1971, 1977; Grigson and Twining, 2002; Grigson et al., 2000; Le Magnen, 1969). These effects are thought to be due, at least in part, to the comparison of the rewarding properties of drugs of abuse and natural rewards, as the appraisal of both is mediated by the mesocorticolimbic dopamine system (Grigson, 1997; Grigon, 2008).

The severity of the problem and difficulty of treatment are further compounded by the fact that addiction is a chronic, relapsing disease that induces long-lasting changes in brain function and these changes interact with numerous environmental factors (O’Brien, 1997). Exposure to drugs themselves, physical or psychological stress, and drug-associated cues all reliably induce relapse to drug seeking and drug taking after a period of abstinence in both animals and humans. Of course, this relapse occurs well after drug-seeking and drug-taking behaviors have been acquired. The data in this thesis will show that several novel factors can be involved in facilitating or preventing the acquisition of drug seeking and drug taking in rats,
including sleep deprivation, a history of bingeing on fat, and environmental enrichment.

Chapters 2 and 3 will discuss the facilitative effects of sleep deprivation on acquisition. Specifically, Chapter 2 will demonstrate that acute sleep deprivation (4-8 h) is capable of augmenting cocaine-induced reinstatement, as well as increasing the rate and efficiency of cocaine seeking and cocaine taking. Chapter 3 will show that, similar to acute sleep deprivation, chronic sleep restriction (30% sleep loss over six days), akin to that experienced by humans (Hale and Do, 2007; Jean-Louis et al., 2000; National Sleep Foundation, 2008), is also capable of increasing cocaine-taking behaviors. Chapter 4 will demonstrate how loss of control over one consummatory behavior (i.e., consumption of fat) can lead to loss of control of another (i.e., cocaine intake). Finally, Chapter 5 will show the protective effects of environmental enrichment on the acquisition of cocaine seeking and cocaine taking.

Collectively, the data in this thesis demonstrate that, while some animals are, by nature, more likely to take drug than others, experience can shift this genetic predisposition leading to devastating vulnerability or marked resilience. Moreover, these manipulations, experienced in adulthood, exerted a robust effect on behavior and, obviously then, on the brain. This suggests that the facilitative and protective effects of these factors and those of drugs of abuse may be mediated, at least in part, via the same neural substrate, the mesocorticolimbic dopamine system, and that this substrate remains highly plastic, even in adulthood. These data have important implications for the treatment, and possibly prevention, of addiction in humans.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance of the means</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BED</td>
<td>binge eating disorder</td>
</tr>
<tr>
<td>BNST</td>
<td>bed nucleus of the stria terminalis</td>
</tr>
<tr>
<td>CORT</td>
<td>corticosterone</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>CRF</td>
<td>corticotrophin releasing factor</td>
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<tr>
<td>CS</td>
<td>conditioned stimulus</td>
</tr>
<tr>
<td>CSR</td>
<td>chronic sleep restriction</td>
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<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
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<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenyl-acetic acid</td>
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<tr>
<td>EEG</td>
<td>electroencephalogram</td>
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<tr>
<td>EMG</td>
<td>electromyogram</td>
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<tr>
<td>FR</td>
<td>fixed ratio</td>
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<td>GABA</td>
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<td>HCl</td>
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<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>HVA</td>
<td>homovanillic acid</td>
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<tr>
<td>ICV</td>
<td>intracerebroventricular</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>IU</td>
<td>international units</td>
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<tr>
<td>i.v.</td>
<td>intravenous</td>
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<tr>
<td>LSD</td>
<td>least significant difference</td>
</tr>
<tr>
<td>MAOA</td>
<td>monoamine oxidase A</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NAc</td>
<td>nucleus accumbens</td>
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<tr>
<td>NE</td>
<td>norepinephrine</td>
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<tr>
<td>NREM</td>
<td>non-rapid eye movement</td>
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<tr>
<td>NSR</td>
<td>non-sleep restricted</td>
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<tr>
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<td>prefrontal cortex</td>
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<td>PR</td>
<td>progressive ratio</td>
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<tr>
<td>REM</td>
<td>rapid eye movement</td>
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<td>SD</td>
<td>sleep deprivation</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>SNA</td>
<td>signaled non-availability</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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Chapter 1

Introduction

ADDITION

Magnitude of the Problem

Substance abuse and addiction have become major health concerns in the United States. It has been reported that 17% of Americans meet the diagnostic criteria for some form of substance dependence, excluding tobacco dependence (Anthony and Helzer, 1991), which is almost double the incidence of depression (9.5%; National Institute of Mental Health, 2000) and 17 times greater than that of schizophrenia (1%; National Institute of Mental Health, 2006). According to the National Survey on Drug Use and Health conducted by the U.S. Department of Health and Human Services in 2008, over 20 million people aged 12 years or older reported using one or more illicit drugs. Of those individuals, 15 million used marijuana, 2 million used cocaine, 1 million used hallucinogens, and 200,000 used heroin. In addition, more than half of Americans aged 12 years or older reported using alcohol and nearly 30% reported using tobacco. Alarmingly, substance abuse is rampant among the adolescent population. Of the 3 million individuals reporting initial use of an illicit drug in the past year, 57% were under the age of 18. Also, 85% of recent initiates of alcohol use were under the age of 21 and 6% of recent initiates of tobacco were under the age of 18. Substance abuse incurs an estimated $484 billion in annual expenses to our nation (diabetes and cancer impose costs of $132 billion and $172 billion, respectively; National Institute on Drug Abuse, 2005).
The cost to society is due, in large part, to the fact that addiction is so difficult to treat. It is a syndrome comprised of several maladaptive behaviors that exploit the existing reward circuitry of the brain. The reward circuitry has been well characterized. It is well established that natural rewards (e.g., food, sex, work, money, caring for one’s offspring) engage the mesocorticolimbic dopamine (DA) system (Wise, 1978; Wise and Rompré, 1989). This complex circuit primarily involves DAergic projections from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens (NAc), amygdala, prefrontal cortex (PFC), and other forebrain regions (see Kelley and Berridge, 2002 for a review). In addition, reciprocal interconnections between these areas, along with the utilization of other neurotransmitters, such as glutamate and γ-aminobutyric acid (GABA), contribute to the complexity of this system (Baldo et al., 2005; Peters and Kalivas, 2006). It is thought that drugs of abuse hijack these very pathways. In fact, electrically-stimulated DA release in the mescorticolimbic pathway directly elicits drug seeking in rats that have been trained to self-administer cocaine (Bradberry et al., 2000; Ito et al., 2000; Phillips et al., 2003). Also, several members of the Fos family of transcription factors have been implicated in the changes in neural plasticity that accompany drug addiction. Specifically, acute self-administration of amphetamine and cocaine has been shown to trigger rapid induction of the proto-oncogene c-fos, which encodes the Fos protein (Graybiel et al., 1990; Young et al., 1991). In addition, chronic drug exposure stimulates the accumulation of ∆FosB in the NAc and striatum (see Nestler, 2004 for a review). Such elevations in ∆FosB have been shown to increase the perceived incentive reward value of drugs of abuse, such as amphetamine (Nye et al, 1995), cocaine (Nye et al., 1995), nicotine (Pich et al., 1997), and morphine (Nye and Nestler, 1996), as well as natural rewards, such as sugar (Freet et al., 2009) and food (Olausson et al., 2006).
Devaluation of Natural Rewards

As a result of the exploitation of this existing neural circuitry, one of the most menacing consequences of addiction involves the devaluation of natural rewards (e.g., food, sex, work, money, caring for one’s offspring) by drugs of abuse. Human studies have shown that addicts are less sensitive to monetary rewards (Goldstein et al., 2007; Wilson et al., 2008), exhibit decreases in efficiency and loss of productivity in the workplace (Jones et al., 1995), and are more likely to provide disrupted or insufficient care to their children (Nair et al., 1997). In addition, human addicts typically maintain insufficient diets, at times to the point of malnourishment, loss of muscle mass, and immunosuppression (Santolaria-Fernández et al., 1995). Likewise, animal studies have shown that female rats exposed to cocaine develop a stronger preference for cocaine-associated stimuli than stimuli associated with their pups (Seip and Morrell, 2007). Rats also suppress intake of a palatable solution (e.g., saccharin) when that solution serves as a cue that predicts access to a drug of abuse. This suppression can be mediated by anticipation of access to both CNS depressants (e.g., morphine and heroin; Cappell and LeBlanc, 1977; Grigson et al., 2000) and CNS stimulants (e.g., cocaine, amphetamine, and nicotine; Cappell and LeBlanc, 1971, 1977; Grigson and Twining, 2002; Le Magnen, 1969). Published data suggest that suppression of CS intake is due, at least in part, to comparison of the two disparate rewards and the resultant devaluation of the lesser valued saccharin reward cue (Grigson, 2008).
Relapse

The severity of the problem (and difficulty of treatment) is further compounded by the fact that addiction is a chronic, relapsing disease that induces long-lasting changes in brain function that interact with numerous environmental factors (O’Brien, 1997). Those interactions, then, make the addict susceptible to relapse. In fact, it has been reported that up to 90% of addicted humans will relapse to drug seeking, even after a prolonged period of abstinence (DeJong, 1994). Three types of relapse have been extensively described in the scientific literature: relapse induced by exposure to environmental stressors, relapse induced by exposure to drug-associated cues, and relapse induced by exposure to drugs themselves (see Shaham et al., 2003 for a review). Drug-induced relapse is the most straightforward of the three. For example, after even a single sip of alcohol, an alcoholic who has maintained abstinence is highly likely to return to his or her full-blown, binge drinking habits. McFarland and Kalivas (2001) have shown that a series of DAergic projections within the PFC and striatum mediate relapse due to drug exposure. Relapse also may be triggered when an abstinent individual is exposed to high levels of stress in his or her workplace or personal life. Interestingly, this type of relapse is not mediated by the hypothalamic-pituitary-adrenal (HPA) axis. Rather, it involves norepinephrine (NE) and corticotrophin releasing factor (CRF) signaling in the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (Erb et al., 2001; Koob and Zorilla, 2010; Leri et al., 2002). However, corticosterone (CORT) has been shown to play a permissive role in a specific type of stress-induced relapse that is induced by food deprivation (Shalev et al., 2003). Finally, abstinent addicts are likely to relapse upon exposure to drug-associated cues. For example, cocaine addicts who are shown images of other individuals smoking cocaine
reported experiencing higher levels of drug cravings and exhibited significant increases in striatal DA levels (Volkow et al., 2006), as well as activation of limbic structures, such as the amygdala (Childress et al., 1999), compared to addicts who were shown neutral, control images. Two types of drug-associated cues may elicit relapse: explicit conditioned stimuli (CSs) or contextual cues. Explicit CSs are discrete stimuli that signal imminent drug reinforcement, whereas contextual cues are more general, multimodal background stimuli that constitute the setting where CS-drug associations are formed (Fuchs et al., 2005). The basolateral amygdala has been shown to mediate relapse elicited by CS exposure (See, 2002), while the dorsomedial PFC has been shown to mediate relapse due to contextual cue exposure (Fuchs et al., 2005).

**The Basic Animal Model**

In order to uncover the neural structures and neurochemistry involved in addiction and relapse, an animal model (most frequently involving rats) of self-administration has been developed (see Schuster and Thompson, 1969 for a review). This behavioral model utilizes the instrumental learning paradigm. As such, the animal is trained to perform a particular behavior (e.g., nose-poking, lever-pressing, or spout-licking) in order to receive a drug reward. During self-administration training, a fixed ratio (FR) schedule of reinforcement is employed where a predetermined number of behavioral responses leads to infusion of the drug. Once self-administration behavior has been acquired and becomes stable, the behavior can then be extinguished using extinction training. During extinction training, the conditions are the same as those used during self-administration training, except behavioral performance is no longer rewarded. Typically, relapse is then tested using the reinstatement model (see Shaham et al.,
The animal is exposed to one of the factors discussed above (e.g., a priming dose of drug, physical or psychological stress, a drug-associated cue) and drug-seeking behaviors are monitored before and after exposure. The rate of responding during reinstatement testing, then, is considered to be a measurement of the animal’s propensity to relapse (Deroche-Gamonet et al., 2004). In addition, the animal’s willingness to work for drug can be tested using a progressive ratio (PR) schedule of reinforcement where the required number of responses needed to receive a drug reward progressively increases, rather than remaining fixed (see Richardson and Roberts, 1996 for a review). Measures of drug seeking, such as goal-directed behavior (i.e., the amount of focus the animal exhibits towards the drug-associated operant) and persistence in responding for drug during extinction training or during periods of signaled drug non-availability, as well as measures of drug taking, such as drug intake and the rate of drug self-administration, can then be quantified. Some paradigms focus on one or two of these measures, while others (Deroche-Gamonet et al., 2004) have used several in the same experiment to quantify “addiction-like” behavior. The experiments presented in this thesis employ both strategies.

**NOVEL BEHAVIORAL FACTORS THAT AFFECT DRUG SEEKING AND DRUG TAKING**

As discussed in the previous section, there are several environmental factors that interact with the neural substrates of addiction, triggering relapse to drug seeking and drug taking, including drugs of abuse, stress, and drug-associated cues. The data presented in this thesis will explore the effects of three novel behavioral factors: sleep deprivation, exposure to a high-fat diet, and environmental enrichment. Sleep deprivation has been shown to increase sensitivity to
reward by lowering the threshold for intracranial self-stimulation in rats, and by increasing the rate of responding for rewarding electrical brain stimulation (Steiner and Ellman, 1972). Furthermore, the clinical literature suggests that sleep deprivation can induce relapse in humans (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994). Intake (especially bingeing) of foods high in fat also has been associated with substance abuse. Binge eating disorders and substance dependence share high comorbidity rates (Conason et al., 2006; Hudson et al., 2007), particularly among individuals suffering from alcohol and cocaine dependence (Brewerton et al., 1995; Bulik et al., 2002; Bushnell, 1994; Johnson et al., 1997; Jonas et al., 1987; Wiederman and Pryor, 1996; Wilson, 1993). Finally, there is a wealth of data demonstrating the protective effects of early environmental enrichment on self-administration and reinstatement of drugs of abuse (Bardo et al., 2001; Chauvet et al., 2009; Green et al., 2002; Stairs et al., 2006).

**Sleep Deprivation**

Sleep deprivation has several neurochemical, cognitive, physical, and behavioral effects on both animals (Andersen et al., 2005a; España and Scammell, 2004; McDermott et al., 2003; Rechtschaffen et al., 1989) and humans (Vgontzas et al., 2004). Most notable are fluctuations in the levels of a number of different monoamines and neuropeptides throughout the brain, which are known to be involved in the regulation of sleep, particularly, DA (Andersen et al., 2005a; España and Scammell, 2004; Maloney et al., 2002), NE (Andersen et al., 2005a), and orexin (España and Scammell, 2004; Estabrooke et al., 2001). Sleep deprivation also causes adrenal hypertrophy (Rechtschaffen et al., 1989) and an increase in CORT levels (España and Scammell,
2004). Furthermore, sleep deprivation has been shown to augment DA activity specifically in the mesocorticolimbic reward system (i.e., in the VTA (Hernández-Peón et al., 1969), NAc (Asakura et al., 1992), and forebrain (Brock et al., 1995; Farooqui et al., 1996)). This augmentation is indicated by increases in DA levels (Hernández-Peón et al., 1969), increases in DA metabolite levels (Asakura et al., 1992; Farooqui et al., 1996), increases in D₁ (Demontis et al., 1990; Hamdi et al., 1993) and D₂ (Brock et al., 1995) DA receptor densities, and decreases in the affinity for DA at reuptake sites (Hamdi et al., 1993). Also, sleep deprivation has been shown to increase sensitivity to DA agonists (Nunes et al., 1994; Tufik, 1981).

Interestingly, these same neurochemicals have been shown to be involved in reward circuits mediating drug seeking, drug taking, and relapse. For example, NE is involved not only in the regulation of sleep, but also in stress-induced relapse (Leri et al., 2002). Also, CORT has been shown to play a permissive role in food deprivation-induced relapse (Shalev et al., 2003).

As described above, DA plays a central role in responding to rewards and is directly affected by sleep deprivation (Andersen et al., 2003; Andersen et al., 2005b; Andersen and Tufik, 2002; Levy Andersen et al., 2003). Finally, orexin, which modulates several brain waking systems (España and Scammell, 2004), also has been demonstrated to play an integral role in the mesocorticolimbic reward system. This is evinced by its signaling capacity in the VTA (Harris et al., 2005; Marcus et al., 2001) and NAc (Trivedi et al., 1998), as well as the overlap of its receptor distribution with that of DA (Fadel and Deutch, 2002) and NE (Baldo et al., 2003). Further, activation of orexin neurons in the lateral hypothalamus (Harris et al., 2005) and direct intracerebroventricular (ICV) infusion of orexin (Boutrel et al., 2005) can reinstate previously extinguished drug-seeking behavior.
**Binge Eating**

Binge eating disorder (BED) is thought by many to be the most common eating disorder, affecting as many as 4% of Americans (American Psychiatric Association, 2000; National Women’s Health Information Center, 2000). Binge eating is operationally defined as the consumption of more food, in a brief amount of time, than most individuals would consume in a similar time period and under similar circumstances (American Psychiatric Association, 2000). As such, binge eating consists of engaging in time-limited bouts of excessive food consumption characterized by subjective loss of control, as well as loss of homeostatic control over an individual meal size. While binge eating and obesity can be co-expressed clinically, nearly 67% of those suffering from some type of binge disorder (including BED, bulimia nervosa, and sub-threshold BED) are not obese (BMI < 30; Hudson et al., 2007).

Binge eating typically involves the intermittent overconsumption of self-restricted foods that are high in fat and/or sugar, such as snacks and desserts (American Psychiatric Association, 2000; Guertin, 1999; Hadigan, et al., 1989; Rosen et al., 1986). As is the case with substance abuse and sleep deprivation, there are several neuroanatomical and neurochemical changes that accompany eating, as well as sucrose and fat consumption in particular. For instance, ghrelin, a hormone that stimulates feeding, has been shown to act directly on DAergic neurons in the VTA, stimulating DA release (Abizaid et al., 2006; Jerlhag et al., 2007). Also, sucrose consumption has been shown to increase NAc neuronal firing rates compared to water consumption (Roop et al., 2002), increase dopamine turnover in the nucleus accumbens (NAc; Hajnal and Norgren, 2002), cause a decrease in D2 receptor binding in the striatum (Bello et al., 2002), and increase the expression of dopamine transporter (DAT) mRNA in the ventral tegmental area (VTA) and
DAT protein binding in the NAc and VTA (Bello et al., 2003). In addition, compulsive intake of foods high in fat also has been shown to cause a downregulation in the expression of D2 receptors in the striatum (Johnson and Kenny, 2010). Again, these sugar and fat consumption-induced changes occur in the mesocorticolimbic DA system. However, what is surprising (and, in fact, alarming), is that these changes mimic the effects of exposure to drugs of abuse (see Koob and Volkow, 2010 for a review).

**Environmental Enrichment**

Certain factors (e.g., exposure to sweets) have been shown to have protective influences on drug-taking and drug-seeking behaviors (Carroll and Lac, 1993; Higgins et al., 1993; Lenoir and Ahmed, 2007; Liu and Grigson, 2005), and much work has been done in the field of environmental enrichment. In fact, environmental enrichment has been shown to reduce the rewarding effects of cocaine, nicotine, and heroin (El Rawas et al., 2008; Green et al., 2003; Solinas et al., 2008a), reduce self-administration of amphetamine (Bardo et al., 2001; Green et al., 2002), enhance the extinction of amphetamine self-administration (Stairs et al., 2006), and attenuate reinstatement of drug seeking induced by drug, drug-associated cues, and stress (Chauvet et al., 2009; Stairs et al., 2006). In addition, environmental enrichment has been shown to cause several neuroanatomical and neurochemical changes in the reward circuitry of the brain. For example, environmental enrichment decreases DAT mRNA levels in the striatum (Bezard et al., 2003) and PFC (Zhu et al., 2005), decreases DAT binding in the striatum (Bezard et al., 2003), and induces expression of brain-derived neurotropic factor (BDNF) in the hippocampus (Zhu et al., 2006) and striatum (Bezard et al., 2003).
In general, it is thought that the neuroprotective effects of environmental enrichment stem from its ability to promote plasticity in the brain. In fact, environmental enrichment has been shown to increase dendritic branching and synapse formation (Black et al., 1989; Kleim et al., 1997). Also, enrichment is capable of attenuating the normal decrease in the number of synapses that accompanies aging (Saito et al., 1994). Finally, several studies have shown that enrichment can stimulate neuronal proliferation in the hippocampus, enhancing learning and memory (Kempermann et al., 1997; Nilsson et al., 1999; Young et al., 1999).

Environmental enrichment can involve any of a number of housing manipulations designed to promote activity and increase the complexity of the environment, including the addition of interactive novel objects (e.g., running wheels, tubes, chew toys, etc.) and social partners. Whether novel objects and social interaction have differential effects on drug-seeking and drug-taking behaviors remains largely uninvestigated, as most studies employ both types of enrichment simultaneously. Some evidence suggests, however, that it may be the availability of novel objects that mediates the effects discussed above, at least in mice. Thus, mice housed in groups with novel objects exhibit higher levels of BDNF in the striatum (Bezard et al., 2003; Turner and Lewis, 2003), less stereotypical behavior (Turner and Lewis, 2003), and decreased DAT mRNA and DAT binding in the striatum (Bezard et al., 2003) compared to mice housed in groups without novel objects.

**SUMMARY**

Although it is clear that these different factors can cause neuroanatomical and neurochemical changes in the mesocorticolimbic DA system, some aspects of their effects on the
acquisition of drug-seeking and drug-taking behaviors remain unknown. While there is evidence that sleep deprivation may be associated with a higher incidence of relapse in humans (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994), as yet, however, no animal model has been developed. Consequently, there has been no opportunity to directly study the effects of sleep deprivation on drug seeking and drug taking, or the underlying neural substrates involved. Therefore, Chapters 2 and 3 will demonstrate the effects of sleep deprivation on cocaine self-administration. Specifically, Chapter 2 will show the facilitative effects of acute sleep deprivation (4-8 h) on cocaine-induced reinstatement, as well as on measures of cocaine-seeking and cocaine-taking using a PR schedule of reinforcement. Chapter 3 will present evidence that chronic sleep restriction, akin to that experienced by humans, also is capable of augmenting the acquisition of cocaine-seeking and cocaine-taking behaviors.

While comorbidity rates between binge eating disorders and substance dependence are high (Brewerton et al., 1995; Bulik et al., 2002; Bushnell, 1994; Conason et al., 2006; Hudson et al., 2007; Johnson et al., 1997; Jonas et al., 1987; Wiederman and Pryor, 1996; Wilson, 1993), use of an animal model of binge eating has been limited and it remains unknown whether it is intake of fat or sugar, per se, or loss of control over intake that result in those alterations. Thus, Chapter 4 will systematically and operationally investigate the relationship between fat bingeing and the development of “addiction-like” behaviors toward cocaine. As such, the data will demonstrate that loss of control over one consummatory behavior (i.e., consumption of fat) can lead to loss of control over another (i.e., cocaine intake).

Finally, although a great deal is known about the protective effects of environmental enrichment on drug seeking and drug taking, the majority of studies provided enrichment during adolescence, not during adulthood, and employed paradigms where drug was administered
passively by the experimenter. No studies have examined the effects of environmental enrichment on the acquisition of self-administration or drug-induced devaluation of a natural reward cue in animals that were enriched as adults. Therefore, Chapter 5 will illustrate the protective effects of environmental enrichment on the acquisition of cocaine-seeking and cocaine-taking behaviors, as well as the effects of enrichment on cocaine-induced devaluation of saccharin in adult rats.
Chapter 2

Acute sleep deprivation increases the rate and efficiency of cocaine self-administration, but not the perceived value of cocaine reward in rats (Puhl et al., 2009).

Chapter 2 will evaluate the effects of acute sleep deprivation on cocaine seeking and self-administration. As discussed in Chapter 1, three types of relapse have been extensively described in the scientific literature: relapse induced by exposure to environmental stressors, relapse induced by exposure to drug-associated cues, and relapse induced by exposure to drugs themselves. The clinical literature suggests that sleep deprivation is another factor that can induce relapse. Both subjective (self-administered questionnaire scores) and objective (polysomnographic sleep parameters) measures of poor sleep quality, in general, have been shown to predict relapse (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994). This problem is compounded by the overall poor sleep quality experienced by the majority of our society. Data collected in the 2008 Sleep in America Poll conducted by the National Sleep Foundation showed that 65% of Americans report experiencing symptoms of a sleep problem several nights per week. Given the alarmingly high addiction statistics discussed in Chapter 1, the population of individuals reporting poor sleep likely overlaps with the substance-dependent population. Yet, no animal models have been developed and relatively little work has been done to ascertain the mechanisms by which sleep deprivation induces relapse.

In addition, several studies purport insomnia as a reliable predictor of relapse in substance-abusing humans (Brower, 2003; Brower et al., 2001; Ford and Kamerow, 1989; Foster and Marshall, 1998; Gillin, 1998; Hohagen et al., 1993; Longo and Johnson, 1998; Malcolm et al., 2007; Teplin et al., 2006). Admittedly, there is a very clear distinction between frank
insomnia and forced sleep deprivation. Insomnia is primarily a disorder of hyperarousal, often linked with high levels of psychological stress and anxiety, among other comorbid conditions. Sleep deprivation refers to the simple suppression or prevention of sleep. However, both conditions represent a profound loss of sleep and poor sleep quality, overall. While the effects of insomnia on relapse cannot simply be generalized to those of sleep deprivation, it seems likely that the two conditions do, in fact, have similar effects on drug-seeking behaviors. Such an inference is not unreasonable given the aforementioned studies showing that both subjective and objective measures of general poor sleep quality also have been shown to predict relapse (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994). Given the impact of insomnia and general poor sleep quality on drug seeking and drug taking reported in the clinical literature, we hypothesized that sleep deprivation would augment cocaine-induced reinstatement and cocaine seeking in drug-experienced rats. The first study, then, used drug-induced reinstatement and progressive ratio testing to investigate the effects of acute sleep deprivation (0, 4, or 8 h) on drug seeking and drug taking in rats that had been trained to self-administer cocaine.

**METHODS**

*Subjects*

This study was conducted in two replications. The subjects were 72 (n=36 for Replication 1 and n=36 for Replication 2) naïve, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC), approximately three months of age at the beginning of the
experiment. Due to complications during surgery, three rats were eliminated from the study. An additional four rats were eliminated due to a loss of catheter patency. The remaining 65 rats underwent self-administration and extinction training (described below). Following behavioral training, 42 rats were chosen for acute sleep deprivation and further experimental testing (described below). These rats were counterbalanced for drug intake and self-administration chamber placement so that all rats could be tested at the same time. Except where otherwise noted, the rats were housed individually in standard wire mesh cages, in a colony room with temperature, humidity, and ventilation controlled automatically. The rats were maintained on a 12-h light-dark cycle, with lights on at 0700 h. They were allowed ad lib access to food (Harlan Teklad, Madison, WI) and water, except where otherwise noted.

**Catheter Construction and Implantation**

*Self-administration catheter.* Intra-jugular catheters were custom-made in our laboratory as described by Grigson and Twining (2002).

*Catheter implantation.* Rats were anesthetized and catheters were implanted into the jugular vein as described by Grigson and Twining (2002) and Liu and Grigson (2005). Following surgery, rats were allowed at least two days to recover. General maintenance of catheter patency involved daily examination and flushing of catheters with heparinized saline (0.2 ml of 30 IU/ml heparin). Catheter patency was verified, as needed, using 0.2 ml of propofol (Diprivan 1%) administered intravenously.
Apparatus

Each rat was trained in one of twelve identical operant chambers (MED Associates, St. Albans, VT) described by Grigson and Twining (2002). Each chamber measured 30.5 cm in length × 24.0 cm in width × 29.0 cm in height, and was individually housed in a light- and sound-attenuated cubicle. The chambers consisted of a clear Plexiglas top, front, and back wall. The side walls were made of aluminum. Grid floors consisted of nineteen 4.8-mm stainless steel rods, spaced 1.6 cm apart (center to center). Each chamber was equipped with two retractable sipper spouts that entered through 1.3-cm diameter holes, spaced 16.4 cm apart (center to center). A stimulus light was located 6.0 cm above each tube. Each chamber was also equipped with a houselight (25 W), a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN), and a speaker for white noise (75 dB). Cocaine reinforcement was controlled by a lickometer circuit that monitored empty spout licking to operate a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT). A coupling assembly attached the syringe pump to the catheter assembly on the back of each rat and entered through a 5.0-cm diameter hole in the top of the chamber. This assembly consisted of a metal spring attached to a metal spacer with Tygon tubing inserted down the center, protecting passage of the tubing from rat interference. The tubing was attached to a counterbalanced swivel assembly (Instech, Plymouth Meeting, PA) that, in turn, was attached to the syringe pump. Events in the chamber and collection of data were controlled on-line with a Pentium computer that used programs written in the Medstate notation language (MED Associates).
**Drug Preparation**

Individual 20-ml syringes were prepared for each self-administration chamber prior to each daily session by diluting 4.0 ml of cocaine HCl stock solution (1.24 g cocaine HCl + 150 ml saline) with 16.0 ml of heparinized saline (0.1 ml 1000 IU heparin/60.0 ml saline) for a dose of 0.33 mg/infusion. This dose of cocaine supports marked and orderly cocaine self-administration behavior (Grigson & Twining, 2001; Liu & Grigson, 2005; Wheeler et al., 2008).

**Data Collection**

In an effort to be consistent with other self-administration data collected in our laboratory (e.g., Grigson & Twining, 2002; Liu and Grigson, 2005), habituation, self-administration training, extinction training, and all experimental testing were conducted during the light phase of the light/dark cycle. As such, it could be argued that all animals experienced some degree of sleep deprivation. However, daily training during the light cycle is standard practice for the development of self-administration behaviors and training sessions were only 60-90 minutes in duration. In addition, sleep deprivation was not forced during the training sessions. The animals were free to sleep in the chambers if they so chose (in fact, most did). Thus, these training sessions imposed little, if any, sleep deprivation.
**Habituation Procedure and Spout Training**

Rats were habituated to the operant chambers for 1 h/day for three days prior to the beginning of self-administration training. During this time, each rat was maintained on a water-deprivation regimen in which they received 1-h daily access to water in the operant chamber from the right spout during the habituation session and 25.0 ml of water in the home cage overnight. Thereafter, rats were returned to ad lib access to water for the duration of the study. See Figure 2.1 for a summary of behavioral training and experimental testing.

![Figure 2.1: Timeline of behavioral training and experimental testing.](image)

**Self-administration and Extinction Procedures**

*Self-administration training.* Self-administration training began immediately following the 3-day habituation phase. Each rat was trained during daily 90-min sessions for 16 days. Specifically, rats were placed in the operant chambers in darkness. Immediately upon initiation of the 90-min session, the white noise was turned on, both spouts advanced into the chamber, and the cue light above the active spout was illuminated. The right spout was termed the “active” spout, while the left spout was termed the “inactive” spout. A fixed ratio (FR) 10
schedule of reinforcement was implemented initially (Days 1-12). During this time, completion of 10 licks on the “active” spout was followed by a single intravenous (i.v.) infusion of 0.33 mg cocaine over six seconds. Drug delivery was signaled by offset of the stimulus light, retraction of the “active” spout, and onset of the tone and houselight. The tone and houselight remained on for a 20-sec timeout period. Responding on the “inactive” spout was without consequence throughout each session. During the final four days of training (Days 13-16), the reinforcement schedule was increased to an FR20 to fully distinguish between active and inactive responding. Following each self-administration training session, the rats were returned to their home cages.

*Extinction training.* Extinction training began immediately following self-administration training, as previously described (Liu and Grigson, 2005), and continued for 14 days. Conditions matched those employed during self-administration training, except completion of the required number of licks on the FR20 schedule of reinforcement was no longer followed by an i.v. infusion of cocaine. Behavior was considered to be extinguished when the number of responses made on the previously active and inactive spouts did not differ significantly for at least three consecutive extinction trials.

*Acute Sleep Deprivation and Experimental Testing Procedures*

*Acute sleep deprivation.* Acute sleep deprivation was conducted using the novel object method described by Cirelli and colleagues (Cirelli et al., 1995; Cirelli & Tononi, 2000; Vyazovskiy et al., 2007). The standard wire mesh cages that served as home cages were not conducive to this method. Therefore, following the fifth day of extinction training, all rats were housed in Plexiglas home cages (46.0 cm in length × 25.0 cm in width × 21.5 cm in height) with
corncob bedding (Harlan Teklad, Madison, WI). They remained in these Plexiglas home cages for the duration of the study, thereby providing ample time for the animals to become habituated to these new home cages prior to the imposition of acute sleep deprivation. During acute sleep deprivation, novel objects were introduced into the Plexiglas home cages of the rats. All rats explored and interacted with these objects, even at the expense of sleep. All animals were under constant investigator supervision for the entire duration of acute sleep deprivation, such that objects could be replaced if their novelty was lost (i.e., if rats stopped attending to those objects). Investigators also introduced folded paper towels into the cages, which prompted interaction by the rats. This approach provides stimulation to the animal, but with less stress than that incurred by other sleep deprivation methods. These methods have been employed reliably for periods of time ranging from 1-24 h (Tononi et al., 1994; Cirelli et al., 1999; Cirelli & Tononi, 2000). Rats were divided into three groups: the SD<sub>0</sub> group (n=14) did not receive any sleep deprivation; the SD<sub>4</sub> group (n=14) received 4 h of total sleep deprivation; and the SD<sub>8</sub> group (n=14) received 8 h of total sleep deprivation.

*Cocaine-induced reinstatement testing.* Twenty-four hours following the final day of extinction training, the rats were acutely sleep deprived, as described, and immediately tested for cocaine-induced reinstatement. Testing occurred during the light phase. During the cocaine-induced reinstatement test, rats were placed in the operant chambers for a single 90-min session, with conditions identical to those employed during extinction training. However, 45 min into the 90-min extinction session, a 0.33 mg infusion of cocaine was passively administered i.v. Responding on the active and inactive spout was measured both before and after the passive infusion.
Progressive ratio testing. An additional method that can be used to assess the impact of acute sleep deprivation on responding for drug is the introduction of a PR schedule of reinforcement to test how hard sleep-deprived rats are willing to work for the drug. Thus, following cocaine-induced reinstatement testing, rats were allowed one day to recover from sleep deprivation. Rats in the same sleep deprivation groups were then subjected to a second bout of acute sleep deprivation. Progressive ratio testing took place immediately thereafter. During PR testing, rats were placed in the operant chambers with conditions identical to those of self-administration training, except the number of active responses required to receive each infusion progressively increased by a multiple of five for up to ten infusions (1, 1+5=6, 6+10=16, 16+15=31, 31+20=51, 51+25=76, 76+30=106, 106+35=141, 141+40=181, 181+45=226). Thereafter, the number of required responses increased by 50 for each successive infusion (226+50=276, 276+50=326, 326+50=376, etc.). During this PR session, rats were allowed to self-administer cocaine (0.33 mg/infusion) until a period of 30 min elapsed without receipt of an infusion. Rate of drug self-administration (inter-infusion interval), as well as goal-directed responding were measured.

Data Analysis

All data were analyzed with Statistica (Version 9, StatSoft, Tulsa, OK) using mixed factorial and one-way analysis of variance (ANOVA) tests, as well as Student’s t-tests. Newman-Keuls post hoc tests were conducted on significant ANOVAs, when appropriate, with $\alpha$ set at 0.05.
RESULTS

When examining the acquisition data, it became evident that there were likely two separate groups of rats. Thus, prior to data analysis, the number of infusions received during the final two days of self-administration training (trials 15-16) was averaged, and the rats were separated into two groups using the median split of those means. Consistent with previous findings (Grigson & Twining, 2002), this allowed for the identification of two distinct sub-populations within our sample population: low drug-takers (n=20) and high drug-takers (n=22). These two sub-populations can be seen in Figure 2.2, which illustrates the bimodal distribution of cocaine self-administration. Thus, during the terminal acquisition period, most rats in the low drug-taking group self-administered 4-9 (median = 5.5, mean = 6) infusions of cocaine/90-min session, while most rats in the high drug-taking group self-administered 18-24 (median = 19, mean = 21) infusions/90-min session.
**Figure 2.2:** Distribution of the number of subjects exhibiting a given number of infusions of cocaine during the terminal acquisition period (i.e., averaged across trials 15-16). The left panel shows the best fit line for the low drug-takers and the right panel shows the best fit line for the high drug-takers. (Low curve: \( y_0 = 0; A = 6; \mu = 7; \sigma = 0.184; \chi^2 = 21.42; \) High curve: \( y_0 = -0.0126; A = 2.62; \mu = 15.479; \sigma = 0.3678; \chi^2 = 27.037; \) Median: 13).

**Acquisition and Extinction**

*Acquisition: Number of responses.* Acquisition of cocaine self-administration was analyzed using a \( 2 \times 2 \times 16 \) mixed factorial ANOVA varying group (low drug-takers or high drug-takers), spout (active or inactive), and trials (1-16). Significant main effects of group, spout, and trial were obtained (see Figure 2.3). Overall, high drug-takers responded more than low drug-takers, \( F(1, 78)=23.53, p < 0.01, \) more active responses were made than inactive responses, \( F(1, 78)=36.55, p < 0.01, \) and responding increased as self-administration trials progressed, \( F(15, 1170)=5.11, p < 0.01. \) In addition, significant two-way interactions were seen between group and spout, as well as between spout and trial. Post hoc tests of the significant
**Figure 2.3:** Mean (+/− SEM) active and inactive responding throughout self-administration and extinction training. Closed symbols represent active responses, while open symbols represent inactive responses. High drug-takers (n=22) are represented by triangles, while circles represent low drug-takers (n=20). * denotes statistical significance (p < 0.05).

The group × spout interaction, F(1, 78)=7.26, p < 0.01, indicated that high drug-takers exhibited more active responses than low drug-takers, overall, p < 0.05. Post hoc analysis of the significant spout × trials interaction, F(15, 1170)=10.90, p < 0.01, revealed that active responding was greater than inactive responding across the 16 trials of acquisition, overall, ps < 0.05. Finally, the group × spout × trial interaction also was significant, F(15, 1170)=5.60, p < 0.01. Post hoc Newman-Keuls tests of this three-way interaction revealed that high drug-takers responded more on the active than the inactive spout on Day 1 and on Days 11-16, ps < 0.01 (see Figure 2.3). However, low drug-takers never displayed this behavior. That is, the differences between active and inactive responding displayed by low drug-takers were not statistically significant throughout the 16 days of self-administration training.

**Acquisition: Response latency.** Acquisition data also were evaluated by examining the effect of group on the latency to self-administer the first infusion of cocaine using a Student’s t-test. In accordance with the infusion data, the high drug-takers also were faster to take the first infusion (i.e., exhibited a shorter latency) than the low drug-takers (p < 0.01; see Figure 2.4).
**Figure 2.4:** Mean (+/- SEM) latency (s) to self-administer the first infusion. The open bar represents the low drug-taking group, while the hatched bar represents the high drug-taking group. * denotes statistical significance (p < 0.01).

*Extinction.* Extinction of self-administration training was analyzed similarly using a 2 × 2 × 14 mixed factorial ANOVA varying group (low drug-takers or high drug-takers), spout (active or inactive), and trials (17-30). Significant main effects of spout and trials were seen (see Figure 2.3). Overall, active responses remained greater than inactive responses, F(1, 80)=28.30, p < 0.01, and responding decreased as extinction trials progressed, F(13, 1040)=35.14, p < 0.01. However, neither the main effect of group, nor the group × spout interaction was significant, Fs < 1. A significant two-way interaction was seen between spout and trial, F(13, 1040)=10.65, p < 0.01, and post hoc tests showed that all rats responded more on the active than the inactive spout.
during trials 17–21, ps < 0.01. In accordance, the group × spout × trials interaction did not attain statistical significance, F < 1. This finding confirmed that the high and the low drug-takers responded similarly on the active and the inactive spouts throughout extinction. Thus, while the low drug-takers failed to make significantly more responses on the active than the inactive spout during acquisition, they exhibited a marked increase in responding on the active spout early in extinction training when the drug was removed. These data suggest that even the low drug-takers were self-administering drug, but simply at a lower level than the high drug-takers.

**Cocaine-induced Reinstatement**

Cocaine-induced reinstatement was analyzed using a 2 × 2 × 3 mixed factorial ANOVA varying group (low drug-takers or high drug-takers), test period (pre-drug or post-drug), and SD condition (SD₀, SD₄, or SD₈; see Figure 2.5). Significant main effects of group and test period were obtained, showing that the high drug-takers responded more than the low drug-takers, overall, F(1, 36)=8.19, p < 0.01, and post-drug responding (i.e., cocaine-induced reinstatement) was greater than pre-drug responding, overall, F(1, 36)=34.21, p < 0.01. The main effect of
Figure 2.5: Mean (+/− SEM) number of infusion attempts made by rats in all three SD conditions (SD₀, SD₄, and SD₈) during the 45 min extinction period before (open bars) and after (hatched bars) the passive infusion of cocaine. Rats with a history of low drug-taking (SD₀: n=7, SD₄: n=6, and SD₈: n=7) can be viewed in the left panel, while rats with a history of high drug-taking (SD₀: n=7, SD₄: n=8, and SD₈: n=7) can be viewed in the right panel. * denotes statistical significance (p < 0.05).

sleep deprivation condition was not statistically significant, F < 1. Significant two-way interactions, however, were found between test period and sleep deprivation condition, F(2, 36)=3.34, p < 0.05, as well as between test period and group, F(1, 36)=4.51, p < 0.05. Post hoc tests of the test period × group interaction showed that while pre-drug responding did not differ between the high and low drug-takers ( p > 0.05), post-drug responding (i.e., cocaine-induced
reinstatement) was greater in the rats with a history of high drug taking compared to those with a history of low drug taking, p < 0.05.

These observations warranted closer inspection of the high and low drug-taker groups, individually. Thus, the low and high drug-takers were analyzed separately using 2 × 3 mixed factorial ANOVAs varying test period (pre-drug or post-drug) and sleep deprivation condition (SD₀, SD₄, or SD₈). When the low drug-taker group was analyzed alone, a significant two-way interaction was obtained between test period and sleep deprivation condition, F(2, 17)=4.06, p < 0.05. Post hoc Newman-Keuls tests revealed that pre-drug responding did not differ across sleep deprivation condition for the low drug-takers, ps > 0.05 (see open bars in left panel of Figure 2.5). However, post-drug responding was significantly greater in the SD₀ group compared to the SD₄ and SD₈ groups, ps < 0.03, which did not differ from one another, ps > 0.05 (see hatched bars in left panel of Figure 2.5). In addition, pre- versus post-drug responding did not differ in either the SD₄ or SD₈ groups, ps > 0.05, while it did differ for rats in the SD₀ group, p < .05. This finding confirms that, unlike the non-sleep-deprived low drug-taking controls, sleep-deprived low drug-takers failed to exhibit cocaine-induced reinstatement (see left panel of Figure 2.5).

A different pattern of behavior emerged for the high drug-takers. Specifically, a similar analysis of the data from the high drug-taker group revealed that the test period × SD condition interaction was not significant, F < 1 (see right panel of Figure 2.5). The main effect of test period, however, was statistically significant, F(1, 19)=40.70, p < 0.01, showing higher post-drug responding than pre-drug responding, regardless of SD condition. Student’s t-tests confirmed that post-drug responding was significantly greater than pre-drug responding for rats in all three SD conditions (SD₀, SD₄, or SD₈), ps < 0.05. Acute sleep deprivation, then, prevented cocaine-
induced reinstatement in the low drug-takers. High drug-takers, on the other hand, were motivated to seek drug following the cocaine challenge whether they were, or were not, acutely sleep-deprived.

**Progressive Ratio Testing**

Total number of infusions and inter-infusion intervals (i.e., the amount of time between infusions) were analyzed for the PR data using $2 \times 3$ mixed factorial ANOVAs varying group (low drug-takers or high drug-takers) and SD condition ($SD_0$, $SD_4$, or $SD_8$). Goal-directed behavior, measured by comparing the number of active and inactive responses as a percentage of total PR responding, also was analyzed using a $2 \times 2 \times 3$ mixed factorial ANOVA varying group (low drug-takers or high drug-takers), spout (active or inactive), and sleep deprivation condition ($SD_0$, $SD_4$, or $SD_8$). Student’s t-tests were employed where necessary.

*Total infusion number.* During PR testing, the high drug-takers exhibited higher break points, overall, than did the low drug-takers. Consequently, the high drug-takers took more infusions than did the low drug-takers during the PR session (see Figure 2.6). This observation was confirmed by a significant main effect of group, $F(1, 36)=20.50$, $p < 0.01$. Sleep deprivation condition, however, had no significant effect on PR responding as indicated by a non-significant main effect of SD condition, $F < 1$, and a non-significant group $\times$ SD condition interaction, $F < 1$. Simply put, all high drug-takers worked for drug, while all low drug-takers did not, and acute sleep deprivation was without effect on this measure. Indeed, even Student’s t-tests failed to reveal significant differences in PR responding between the $SD_0$ group and the $SD_4$ group, $p > 0.05$, or between the $SD_0$ group and the $SD_8$ group, $p > 0.05$, for the high drug-takers. Acute
sleep deprivation, then, did not significantly increase or significantly decrease a drug-experienced rat’s willingness to work for drug across a protracted PR session.

**Figure 2.6:** Mean (+/- SEM) number of infusions self-administered by low and high drug-taking rats across the three SD conditions during PR testing. Low drug-takers (SD0: n=7, SD4: n=6, and SD8: n=7) can be viewed in the left panel and high drug-takers (SD0: n=7, SD4: n=8, and SD8: n=7) in the right panel.

**Inter-infusion interval.** As indicated, we also were interested in the rate at which the rats took drug when given the opportunity. When evaluating inter-infusion intervals across the entire session, acute sleep deprivation had a clear and marked facilitative effect (see Figure 2.7). The results of a $2 \times 3$ mixed factorial ANOVA varying group (low drug-takers or high drug-takers) and SD condition (SD0, SD4, or SD8) showed that the main effect of SD condition was
significant, F(2, 36)=8.74, p < 0.01, indicating that, overall, inter-infusion intervals were shorter for sleep-deprived versus non-sleep-deprived rats. Post hoc Newman-Keuls tests revealed that inter-infusion intervals for the SD<sub>0</sub> group were significantly longer than inter-infusion intervals for the SD<sub>4</sub> and SD<sub>8</sub> groups, ps < 0.05, overall, which did not differ from one another, p > 0.05.

![Figure 2.7: Mean (± SEM) inter-infusion intervals for low and high drug-taking rats across all SD conditions during PR testing. Low drug-takers (SD<sub>0</sub>: n=7, SD<sub>4</sub>: n=6, and SD<sub>8</sub>: n=7) are shown in the left panel and high drug-takers (SD<sub>0</sub>: n=7, SD<sub>4</sub>: n=8, and SD<sub>8</sub>: n=7) are shown in the right panel. * denotes statistical significance (p < 0.05).](image)

Thus, acute sleep deprivation increased the rate at which the rats self-administered the drug when tested on a PR schedule of reinforcement. The group × SD condition interaction, however, was
not significant, \( F < 1 \), indicating that the facilitative effect of sleep deprivation on the rate of drug infusion was the same for the low and the high drug-takers.

**Goal-directed behavior.** Goal-directed behavior was analyzed by comparing active and inactive responding during PR testing, both represented as percentages of the total number of responses made during the testing period. A significant main effect of spout was seen, \( F(1, 36)=118.61, p < 0.01 \), indicating that, overall, more responses were made on the active spout than on the inactive spout (see Figure 2.8). This high level of active responding was neither

![Figure 2.8](image-url)

**Figure 2.8:** Goal-directed behavior of low (Left panel: \( SD_0: n=7, SD_4: n=6, \) and \( SD_8: n=7 \)) and high (Right panel: \( SD_0: n=7, SD_4: n=8, \) and \( SD_8: n=7 \)) drug-taking rats across all SD conditions presented as the comparison of active and inactive responses (both represented as percentages of the total number of responses made) during PR testing. * denotes statistical significance (\( p < 0.05 \)).
affected by group \( (\text{group} \times \text{spout interaction}, F < 1) \) nor by SD condition \( (\text{SD condition} \times \text{spout interaction}, F < 1) \). Even so, Student’s t-tests revealed something interesting. That is, for the low drug-takers in the SD\(_0\) group, there was no difference between active and inactive responding, \( p > 0.05 \) (see Figure 2.8, left panel). This was not the case, however, for the low drug-takers following acute sleep deprivation. Thus, while the non-sleep-deprived low drug-takers failed to exhibit significant goal-directed behavior, just 4-8 h of acute sleep deprivation led low drug-takers to make significantly more responses on the active versus the inactive spout, \( p < 0.05 \). Similar goal-directed behavior was seen for the high drug-takers, but this effect was significant across all SD conditions, \( p < 0.05 \), (see Figure 2.8, right panel). These results demonstrate that acute sleep deprivation focuses the behavior of low drug-taking rats on the active (i.e., drug-associated) spout. A full overview of the results presented in Chapter 2 can be viewed in Table 2.1.

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**Table 2.1:** Behavioral effects of acute sleep deprivation. Plus (+) and minus (−) signs indicate the presence or absence of behavior, respectively. Arrows indicate an increase or decrease in the variable in question, as well as the magnitude of that change (e.g., ↑↑ indicates an increase of greater magnitude than ↑).
DISCUSSION

The existence of distinct low and high drug-taking sub-populations of outbred Sprague-Dawley rats has been well documented. Piazza et al. (1989) were the first to characterize individual differences in the vulnerability to self-administer substances of abuse on the basis of locomotor response following placement into a novel environment. Two groups were identified: low responders (who exhibited low levels of locomotor activity) and high responders (who exhibited high levels of locomotor activity). When tested with drug self-administration (i.e., amphetamine or cocaine), the high responders self-administered more drug than did the low responders (Piazza et al., 1989; Piazza et al., 2000) and they exhibited a greater dopamine response in the nucleus accumbens following the administration of cocaine (Hooks et al., 1991). Thus, the high responders were thought to be more sensitive to the rewarding properties of drug compared to the low responders. In accordance, the high responders were initially faster at acquiring drug self-administration than the low responders (i.e., high responders exhibited statistically higher active than inactive responding sooner than low responders). However, that group difference was attenuated as self-administration training progressed and active responding made by the low responders increased (Piazza et al., 1989).

In general, these findings are consistent with the data presented in Chapter 2. In addition, Grigson and Twining (2002) reported that outbred male Sprague-Dawley rats given daily saccharin-cocaine (FR10 0.33 mg/infusion 1h) pairings also could be divided into two separate groups: large suppressers and small suppressers. Large suppressors greatly reduced intake of the saccharin cue following saccharin-cocaine pairings, while small suppressors did not. Importantly, when given the opportunity to self-administer cocaine, the rats that most greatly
avoided the saccharin cue (i.e., the large suppressers), self-administered 3-4 times more cocaine than did the small suppressors. Greater avoidance of the drug-associated taste cue, then, was correlated with greater drug-taking behavior, and some rats were more sensitive than others. Of course, this behavior develops across training trials and, by definition, cannot be used, \textit{a priori}, to predict who will and will not take drug. Even so, these group differences emerge relatively early and are sustained throughout training. As such, the paradigm is useful for addressing questions related to the acquisition of drug-taking behavior, maintenance of drug-taking behavior, and relapse as well. Finally, it should be noted that, although it is tempting to conclude that the high and low drug-takers in the saccharin-cocaine paradigm (Grigson & Twining, 2002) are the same as those revealed in the present paradigm (cocaine self-administration only), we cannot know this. Thus, we draw conclusions across the two paradigms with some caution.

Once divided on the basis of terminal cocaine intake, the high drug-takers in the present study took more drug, by definition, than did the low drug-takers (approximately 20 versus 6 infusions/90 min session, respectively). The high drug-takers also initiated the first infusion more quickly and they made significantly more responses on the active than the inactive spout. The low drug-takers, on the other hand, failed to make significantly more responses on the active than the inactive spout during acquisition. This, however, should not be taken as evidence that the low drug-takers were unmotivated to take drug, because, when shifted to extinction, responses on the previously active spout increased considerably for these low drug-takers. Indeed, during the first five days of extinction, the number of responses made on the previously active spout by the low drug-takers was significantly higher than the number made on the previously inactive spout, and these numbers did not differ from those made by the high drug-takers during the same time period. Thus, during acquisition, high drug-takers are motivated to
take a high number of infusions and low drug-takers are motivated to take a low number of infusions.

When tested in a non-sleep-deprived state, both the low and the high drug-takers exhibited marked drug-induced reinstatement, which did not differ between the two groups. Acute sleep deprivation (4 or 8 h) had no significant effect on this behavior in the high drug-takers. All high drug-takers exhibited marked drug-induced reinstatement, regardless of sleep deprivation condition. In the low drug-takers, however, acute sleep deprivation (4 or 8 h) fully prevented drug-seeking behavior following the cocaine challenge. When observing the behavior of the rats, the explanation for these data was obvious. Rather than seek a drug that was not available (the rats received only a single, passive infusion during cocaine-induced reinstatement testing), sleep-deprived low drug-takers chose to sleep. Competing biological motivations, then, lie at the crux of these effects. High drug-takers are highly motivated to seek and take drug. In the case of our high drug-taking rats, the motivation to seek drug overrode the motivation to sleep, even when tested after 4 or 8 h of acute sleep deprivation. Low drug-takers, on the other hand, do not possess the same high level of motivation for drug. Thus, in the absence of drug, the motivation to sleep overpowered the motivation to seek drug. This general pattern of behavior is interesting when considering the behavior of low drug-takers in the saccharin-cocaine paradigm (Grigson & Twining, 2002). In that case, when saccharin predicted the opportunity to self-administer drug, thirsty low drug-takers continued to consume a great deal of the saccharin cue while thirsty high drug-takers did not. Low drug-takers, then, may be less responsive to drug and more responsive to alternative natural rewards (like fluid or sweets when thirsty or hungry, or sleep when sleep-deprived). Perhaps similar individual differences in the motivation for drug
also affect choice when involving other competing motivations, such as caring for one’s offspring (Seip & Morrell, 2007).

Although the cocaine-induced reinstatement paradigm was informative, it does not mimic relapse situations encountered by human addicts. That is, when human addicts relapse, the drug is either readily available or it can be acquired. Thus, we re-evaluated the impact of acute sleep deprivation on responding when drug was present using a PR schedule of reinforcement. As was evident with FR testing during acquisition, the high drug-takers worked harder on the PR schedule for drug than did the low drug-takers. In general, despite the increased workload, both the high and the low drug-takers maintained the same number of infusions that they were accustomed to self-administering when working on the FR schedules of reinforcement (i.e., approximately 20 vs. 6 infusions/session, respectively). Interestingly, the low drug-takers maintained this infusion number on the PR schedule even when sleep-deprived. This finding suggests that acute sleep deprivation did not increase break point (i.e., did not increase the apparent perceived incentive value of the drug) for the low drug-takers, and the ratio requirement was, presumably, not a strain for these rats. The high drug-takers, on the other hand, tended to exhibit lower break points when sleep-deprived. This trend toward a decrease in break point could indicate that acute sleep deprivation decreased the perceived value of the cocaine reward for the high drug-takers or, alternatively, that the drive for sleep simply interfered with completing the higher ratio requirements.

We suspect the latter because, on the whole, the evidence suggests that acute sleep deprivation increases, rather than decreases, the drive to self-administer cocaine. First, all sleep-deprived rats (low and high drug-takers) self-administered cocaine at a faster rate than did their non-sleep-deprived counterparts, as indicated by a significant decrease in inter-infusion interval.
Of course, by definition, inter-infusion intervals increase as a function of the number of infusions self-administered across the PR test (i.e., as the number of infusions being self-administered increases, more licks are required/infusion and, thus, more time is required to reach the next infusion). Even so, the facilitative effect of sleep deprivation on inter-infusion interval cannot be fully accounted for by mere differences in the number of infusions administered, because inter-infusion intervals were shorter for sleep-deprived low and high drug-takers even though the total number of infusions obtained was not significantly reduced relative to non-sleep-deprived controls. Second, acute sleep deprivation also served to focus drug-seeking behavior when responding for drug on the PR schedule. Of particular interest is the finding that 4 or 8 h of acute sleep deprivation led to marked goal-directed behavior in the low drug-taking population of rats. In accordance, other data have linked an increase in goal-directed behavior to an increase in motivation (Kuntz et al., 2008). Therefore, the increases in the motivation to self-administer drug, resulting from acute sleep deprivation, evident in both low and high drug-taking rats in the present study, appear to reflect an increase in drive (i.e., as evidenced by an increase in rate of infusion among low and high drug-takers, and an increase in the goal-directed nature of the drug-seeking behavior exhibited by the low drug-takers) rather than an increase in the perceived incentive value of the drug (i.e., as evidenced by a failure to significantly alter PR responding for drug). Again, competing biological motivations appear to explain these effects. When drug is available, the motivation to sleep appears to drive the motivation to take drug at a faster rate and to seek drug more efficiently (i.e., the motivation to sleep and the motivation to seek and take drug coexist, and the animals strive to satisfy the need to take drug so that the need to sleep can then also be satisfied).
The facilitative effect of sleep deprivation on drug-seeking and drug-taking behavior, in the presence of the drug, is consistent with other data showing a sleep-deprivation-induced increase in responding for a reward. Specifically, sleep deprivation also increased the rate of responding for rewarding electrical brain stimulation and lowered the threshold for intracranial self-stimulation (Steiner & Ellman, 1972). In the present case, however, it appeared that the increase in the rate of responding for drug related not only to the drive for the drug, but also to the drive to sleep. An interesting study of night eating syndrome in humans conducted by O’Reardon et al. (2006) may shed some light on these results. Night eating syndrome is an eating disorder characterized by nocturnal awakenings, during which the individual engages in ingestive behaviors. This syndrome may relate to the drug-seeking behaviors we observed in sleep-deprived rats, since it too involves competing biological motivations between the drive to eat and the drive to sleep. Thus, in the case of night eating syndrome, the motivation or desire to eat is so powerful that individuals awaken during the night in order to satisfy their hunger. When individuals suffering from this disorder were asked to keep a diary of the times they woke during the night to eat, and their associated feelings, many subjects failed to report nighttime eating episodes or to record how they were feeling because they began eating almost immediately after awakening and, thereafter, quickly returned to sleep. In essence, the patients did not have time to record their data because they were racing to eat and then rapidly returned to sleep. Similar pressures may have dictated the behavior of our sleep-deprived rats that very rapidly took the “requisite” amount of drug and then promptly fell asleep.

Finally, while acute sleep deprivation may be responsible for the effects discussed here, the possibility must be considered that these effects may not be specific to sleep deprivation, per se. Thus, it may be the case that they are a more general consequence of the creation of a
biological state of deprivation. In fact, it has been shown that acute food deprivation induces reinstatement of heroin and cocaine seeking in rats (Shalev et al., 2000a; Shalev et al., 2000b) and, also, that chronic food restriction augments the central rewarding effects of cocaine in rats (Carr et al., 2000). Thus, rats in need of food, water, or salt, for example, also may rapidly take drug before turning to satisfy the alternative need state. This more general hypothesis remains to be tested.

As previously stated in Chapter 1, 17% of Americans meet the diagnostic criteria for some form of substance dependence (Anthony and Helzer, 1991) and 90% of these individuals will relapse even after prolonged periods of abstinence (DeJong, 1994). Like substance abuse and addiction, sleep deprivation also is ubiquitous in our society. Sixty-five percent of Americans report experiencing symptoms of a sleep problem several nights per week (National Sleep Foundation, 2008 Sleep in America Poll). Finally, the problems associated with each of these conditions are greatly exacerbated when these two diagnoses intersect. For example, in the rat population, high responders in the locomotor task (i.e., those likely to be high drug-takers) exhibited greater amounts of wakefulness and less slow wave sleep compared to low responders (Bouyer et al., 1998). Similarly, abstinence and withdrawal in humans are associated with difficulty sleeping and frank insomnia (Malcolm et al., 2007) and it is now clear that sleep deprivation causes relapse in humans (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994). Along with these reports, the present data show that even acute sleep deprivation markedly increases the rate of drug-taking in low and high drug-takers and the sharpening of goal-directed behavior in the rats that, otherwise, would not exhibit goal-directed behavior (i.e., in rats with a history of low drug-taking). Given that humans suffer primarily from chronic, rather than acute, sleep deprivation, future studies must examine the effect of both
acute and chronic sleep deprivation on acquisition, maintenance, and reinstatement of drug-seeking and drug-taking behavior, and must begin to examine the underlying neural correlates. In addition, it will be important to assess the impact of both acute and chronic sleep deprivation using a CNS depressant, such as an opiate (e.g., heroin or morphine). Finally, given the prevalence of chronic sleep deprivation in the adolescent population (Carskadon, 1990) and the adolescent’s propensity for drug-taking behavior, future studies also must examine the impact of chronic sleep deprivation on both acquisition and reinstatement of drug-taking behavior in the adolescent rat. The study outlined in Chapter 3 begins by assessing the impact of chronic sleep deprivation on responding for cocaine in the adult rat.
Chapter 3

Chronic sleep restriction enhances the acquisition of cocaine self-administration on both fixed ratio and progressive ratio schedules of reinforcement.

The majority of the scientific literature on sleep deprivation (including the studies discussed above) focuses on acute sleep deprivation (i.e., a discrete block of sleep deprivation usually 1-24 h in duration). While these studies have been extremely important in identifying the cognitive, physical, and behavioral effects resulting from sleep deprivation, the problem of sleep deprivation in the United States is largely one of chronicity (National Sleep Foundation, 2008). Thus, even though acute sleep loss (e.g., “pulling an all-nighter” to finish a work- or school-related project, staying out late on the weekends, etc.) is troublesome, the majority of Americans report maintaining 6 h of sleep or less per night (a 25% reduction of the recommended 8 h) over the course of months, or even years (Hale and Do, 2007; Jean-Louis et al., 2000). Therefore, a model of chronic sleep restriction is more appropriate for the investigation of the impact of sleep deprivation on substance abuse and addiction. Unfortunately, a limited number of studies have employed the chronic sleep restriction method and none have assessed the impact of chronic sleep deprivation on responding for abused substances. Animal studies have shown that chronic sleep restriction has adverse effects on immune function and stress reactivity. Specifically, rats exposed to chronic sleep restriction exhibited a suppressed HPA stress response to novel (Meerlo et al., 2002) and conditioned (Novati et al., 2008) stressors, as well as immunosuppression (Zager et al., 2007). It is thought that these neuroendocrine effects are largely due to a reduced sensitivity of serotonin-1A receptors, similar to what is seen in depression (Meerlo et al., 2008 Novati et al., 2008; Roman et al., 2005b). In addition, chronic sleep restriction also has been shown to decrease the proliferation of neurons in the hippocampus (Roman et al., 2005a).
As discussed in Chapter 2, the clinical literature suggests that sleep deprivation is another factor that can induce relapse and, as described, both subjective (self-administered questionnaire scores) and objective (polysomnographic sleep parameters) measures of poor sleep quality have been shown to predict relapse in humans (Brower, 2001; Clark et al., 1998; Foster and Peters, 1999; Gillin et al., 1994). In addition, we have demonstrated that acute sleep deprivation increases the rate and efficiency of cocaine self-administration during PR testing, even in rats that maintain low levels of drug intake (i.e., low drug-takers). However, Chapter 3 will evaluate the effects of chronic sleep deprivation, akin to that experienced by humans, on the acquisition of cocaine-seeking and cocaine-taking behaviors in a rodent model.

METHODS

Subjects

This study was conducted in two replications. The subjects were 20 (n=10 for Replication 1 and n=10 for Replication 2) naïve, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC), approximately three months of age at the beginning of the experiment. Due to complications during surgery, four rats were eliminated from the study, leaving 16 for behavioral training and experimental testing (described below). Except where otherwise noted, the rats were housed individually in standard wire mesh cages, in a colony room with temperature, humidity, and ventilation controlled automatically. The rats were maintained on a 12-h light/dark cycle, with lights on at 0700 h. They were allowed ad lib access to food (Harlan Teklad, Madison, WI) and water, except where otherwise noted.
**Catheter Construction and Surgical Procedures**

*Self-administration catheter.* Intra-jugular catheters were custom-made in our laboratory as described by Twining et al. (2009).

*Catheter and EEG/EMG electrode implantation.* Rats were anesthetized and catheters were implanted into the jugular vein, as described by Twining et al. (2009). Immediately thereafter, EEG and EMG recording electrodes were implanted as described by Fang and Fishbein (1996). Briefly, four stainless steel electrodes were implanted in the frontal and parietal bones for EEG recording, and three EMG recording electrodes made of stainless steel wire were inserted into the dorsal muscle of the neck. The electrodes and attached wires were fixed to the skull with dental cement. Following surgery, rats were allowed at least two weeks to recover. General maintenance of catheter patency involved daily examination and flushing of catheters with heparinized saline (0.2 ml of 30 IU/ml heparin). Catheter patency was verified, as needed, using 0.2 ml of propofol (Diprivan 1%) administered intravenously.

**Chronic Sleep Restriction Apparatus**

Chronic sleep restriction was conducted in special chambers that implement a modification of the treadmill method (the disc treadmill method) developed by the Fang laboratory (Pennsylvania State University College of Medicine, Department of Psychiatry; see Figure 3.1). These chambers consist of an open-top and open-bottom Plexiglas cylinder (35.0 cm in diameter and 45.0 cm high) and a chamber bottom that is attached to a bidirectional motor. The cylinder is suspended above the chamber bottom filled with corncob bedding (Harlan...
Teklad, Madison, WI), so that the two do not turn concurrently. A metal panel (37.5 cm in diameter and 5.0 cm high) is attached to the bottom of the cylinder that divides the chamber into two equal parts. The animals can cross this panel easily and are free to occupy either side of the chamber.

![Figure 3.1: Chronic sleep restriction chamber.](image)

**Cocaine Self-Administration Apparatus**

Each rat was trained in one of twelve identical operant chambers (MED Associates, St. Albans, VT) described by Twining et al. (2009). Each chamber measured 30.5 cm in length × 24.0 cm in width × 29.0 cm in height, and was individually housed in a light- and sound- attenuated cubicle. The chambers consisted of a clear Plexiglas top, front, and back wall. The
side walls were made of aluminum. Grid floors consisted of nineteen 4.8-mm stainless steel rods, spaced 1.6 cm apart (center to center). Each chamber was equipped with three retractable sipper spouts that entered through 1.3-cm diameter holes, spaced 16.4 cm apart (center to center). A stimulus light was located 6.0 cm above each tube. Each chamber also was equipped with a houselight (25 W), a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN), and a speaker for white noise (75 dB). Cocaine reinforcement was controlled by a lickometer circuit that monitored empty spout licking to operate a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT). A coupling assembly attached the syringe pump to the catheter assembly on the back of each rat and entered through a 5.0-cm diameter hole in the top of the chamber. This assembly consisted of a metal spring attached to a metal spacer with Tygon tubing inserted down the center, protecting passage of the tubing from rat interference. The tubing was attached to a counterbalanced swivel assembly (Instech, Plymouth Meeting, PA) that, in turn, was attached to the syringe pump. Events in the chamber and collection of data were controlled on-line with a Pentium computer that used programs written in the Medstate notation language (MED Associates).

**Drug Preparation**

Individual 20-ml syringes were prepared for each self-administration chamber prior to each daily session by diluting 4.0 ml of cocaine HCl stock solution (1.24 g cocaine HCl + 150 ml saline) with 16.0 ml of heparinized saline (0.1 ml 1000 IU heparin/60.0 ml saline) for a dose of 0.33 mg/infusion.
Data Collection

Chronic sleep restriction, habituation training, self-administration training, and progressive ratio testing were conducted during the light phase of the light/dark cycle.

Habituation Procedure and Spout Training

Prior to the beginning of the self-administration training, the rats were moved from the wire mesh cages to the chronic sleep restriction chambers (hereafter referred to as the home cage), where they remained for the duration of the study. They were then habituated to the operant chambers for 1 h/day for three days. During this time, each rat was maintained on a water-deprivation regimen in which they received 1-h daily access to water in the operant chamber from the right spout during the habituation session and 25.0 ml of water in the chronic sleep restriction chamber overnight. Thereafter, rats were returned to ad lib access to water for the duration of the study.

Chronic Sleep Restriction

Immediately following the three-day habituation phase, EEG and EMG thresholds for sleep were established and adjusted for each individual animal via test recordings. EEG and EMG signals were fed into Grass NeuroData (Model 15) amplifiers through cable and computator systems. The signals were amplified and properly filtered. The EEG and EMG signals were then digitized at 128 Hz and saved to the hard drive under the control of a computer
program, as described by Fang et al. (1997). Chronic sleep restriction occurred in two 4-day cycles, with two recovery days occurring between each cycle. One group (CSR; n=8) was deprived of approximately 30% of their daily sleep, roughly evenly distributed across a 24 h period. Another group (NSR; n=8) served as chamber-matched controls. The EEG and EMG signals of the rats were continuously recorded, also as described by Fang et al. (1997). The entire sleep restriction period was divided into multiple 120-min blocks. CSR animals were allowed to sleep for 60% of their light phase baseline sleep time and 20% of their dark phase baseline sleep time. EEG and EMG information were computed and updated every two seconds. Once the computer program detected the onset of non-rapid eye movement (NREM) sleep (high amplitude EEG and low amplitude EMG) in a CSR animal, the chamber bottom rotated (one-second pulses every two seconds at a rate of 6.0 RPM) until the animal awakened. Rats were awakened as they felt the rotation of the chamber bottom or as they came in contact with the suspended panel in the chamber. The total number of times the motor turned on was matched between the CSR and control (i.e., NSR) groups. For the control rats, the rotations of the chamber bottoms were dispersed throughout the last 12 min of each 120-min recording block, providing ample time for sleep. The results of chronic sleep restriction were verified by visual scoring of sleep stages in 10-sec segments and EEG power spectra were calculated, both as described previously (Fang and Fishbein, 1996; Fang et al., 1997).

**Self-Administration Training Procedure**

Self-administration training began immediately following the three-day habituation phase, and was conducted concurrently with chronic sleep restriction. Each rat was trained
during daily 90-min fixed ratio sessions for six days (two cycles of three fixed ratio training days separated by one progressive ratio test day and two recovery days). See Figure 3.2 for a summary of behavioral training and experimental testing. Specifically, rats were placed in the

**Figure 3.2:** Timeline of behavioral training and experimental testing.

<table>
<thead>
<tr>
<th>Habituation</th>
<th>Baseline</th>
<th>FR5</th>
<th>PR</th>
<th>Recovery</th>
<th>FR5</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>1 day</td>
<td>3 days</td>
<td>1 day</td>
<td>2 days</td>
<td>3 days</td>
<td>1 day</td>
</tr>
</tbody>
</table>

Chronic Sleep Restriction

operant chambers in darkness. Immediately upon initiation of the 90-min session, the white noise was turned on, two empty spouts advanced into the chamber, and the cue light above the active spout was illuminated. The right spout was termed the “active” spout, while the left spout was termed the “inactive” spout. A fixed ratio (FR) 5 schedule of reinforcement was implemented. During this time, completion of 5 licks on the “active” spout was followed by a single intravenous (i.v.) infusion of 0.33 mg cocaine over six seconds. Drug delivery was signaled by offset of the stimulus light, retraction of the “active” spout, and onset of the tone and houselight. The tone and houselight remained on for a 20-sec timeout period. Responding on the “inactive” spout was without consequence throughout each session. Following each self-administration training session, the rats were returned to their home cages. Latency to the first infusion, infusion number, and goal-directed behavior (calculated by subtracting the total number of inactive responses from the total number of active responses) were evaluated.
Progressive Ratio Testing

In addition to fixed ratio training, a progressive ratio (PR) schedule of reinforcement was implemented to test the impact of chronic sleep restriction on the rats’ willingness to work for drug. Thus, following the third day of FR training in each training block, PR testing was conducted (see Figure 3.1). During PR testing, rats were placed in the operant chambers with conditions identical to those of self-administration training, except the number of active responses required to receive each infusion progressively increased by a multiple of five for up to ten infusions (1, 1+5=6, 6+10=16, 16+15=31, 31+20=51, 51+25=76, 76+30=106, 106+35=141, 141+40=181, 181+45=226). Thereafter, the number of required responses increased by 50 for each successive infusion (226+50=276, 276+50=326, 326+50=376, etc.; see Chapter 2 and Puhl et al., 2009). During this PR session, rats were allowed to self-administer cocaine (0.33 mg/infusion) until a period of 30 min elapsed without receipt of an infusion. Break point (the highest ratio completed) and goal-directed behavior were measured.

Data Analysis

All data were analyzed with Statistica (Version 9, StatSoft, Tulsa, OK) using repeated measures analysis of variance (ANOVA) tests, as well as Student’s t-tests. Newman-Keuls post hoc tests were conducted on significant ANOVAs, when appropriate, with \( \alpha \) set at 0.05.
RESULTS

*C*ho*nic* Sleep *R*estriction

*Time spent in wakefulness, NREM sleep, and REM sleep.* Time spent in wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep was analyzed during the single baseline recording day and during the six complete 24-hr recording days concurrent with self-administration training. Student’s t-tests revealed that the NSR and CSR groups did not differ in time spent in wakefulness, NREM sleep, or REM sleep during baseline recording (ps > 0.05; see Figure 3.3, left panel). However, CSR rats spent significantly more time in wakefulness and significantly less time in NREM sleep during self-administration training than NSR rats (ps < 0.05; see Figure 3.3, right panel). Also, while time spent in wakefulness, NREM sleep, and REM sleep did not differ between baseline recording and self-administration training for the NSR group, CSR rats spent significantly more time in wakefulness and significantly less time in NREM sleep during self-administration training compared to baseline (ps < 0.03). These data show that CSR rats were chronically sleep-deprived during self-administration training, while NSR rats were not.
Figure 3.3: Mean (+/- SEM) time spent in wakefulness, NREM sleep, and REM sleep. Left panel. Mean (+/- SEM) time spent in wakefulness, NREM sleep, and REM sleep during baseline recording. Open bars represent wakefulness, single-hatched bars represent NREM sleep, and cross-hatched bars represent REM sleep. Right panel. Mean (+/- SEM) time spent in wakefulness, NREM sleep, and REM sleep during the six complete days of 24-hr recording during self-administration training. Open bars represent wakefulness, single-hatched bars represent NREM sleep, and cross-hatched bars represent REM sleep. * denotes statistical significance (p < 0.05) between the NSR and CSR group during self-administration training. # denotes statistical significance (p < 0.03) for the CSR group between baseline and self-administration training.

Cumulative time differences in wakefulness and NREM sleep. In addition, the cumulative time differences in wakefulness and NREM sleep between baseline recording and the six complete 24-hr recordings days during self-administration training were calculated. Student’s t-tests revealed that CSR rats spent significantly more time in wakefulness and
significantly less time in NREM sleep across self-administration training days than NSR rats (ps < 0.05; see Figure 3.4). These data indicate that CSR rats cumulatively lost approximately 10 h of NREM sleep throughout self-administration training, while NSR rats lost none.

Figure 3.4: Mean (+/- SEM) cumulative differences in time spent in wakefulness and NREM sleep between baseline recording and the six complete 24-h recording days during self-administration training in NSR (left panel) and CSR (right panel) rats. Open bars represent wakefulness and hatched bars represent NREM sleep. * denotes statistical significance (p < 0.05).

Cocaine Self-Administration

Latency to first infusion. A 2 × 6 repeated measures ANOVA varying group (NSR or CSR) and trials (1-6) conducted on the latency to self-administer the first infusion across trials
failed to reach statistical significance for all parameters (group: F(1, 14)=4.51, p=0.05; trials: F < 1; group × trials: F(5, 70)=1.70, p=0.14). However, a Student’s t-test revealed a significant difference between the NSR and CSR groups (p < 0.05; see Figure 3.5), indicating that CSR rats were faster to self-administer the first infusion of cocaine during FR training than NSR rats.

**Figure 3.5**: Mean (+/− SEM) latency (s) to self-administer the first infusion during FR training. The open bar represents the NSR group and the hatched bar represents the CSR group. * denotes statistical significance (p < 0.05).

**Number of infusions.** A 2 × 6 repeated measures ANOVA varying group (NSR or CSR) and trials (1-6) conducted on the number of infusions self-administered across trials also failed to reach statistical significance for all parameters (group: F(1, 14)=1.69, p=0.21; trials: F(5,
Again, a Student’s t-test revealed a significant difference between the NSR and CSR groups (p < 0.01; see Figure 3.6), indicating that CSR rats self-administered more cocaine during FR training than NSR rats.

**Goal-directed behavior.** A 2 × 6 repeated measures ANOVA varying group (NSR or CSR) and trials (1-6) conducted on goal-directed behavior revealed a significant main effect of trials, F(5, 70)=2.48, p < 0.05, however, main effect of group, F(1, 14)=1.27, p=0.28, and group × trials interaction, F(5, 70)=1.13, p=0.35, did not attain statistical significance. Finally, a

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**Figure 3.6:** Mean (+/- SEM) number of cocaine infusions self-administered during FR training. The open bar represent the NSR group and the hatched bar represents the CSR group. * denotes statistical significance (p < 0.01).
Student’s t-test revealed a significant difference between the NSR and CSR groups (p < 0.05; see Figure 3.7), indicating that CSR rats were more focused on the cocaine-associated spout during FR training than NSR rats.

**Figure 3.7:** Mean (+/- SEM) goal-directed responding (total active responses minus total inactive responses) during FR training. The open bar represents the NSR group and the hatched bar represents the CSR group. * denotes statistical significance (p < 0.05).

**Progressive Ratio Testing**

*Break point.* Consistent with the FR data, a 2 × 2 repeated measures ANOVA varying group (NSR or CSR) and trials (1-2) conducted on the break point failed to reach statistical significance for all parameters (group: F(1, 14)=4.11, p=0.06; trials: F(1, 14)=1.83, p=0.20;
group × trials: F < 1), while a Student’s t-test revealed a significant difference between the NSR and CSR groups (p < 0.01; see Figure 3.8). These results indicate that CSR rats were willing to

![Bar chart showing mean PR infusions for NSR and CSR groups.](image)

**Figure 3.8**: Mean (+/- SEM) cocaine infusions self-administered during PR testing. The open bar represents the NSR group and the hatched bar represents the CSR group. * denotes statistical significance (p < 0.01).

work harder for drug than NSR rats.

**Goal-directed behavior.** Likewise, a 2 × 2 repeated measures ANOVA varying group (NSR or CSR) and trials (1-2) conducted on goal-directed behavior also failed to reach statistical significance for all parameters (group: F(1, 14)=2.89, p=0.11; trials: F(1, 14)=2.38, p=0.15; group × trials: F(1, 14)=2.26, p=0.15). However, again, a Student’s t-test revealed a significant difference between the NSR and CSR groups (p < 0.05; see Figure 3.9), indicating that CSR rats
Figure 3.9: Mean (+/− SEM) goal-directed responding (total active responses minus total inactive responses) during PR testing. The open bar represents the NSR group and the hatched bar represents the CSR group. * denotes statistical significance (p < 0.05).

were more focused on the cocaine-associated spout during PR training than NSR rats.

DISCUSSION

These findings are consistent with the results of the study described in Chapter 2. Not only does the current study support those previous findings, but it also furthers our knowledge of the interaction between sleep deprivation and substance abuse. While, Chapter 2 investigated the effects of acute sleep deprivation on cocaine-induced reinstatement and PR responding
subsequent to FR training and acquisition, the current study explored the effects of chronic sleep restriction (more akin to the type of sleep deprivation experienced by humans) imposed concurrently with acquisition of self-administration training. As such, we were able to directly investigate the effects of sleep deprivation on acquisition of drug-seeking and drug-taking behaviors. Thus, these data are the first to demonstrate that chronic sleep restriction (approximately 30% sleep loss over the course of eight days separated by a two-day recovery period) greatly enhances the rate and quantity of cocaine self-administration and goal-directed responding using both FR and PR schedules of reinforcement early in training.

These results are not surprising given the neurochemical consequences of sleep deprivation discussed in Chapter 1. Namely, sleep deprivation has been shown to augment DA activity in the mesocorticolimbic reward system (Asakura et al., 1992; Brock et al., 1995; Farooqui et al., 1996; Hernández-Peón et al., 1969). This augmentation is indicated by increases in DA levels (Hernández-Peón et al., 1969), increases in DA metabolite levels (Asakura et al., 1992; Farooqui et al., 1996), increases in DA receptor densities (Brock et al., 1995; Demontis et al., 1990; Hamdi et al., 1993), and decreases in the affinity for DA at reuptake sites (Hamdi et al., 1993). Accordingly, sleep deprivation also has been shown to increase sensitivity to DA agonists (Nunes et al., 1994; Tufik, 1981). Along with the current data, these studies suggest that sleep deprivation may augment the perceived incentive reward value of cocaine by enhancing and prolonging the effects of DA in the brain’s reward circuit. It remains to be seen whether these results can be generalized to natural rewards (e.g., saccharin or sucrose), which also are processed by the mesocorticolimbic DA system. In support of such a possibility, obesity has recently been linked to disrupted sleep patterns in adolescents (Shaikh et al., 2009) and
adults (Adámková et al., 2009; Cizza et al., 2010). Thus, chronic sleep deprivation may augment the perceived reward value of food as well as drug.

Admittedly, the current study employed a relatively easy FR schedule of reinforcement (four times lower than the terminal schedule employed during the previous study). As such, it could be argued that the results obtained were a function of the ease of acquiring drug. However, this explanation seems unlikely for two reasons. First, while the FR schedule was relatively easy, NSR rats still failed to acquire self-administration behaviors within 6 sessions (i.e., they self-administered very low levels of drug). Also, during PR testing, CSR rats were, on average, making nearly 400 responses to receive a single cocaine infusion (with some rats making well over 1000 responses for a single infusion), indicating that they were very willing to work for drug. In addition, the disk treadmill method of chronic sleep restriction employed by the current study primarily affects NREM sleep, leaving REM sleep relatively undisturbed (see Figure 3.3). Therefore, it remains to be seen whether NREM and REM sleep deprivation affect the acquisition of drug seeking and drug taking differently.

In summary, these findings, in conjunction with previous work (Puhl et al., 2009), suggest that sleep deprivation augments the acquisition of cocaine seeking and cocaine taking. With substance abuse and sleep deprivation rampant in our society, these results have important implications for the prevention and treatment of addiction in humans. This is the first study to directly investigate the behavioral influences of sleep deprivation on acquisition of drug self-administration. As such, it offers a valuable animal model that can be used to further investigate the effects of chronic sleep restriction on drug-seeking and drug-taking behaviors. Specifically, the effects of chronic sleep restriction on the maintenance and reinstatement of drug seeking and drug taking remain unknown. In addition, the effects of chronic sleep restriction on the intake of
natural rewards (e.g., saccharin and sucrose) have not been investigated, although the link between poor sleep and obesity is beginning to be explored in humans. Also, it will be important to assess the impact of acute sleep deprivation and chronic sleep restriction using a CNS depressant, such as an opiate (e.g., heroin or morphine). Finally, recent evidence suggests that sleep problems in adolescence can predict substance abuse (Wong et al., 2010). Given the prevalence of chronic sleep deprivation in the adolescent population (Carskadon, 1990; Eaton et al., 2010), future studies also must examine the impact of chronic sleep deprivation on both acquisition and reinstatement of drug-taking behavior in the adolescent rat.
Chapter 4

A history of bingeing on fat enhances cocaine seeking and taking in rats.

Recently, addiction to food (especially foods high in sugar and fat) has emerged as a topic of interest. Of particular concern are intermittent, excessive, dysfunctional appetitive behaviors such as binge eating (National Institutes of Health, 2004). As discussed in Chapter 1, binge eating is operationally defined as the consumption of more food, in a brief amount of time, than most individuals would consume in a similar time period and under similar circumstances (American Psychiatric Association, 2000). Likewise, substance dependence is operationally defined as compulsive seeking and use or consumption of a substance, in lieu of all other activities, and despite adverse consequences (American Psychiatric Association, 2000). Thus, both disorders are characterized by a loss of control over consummatory behaviors that is thought to result in long-lasting neuronal alterations. This may contribute to high comorbidity rates among these conditions (Conason et al., 2006; Hudson et al., 2007), particularly among individuals suffering from alcohol and cocaine dependence (Brewerton et al., 1995; Bulik et al., 2002; Bushnell, 1994; Johnson et al., 1997; Jonas et al., 1987; Wiederman and Pryor, 1996; Wilson, 1993). In addition, expression of bulimic symptoms has been shown to predict the onset of Alcohol Use Disorder (Franko et al., 2005).

As such, the possibility that one maladaptive behavior (e.g., compulsive food intake) may serve as a gateway for the development of the other (e.g., drug addiction) has been proposed. A similar progression in use of increasingly serious substances of abuse has been characterized (Degenhardt et al., 2008). Substantial behavioral evidence linking either sugar or fat intake with the intake of drugs of abuse lends support to this idea. For example, a correlation between
preference for sweets and drugs of abuse has been identified in humans (Pelchat, 2002). In rats, diets promoting sucrose consumption have been shown to enhance behavioral sensitization to amphetamine (Avena and Hoebel, 2003) and cocaine (Gosnell et al., 2005), as well as increase the self-administration of amphetamine (DeSousa et al., 2000), cocaine (Gosnell et al., 2000), and the consumption of ethanol (Avena et al., 2004). Furthermore, abrupt discontinuation of diets promoting sucrose consumption has been shown to produce opiate-like withdrawal symptoms (Colantuoni et al., 2002) and to exacerbate the expression of morphine withdrawal (Schoenbaum et al., 1990). Likewise, diets promoting fat consumption have been shown to increase ethanol consumption (Carrillo et al., 2004; Krahn et al., 1991).

While chronic intake of sugar, fat, and drugs of abuse have been shown to exert similar effects on the mesocorticolimbic dopamine system (see Chapter 1), it is still unknown whether it is intake, per se, or loss of control over intake that result in those alterations. For example, do addicts simply have “addictive personalities” that predispose them to “addiction-like” behaviors in many venues (e.g., eating, drinking, drug taking, gambling), or does experience with one type of addiction change the brain, thereby making the individual more prone to other addictions? To shed some light on this “nature versus nurture” debate, the study described in Chapter 4 was designed to systematically and operationally investigate whether loss of control over the intake of fat will predispose subjects to the loss of control over the self-administration of a drug of abuse, in this case, cocaine. To do so, we employed two separate behavioral paradigms. The first was the limited access protocol developed by Corwin et al. (1998), which was used to promote fat-bingeing behaviors. The second paradigm employed was the extended-access drug self-administration protocol described by Deroche-Gamonet et al. (2004) which assesses the compulsive drug-seeking and -taking behaviors that result from chronic drug exposure, such as
persistent responding for drug during periods of signaled non-availability (SNA) and the motivation to and preoccupation with obtaining and consuming drug during progressive ratio (PR) testing. Concomitant use of these two paradigms allows for the direct assessment of the loss of control over fat intake and possible subsequent loss of control over cocaine intake. We hypothesized that rats with the most restricted access to the high-fat diet would develop fat-bingeing behaviors and, consequently, that rats with a history of fat-bingeing would exhibit more robust “addiction-like” behaviors toward cocaine.

METHODS

Subjects

This study was conducted in two replications. The subjects were 81 (n=36 for Replication 1 and n=53 for Replication 2) naïve, male Sprague-Dawley rats (Harlan, Indianapolis, IN), 60 days of age at the beginning of the experiment. Due to complications during surgery, four rats were eliminated from the study. An additional eight rats were eliminated due to loss of catheter patency, leaving 69 rats for self-administration training and experimental testing (described below). All rats were housed individually in hanging wire mesh cages in a colony room with temperature, humidity, and ventilation controlled automatically. They were allowed ad lib access to a nutritionally complete commercial laboratory rodent chow (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN; percent of calories as protein: 28.05%, fat: 12.14%, carbohydrate: 59.81%; 3.3 kcal/g) and water, except where otherwise noted. In addition, the rats were maintained on a 12-h light/dark cycle, with lights on at 0700 h.
Limited Access Protocol

Prior to the beginning of the experiment, all rats (n=69) underwent a one-week adaptation period, during which they were given ad lib access to chow and water. They were then given overnight access to an optional source of dietary fat (Crisco® All-Vegetable shortening, J. M. Smucker Co., Orrville, OH). Then, using the limited access protocol developed by Corwin et al. (1998), rats were maintained on a nutritionally complete diet of chow and water throughout. In addition, they were then given varying degrees of restricted access to the same optional source of dietary fat. Rats were maintained on one of four dietary protocols for a period of six weeks: no access to the optional fat (Chow; n=9), continuous access to the optional fat (Ad Lib; n=19), daily 1-h access to the optional fat (Daily; n=20), or 1-h access to the optional fat on Monday, Wednesday, and Friday (MWF; n=21), and groups were counterbalanced on the basis of their body weight, three-day chow intake, and initial overnight fat intake. Body weight and intake of shortening (1-h and 24-h) were measured. Immediately following this six-week period, all rats were transported to the Grigson laboratory in the Department of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, where they remained for the duration of the study. From this point until the completion of the study, access to the optional fat was no longer provided.

Catheter Construction and Implantation

Self-administration catheter. Intra-jugular catheters were custom-made in our laboratory as described by Twining et al. (2009).
**Catheter implantation.** Rats were anesthetized and catheters were implanted into the jugular vein as described by Twining et al. (2009). Following surgery, rats were allowed at least two days to recover. General maintenance of catheter patency involved daily examination and flushing of catheters with heparinized saline (0.2 ml of 30 IU/ml heparin). Catheter patency was verified, as needed, using 0.2 ml of propofol (Diprivan 1%) administered intravenously.

**Apparatus**

Each rat was trained in one of twelve identical operant chambers (MED Associates, St. Albans, VT) described by Grigson and Twining (2002) and Twining et al. (2009). Each chamber measured 30.5 cm in length × 24.0 cm in width × 29.0 cm in height, and was individually housed in a light- and sound-attenuated cubicle. The chambers consisted of a clear Plexiglas top, front, and back wall. The side walls were made of aluminum. Grid floors consisted of nineteen 4.8-mm stainless steel rods, spaced 1.6 cm apart (center to center). Each chamber was equipped with three retractable sipper spouts that entered through 1.3-cm diameter holes, spaced 16.4 cm apart (center to center). A stimulus light was located 6.0 cm above each tube. Each chamber was also equipped with a houselight (25 W), a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN), and a speaker for white noise (75 dB). Cocaine reinforcement was controlled by a lickometer circuit that monitored empty spout licking to operate a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT). A coupling assembly attached the syringe pump to the catheter assembly on the back of each rat and entered through a 5.0-cm diameter hole in the top of the chamber. This assembly consisted of a metal spring attached to a metal spacer with Tygon tubing inserted down the center, protecting passage of the tubing from
rat interference. The tubing was attached to a counterbalanced swivel assembly (Instech, Plymouth Meeting, PA) that, in turn, was attached to the syringe pump. Events in the chamber and collection of data were controlled on-line with a Pentium computer that used programs written in the Medstate notation language (MED Associates).

**Drug Preparation**

Individual 20-ml syringes were prepared for each rat prior to each daily session by diluting cocaine HCl stock solution (1.24 g cocaine HCl + 150 ml saline) with heparinized saline (0.1 ml 1000 IU heparin/60.0 ml saline) for a dose of 0.8 mg/kg, the same dose used by Deroche-Gamonet et al. (2004).

**Data Collection**

Habituation, self-administration training, and progressive ratio testing were conducted during the light phase of the light/dark cycle.

**Habituation Procedure and Spout Training**

Rats were water-deprived for approximately 16 h and then were habituated to the operant chambers during a single 15-min session the day before the beginning of self-administration training. During this session, water was available in the right (“active”) spout within the operant
chamber, while the left ("inactive") spout was empty. Thereafter, rats were returned to ad lib access to water for the duration of the study.

**Self-Administration Training Procedure**

Self-administration training began immediately following the habituation phase. Each rat was trained during daily 150-min sessions, as described by Deroche-Gamonet et al. (2004), for 39 days (see Figure 4.1b). Each 150-min session consisted of three drug periods, separated by two signaled non-availability (SNA) periods (see Figure 4.1a). Specifically, rats were placed in the operant chambers in darkness. Immediately upon initiation of the 150-min session, the white noise was turned on, the right and left empty spouts advanced into the chamber, and the cue light above the right spout was illuminated. Rats were then allowed to self-administer cocaine (0.8 mg/kg) for 40 minutes. The right spout was termed the “active” spout, while the left spout was

![Figure 4.1: Overview of daily fixed ratio (FR) training sessions and timeline of behavioral training and experimental testing. a) Each daily 150-min FR training trial was divided into three 40-min active drug periods that were separated by two 15-min signaled drug non-availability (SNA) periods. b) During the 39 days of training, FR1 (Trials 1-3), FR5 (Trials 4-22), and FR20 (Trials 23-39) schedules of reinforcement were used. Progressive ratio (PR) tests were conducted intermittently (approximately every 7 days).](image-url)
termed the “inactive” spout. A 15-min SNA period followed the 40-min drug period, during which the cue light above the right spout was turned off, a light on the chamber wall opposite the spouts was illuminated, and the infusion pump was turned off. Responding on the “active” spout was without consequence during SNA periods. During the 40-min drug periods, a fixed ratio (FR) 1 schedule of reinforcement was implemented initially (Trials 1-3), whereby completion of a single lick on the “active” spout was followed by a single intravenous (i.v.) infusion of cocaine over six seconds. Drug delivery was signaled by offset of the stimulus light and onset of the tone and houselight. The tone and houselight remained on for a 20-sec timeout period. Responding on the “inactive” spout was without consequence throughout each 150-min session. The reinforcement schedule was increased to FR5 (Trials 4-22) and then to FR20 (Trials 23-39) to fully distinguish between active and inactive responding. Following each self-administration training session, the rats were returned to their home cages. The number of infusions self-administered during drug periods was evaluated throughout self-administration training. In addition, terminal responding (i.e., responding across FR20 trials) also was assessed. Thus, the number of infusions self-administered and terminal goal-directed behavior (calculated by subtracting the total number of inactive responses made from the total number of active responses made) were evaluated across FR20 trials. Responding was evaluated similarly and independently across the intervening SNA periods.

**Progressive Ratio Testing**

In addition to fixed ratio training, a progressive ratio (PR) schedule of reinforcement, as described by Deroche-Gamonet et al. (2004), was implemented intermittently to test the impact
of a history of fat bingeing on the rats’ willingness to work for the drug. Thus, PR testing was conducted approximately every seven days (see Figure 4.1b). During PR testing, rats were placed in the operant chambers with conditions identical to those of self-administration training, except the number of active responses required to receive the first infusion started at 10 and then progressively increased by a multiple of 10 (except for the third infusion where there is only an increase of 5) every third infusion (10, 10, 10+5=15, 15, 15+10=25, 25, 25+10=35, etc.). During this PR session, rats were allowed to self-administer cocaine (0.8 mg/kg) until a period of 30 min elapsed without receipt of an infusion. Terminal (i.e., during the final PR test) break point (the highest ratio completed) and terminal goal-directed behavior were measured. This PR test and the dose of cocaine employed are in keeping with those used by Deroche-Gamonet et al. (2004).

Data Analysis

All data were analyzed with Statistica (StatSoft, Tulsa, OK) using repeated measures and one-way analysis of variance (ANOVA) tests. Newman-Keuls post hoc tests were conducted on significant ANOVAs, when appropriate, with \( \alpha \) set at 0.05.

RESULTS

Fat bingeing

Initial overnight fat intake. Prior to the beginning of the experiment, all groups (Chow, Ad Lib, Daily, and MWF) were matched on the basis of their initial overnight shortening intake.
Accordingly, a one-way ANOVA revealed no significant differences between groups, $F(3, 65)=1.12$, $p > 0.05$ (see Figure 4.2).

**Figure 4.2:** Mean (+/- SEM) initial overnight shortening intake. The open bar represents shortening intake in the Chow group, the forward-hatched bar represents shortening intake in the Ad Lib group, the backward-hatched bar represents shortening intake in the Daily group, and the cross-hatched bar represents shortening intake in the MWF group.

*Fat intake.* A one-way ANOVA conducted on terminal (Week 6) intake during the 1-h shortening access period revealed a significant main effect of Group, $F(3, 65)=78.18$, $p < 0.01$ (see Figure 4.3). Post hoc Newman-Keuls tests indicated that the 1-h intake of the Daily group
Figure 4.3: Mean (+/- SEM) 1-h intake during the 1-h shortening access period of Week 6 of access to optional fat. The open bar represents chow intake in the chow only group during the 1-h period in which the other groups had access to shortening, the forward-hatched bar represents shortening intake in the Ad Lib group, the backward-hatched bar represents shortening intake in the Daily group, and the cross-hatched bar represents shortening intake in the MWF group. * and ** denote statistical significance (ps < 0.01).

exceeded that of the Chow and Ad Lib groups (ps < 0.01) and that the 1-h intake of the MWF group was greater than that of all other groups (ps < 0.01). These data indicate that MWF rats (the group with the most restricted access) clearly developed a binge-type loss of control over the intake of fat, consuming more than any of the other groups during the 1-h fat access period. In fact, when analyzing the 24-h intakes of the Ad Lib group, a one-way ANOVA also revealed a significant main effect of Group, F(2, 57)=18.82, p < 0.01 (see Figure 4.4). Post hoc Newman-
Figure 4.4: Mean (+/− SEM) intake of shortening during Week 6 of access to optional fat. The forward-hatched bar represents 24-h shortening intake in the Ad Lib group, the backward-hatched bar represents 1-h shortening intake in the Daily group, and the cross-hatched bar represents 1-h shortening intake in the MWF group. * and ** denote statistical significance (p < 0.01 and ps < 0.02, respectively).

Keuls tests indicated that the 24-h intake of the Ad Lib group was greater than the 1-h intake of the Daily group (p < 0.01) and that the 1-h intake of the MWF group was greater than both the 1-h intake of the Daily group and the 24-h intake of the Ad Lib group (ps < 0.02). These data show that MWF rats consumed more fat in a 1-h period than Ad Lib rats did in a 24-h period. This finding is remarkable, given the fact that the Ad Lib group had access to the optional fat seven days a week, while the MWF group only had access three days a week. Despite the 1-h
and 24-h fat intake data, a one-way ANOVA conducted on overall fat intake (i.e., averaged across the entire six-week period of maintenance on the special dietary protocols) revealed an interesting significant main effect of Group, F(2, 57)=94.08, p < 0.01 (see Figure 4.5). Post hoc Newman-Keuls tests indicated that the total intake of the Daily group was greater than that of the MWF group (p < 0.01) and that the total intake of the Ad Lib group was greater than all other groups (ps < 0.01). These data show that the Ad Lib group consumed the most total fat across the entire six weeks of maintenance on the dietary protocol, while the MWF group consumed the least. This finding may seem contradictory to the other intake data, however, they make sense
given the differing access to optional fat provided to each group. The Ad Lib group had continuous access 24 h per day, seven days per week, while the MWF group only had 1-h access three days per week. In spite of the differences in overall fat consumption, significant differences in body weight did not develop (data not shown). These group differences in overall fat consumption allow us to separate the impact of high fat intake from that of binge intake.

*Escalation in fat intake.* A one-way ANOVA conducted on the difference in 1-h shortening intake from Week 1 to Week 6 of access to optional fat, revealed a significant main effect of Group, F(2, 56)=79.31, p < 0.01 (see Figure 4.6). This measure was used as an index of

![Figure 4.6](image)

**Figure 4.6:** Mean (+/- SEM) change in shortening intake from Week 1 to Week 6 of access to optional fat. The forward-hatched bar represents change in 24-h shortening intake in the Ad Lib group, the backward-hatched bar represents change in 1-h shortening intake in the Daily group, and the cross-hatched bar represents change in 1-h shortening intake in the MWF group. * and ** denote statistical significance (ps < 0.01).

escalation in fat intake. Thus, post hoc Newman-Keuls tests indicated that fat intake escalated more in the Daily group than the Ad Lib group (p < 0.01) and that fat intake escalated more in
the MWF group than all other groups (ps < 0.01). These data demonstrate that intake of fat quickly escalated in rats that were maintained on restricted access diets.

**Cocaine Intake During FR Active Drug Periods**

Upon analysis of the data from the FR5 trials, the results of a 4 × 19 repeated measures ANOVA varying group (Chow, Ad Lib, Daily, or MWF) and trials (4-22) revealed a significant main effect of Trials, F(18, 954)=2.50, p < 0.01. Analysis of the FR20 trials using a 4 × 17 repeated measures ANOVA varying group (Chow, Ad Lib, Daily, or MWF) and trials (23-39) also revealed a significant main effect of trials, F(16, 640)=1.63, p = 0.05, as well as a significant group × trials interaction, F(48, 640)=1.63, p < 0.01. Post hoc Newman-Keuls tests of the two-way interaction, however, yielded no meaningful results.

Overall, a history of fat exposure tended to cause an increase in cocaine self-administration during FR5 trials (i.e., Ad Lib, Daily, and MWF rats tended to self-administer more cocaine than Chow rats; see the center panel of 4.7), even though these results did not reach statistical significance. Moreover, when the schedule of reinforcement was increased to
**Figure 4.7:** Mean (+/- SEM) cocaine infusions self-administered during the 40-min signaled active drug periods. Circles represent the Chow group, squares represent the Ad Lib group, diamonds represent the Daily group, and triangles represent the MWF group.

FR20, those effects were attenuated. However, by the end of FR20 training, the MWF group (the fat-bingeing group) began to emerge as the group self-administering the most cocaine (see the right panel of Figure 4.7). Again, this trend did not attain statistical significance. While differences in the overall intake of cocaine may be intuitively expected, the absence of an effect during FR sessions is consistent with Deroche-Gamonet (2004), where differences in drug intake did not manifest until evaluated with SNA responding and PR breakpoint.
Compulsive Drug Seeking During Terminal SNA Periods

One of the revealing manipulations in the Deroche-Gamonet paradigm was SNA responding. In accordance, throughout FR trials SNA responding was tracked daily. Here we report on SNA responding during the 17 FR20 trials. In a first-pass analysis (here and elsewhere), we compared responding by the Chow, Ad Lib, and Daily groups in a 3 × 17 repeated measures ANOVA varying group (Chow, Ad Lib, or Daily) and trials (23-39). The results of this analysis revealed that the responding of the Chow, Ad Lib, and Daily groups did not differ from one another (F < 1). Therefore, the groups were combined and compared to the MWF group.

SNA Infusion Attempts. Infusion attempts made during FR20 SNA periods were analyzed using a 2 × 17 repeated measures ANOVA varying group (Non-MWF or MWF) and trials (23-39). The results of the analysis revealed a significant main effect of Group, F(1, 43)=6.17, p < 0.02, indicating that the rats in the MWF group made more infusion attempts during the FR20 SNA periods than all other groups (see Figure 4.8). Also, there was a significant main effect of
**Figure 4.8:** Mean (+/- SEM) infusion attempts made during the 15-min signaled non-availability (SNA) periods across FR20 (trials 23-39). The open bar represents the Chow group, the forward-hatched bar represents the Ad Lib group, the backward-hatched bar represents the Daily group, and the cross-hatched bar represents the MWF group. * denotes statistical significance (p < 0.02).

Trials, F(16, 688)=4.24, p < 0.01, as well as a significant Group × Trials interaction, F(16, 688)=2.35, p < 0.01. Post hoc Newman-Keuls tests on the two-way interaction yielded no meaningful results.

**Goal-directed behavior.** Goal-directed responding (total active minus total inactive responses) during FR20 SNA periods also was analyzed using a 2 × 17 repeated measures ANOVA varying group (Non-MWF or MWF) and trials (23-39). The results of the analysis revealed a significant main effect of Group, F(1, 43)=7.12, p < 0.02, indicating that rats in the MWF group focused more attention on cocaine-associated spout during the FR20 SNA periods than the other groups combined (see Figure 4.9). In addition, a significant main effect of Trials, F(16, 688)=4.87, p < 0.01, and a significant Group × Trials interaction, F(16, 688)=1.72, p <
Figure 4.9: Mean (+/- SEM) goal-directed responding (total active responses minus total inactive responses) made during the 15-min signalled non-availability (SNA) periods of the FR20 trials (trials 23-39). The open bar represents the Chow group, the forward-hatched bar represents the Ad Lib group, the backward-hatched bar represents the Daily group, and the cross-hatched bar represents the MWF group. * denotes statistical significance (p < 0.02).

0.05, were also found. Post hoc Newman-Keuls tests on the two-way interaction yielded no meaningful results. Taken together, these data indicate that MWF rats persisted in responding for cocaine, even when it was signaled that cocaine was no longer available.

Motivation to Work for Cocaine During Terminal PR Testing

The willingness to work for cocaine was assessed using a PR schedule of reinforcement. Again, the results of a one-way ANOVA revealed that the Chow, Ad Lib, and Daily groups did not differ from one another (F < 1). Therefore, they were combined and compared to the MWF group.

Break point. A one-way ANOVA conducted on the number of cocaine infusions self-administered during the final PR test (PR test # 6) revealed a significant main effect of Group, F(1, 43)=11.02, p < 0.01, indicating that rats in the MWF group exhibited higher break points (i.e., worked harder) for cocaine during the final PR test than all other groups combined (see Figure 4.10).
Figure 4.10: Mean (+/- SEM) cocaine infusions self-administered during the final PR test. The open bar represents the Chow group, the forward-hatched bar represents the Ad Lib group, the backward-hatched bar represents the Daily group, and the cross-hatched bar represents the MWF group. * denotes statistical significance (p < 0.01).

Goal-directed behavior. Goal-directed responding (total active minus total inactive responses) during the final PR test (PR test # 6) was analyzed using a one-way ANOVA. The results of the analysis revealed a significant main effect of Group, F(1, 43)=9.51, p < 0.01, indicating that rats in the MWF group focused more attention on the cocaine-associated empty spout during the final PR test than all other groups (see Figure 4.11). In addition, a significant Group × Trials interaction, F(5, 215)=4.32, p < 0.01, was also found. Post hoc Newman-Keuls
Figure 4.11: Mean (+/- SEM) goal-directed responding (total active responses minus total inactive responses) made during the final PR test. The open bar represents the Chow group, the forward-hatched bar represents the Ad Lib group, the backward-hatched bar represents the Daily group, and the cross-hatched bar represents the MWF group. * denotes statistical significance (p < 0.01).

Tests on the two-way interaction yielded no meaningful results. Collectively, these data indicate that MWF rats had greater motivation to seek and take drug than all other groups.

“Addiction-like” Behaviors

Similar to Deroche-Gamonet et al. (2004), behavioral measures of cocaine seeking and cocaine taking were used to determine “addiction-like” behavior scores for each animal. First,
all rats were ranked on the basis of the number of responses made on the “active” empty spout during SNA periods of FR20 trials (trials 23-39) and also on the basis of the number of infusions self-administered during the final PR test. Those rats that ranked in the top 33rd percentile for each measure were considered positive for that particular criterion. All rats, then, received an “addiction-like” behavior score of 0, 1, or 2, depending on the number of criteria they met. Nineteen percent of all the animals (13 out of 69) scored a 2. Interestingly, of those animals that scored a 2, 46% were from the MWF group, while only 8% were from the Chow group, 15% were from the Ad Lib group, and 31% were from the Daily group (see Figure 4.12). These data illustrate that a history of bingeing on fat (a naturally rewarding substance) can predispose rats to

![Figure 4.12: Percentages of animals with an “addiction-like” behavior score of 2. The open bar](image-url)
represents the Chow group, the forward-hatched bar represents the Ad Lib group, the backward-hatched bar represents the Daily group, and the cross-hatched bar represents the MWF group.

the expression of “addiction-like” behaviors toward a substance of abuse (in this case, cocaine).

DISCUSSION

Binge eating is operationally defined as the consumption of more food, in a brief amount of time, than most individuals would consume in a similar time period and under similar circumstances (American Psychiatric Association, 2000). As such, binge eating consists of engaging in time-limited bouts of excessive food consumption characterized by subjective loss of control, as well as loss of homeostatic control over an individual meal size. Likewise, substance dependence is operationally defined as compulsive seeking and use or consumption of a substance, in lieu of all other activities, and despite adverse consequences (American Psychiatric Association, 2000). Thus, both disorders are characterized by a loss of control that is thought to result in long-lasting neuronal alterations.

While epidemiological studies in humans are useful for identifying associations between binge eating and substance abuse, the development and use of animal models is critical for systematically identifying the maladaptive behaviors and underlying neural mechanisms involved in the expression of these disorders. The limited access protocol reliably produces binge-type eating of fat in rats (Corwin, 2004; Corwin et al., 1998; Davis et al., 2007; Dimitriou et al., 2000; Thomas et al., 2002; Wojnicki et al., 2008a, b). In addition, under this model, rats exhibit compulsive fat-seeking and -consuming behaviors that are reminiscent of the drug-seeking and -taking behaviors displayed in drug self-administration models. For example, as
shown here, intake of fat escalates to a greater extent in fat-bingeing rats (Corwin et al., 1998; Dimitriou et al., 2000), much like the escalation of intake reported with drugs of abuse that is used as a behavioral indicator of drug addiction (Ahmed et al., 2002). Also, when previously restricted rats are given prolonged access to fat, bingeing rats consume more than controls, similar to the increase in self-administration of cocaine under conditions of prolonged access in “addiction-prone” rats (Deroche-Gamonet et al., 2004). Finally, progressive ratio (PR) responding for fat increases across time (Wojnicki et al., 2006) and fat-bingeing rats exhibit higher breakpoints for fat compared to controls (Wojnicki et al., 2010). This is akin to the escalation of intake and the ultimately higher breakpoints seen when rats are tested under PR schedules of reinforcement for cocaine (Deroche-Gamonet et al., 2004; Roberts et al., 2007).

Consistent with previous findings, the current study demonstrates that restricted access to fat in non-food-deprived rats leads to the development of fat-bingeing behaviors. Not only did the MWF group (the fat-bingeing group) consume more fat than the Chow, Ad Lib, and Daily groups in a given 1-h period, they also consumed more fat in a 1-h period than the Ad Lib group did in an entire 24-h period. Interestingly, a simple history of exposure to a diet high in fat (whether Ad Lib, Daily, or MWF) resulted in the tendency to self-administer more cocaine when the reinforcement schedule was easy (i.e., FR5). Given that foods (especially highly palatable foods, such as those rich in fat and sugar) and drugs of abuse both interact with the same neuroanatomical reward structures and circuitry, the FR5 results are not surprising. However, when shifted to a more difficult reinforcement schedule (e.g., FR20), the MWF group tended to self-administer more cocaine.

This finding is in keeping with other data obtained from this group when challenged for “addiction-like” behaviors. Specifically, the MWF group exhibited high levels of responding for
drug and they focused more exclusively on the drug-associated spout during terminal SNA periods, compared to all other groups. This high level of responding, as well as the highly goal-directed nature of that responding, despite signaling that drug was no longer available, are the epitome of the compulsive drug-seeking seen among human drug addicts. Furthermore, the MWF group also exhibited higher breakpoints and greater goal-directed responding during terminal PR testing. This high motivation to seek and take drug is also characteristic of drug addiction (American Psychiatric Association, 2000). The fact that these compulsive drug-seeking and -taking behaviors appear after prolonged, chronic self-administration training is consistent with previous findings (Deroche-Gamonet et al., 2004). In this case, however, the expression of “addiction-like” behaviors for cocaine is not attributable to genetic differences, per se, because initial overnight fat intakes did not differ between groups, and rats exhibiting “addiction-like” behaviors for cocaine exceeded 17% of the study population and were not evenly distributed across the four groups. Instead, these “addiction-like” behaviors occurred almost exclusively in the rats with a history of fat bingeing. In fact, when “addiction-like” behavior scores were calculated, nearly 50% of the rats from the MWF group were positive for both criteria. Also, it is interesting to note that, while the rats in the Ad Lib group consumed far more fat than all other groups overall, rats with a history of Ad Lib access were three times less likely than the MWF group and two times less likely than the Daily group, both of which had some degree of restricted access to fat, to exhibit “addiction-like” behavior for cocaine. In addition, all rats with a history of having consumed fat were more likely to exhibit “addiction-like” behavior for cocaine compared to the Chow group. Collectively, these data show that loss of control over one consummatory behavior (in this case, intake of fat) predisposes the loss of control over another (intake of drug).
In summary, these findings provide direct evidence that food intake can lead to addiction and offer an operational explanation for the co-morbid expression of binge-type disorders and substance abuse. This suggests that, due to the overlap among the neural substrates involved in the processing of the rewarding nature of foods (especially highly palatable foods, such as those rich in fat and sugar) and drugs of abuse, the brain essentially cannot tell the difference between excessive dysfunctional food intake and compulsive drug abuse. Unfortunately, once the neural mechanisms mediating addiction have been “turned on” in the brain, the individual is much more likely to develop dysregulated, compulsive responding in other motivated behaviors. Therefore, when one such behavior spirals out of control, the brain’s reward circuitry is altered, predisposing the development of similar dysfunctional intake behaviors for other rewarding stimuli. Accordingly, the data from the present study highlight the critical importance of behavior and experience in shaping the aberrant consummatory behaviors that are born of addiction. These results also offer a means of better understanding parameters that may increase vulnerability to, or conversely, prevention or rescue from, the development of such “addiction-like” behaviors. As such, it is important to be mindful of the turnstile nature of these behaviors, especially in clinical settings, focused on treatment and relapse prevention.
Chapter 5

Environmental enrichment protects against the acquisition of cocaine taking and seeking in adult male rats, but does not attenuate avoidance of a drug-associated saccharin cue.

While extremely valuable, the environmental enrichment studies discussed in Chapter 1 contain methodological considerations that did not lend themselves to investigate the impact of environmental enrichment on acquisition of drug self-administration behavior in adults. First, most of the studies described above exposed the subjects to enrichment during adolescence, not during adulthood (Bardo et al., 2001; Bezard et al., 2003; El Rawas et al., 2008; Green et al., 2002; Green et al., 2003; Solinas et al., 2008a; Stairs et al., 2006; Zhu et al., 2006). Second, many of the studies that were conducted in adult animals employed paradigms where drug was administered passively by the experimenter (e.g., conditioned place preference or sensitization), rather than self-administered (Bezard et al., 2003; El Rawas et al., 2008; Green et al., 2003; Solinas et al., 2008a). Third, those studies that did use active drug self-administration paradigms in adult rats, did not institute environmental enrichment until after the acquisition phase (Bardo et al., 2001; Chauvet et al., 2009; Stairs et al., 2006). Finally, no environmental enrichment studies have been conducted to determine whether environmental enrichment will reduce not only responding for drug, but avoidance (i.e., devaluation) of a drug-associated natural reward cue as well.

Thus, in the study described in Chapter 5, we were interested in determining whether the protective effects of environmental enrichment discussed above could be extended to rats that were placed in enriched environments as adults and then trained in an active self-administration paradigm thereafter. In addition, in an effort to assess the effect of environmental enrichment on drug-induced devaluation of natural rewards, access to cocaine was signaled by the availability
of an otherwise palatable saccharin cue (Grigson and Twining, 2002). We hypothesized that non-enriched rats in the saccharin-cocaine condition would readily self-administer cocaine and exhibit high levels of goal-directed behavior towards the cocaine-associated operant. Housing in the enriched environment, on the other hand, was expected to prevent cocaine self-administration and to cause a reduction in goal-directed behavior. Finally, we hypothesized that non-enriched rats would avoid intake of the saccharin cue when it predicted imminent access to cocaine and that environmental enrichment would prevent that suppression.

METHODS

Subjects

This study was conducted in two replications. The subjects were 80 (n=40 for Replication 1 and n=40 for Replication 2) naïve, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC), approximately three months of age (300-400 g in weight) at the beginning of the experiment. Due to complications during surgery, six rats were eliminated from the study. An additional 15 rats were eliminated due to loss of catheter patency, leaving 59 rats for behavioral training and experimental testing (described below). The rats were maintained on a 12-h light-dark cycle, with lights on at 0700 h. They were allowed ad lib access to food (Harlan Teklad, Madison, WI) and water, except where otherwise noted.
Housing Conditions

All rats were received from the provider and immediately placed in quarantine, where they remained for a period of one week. After quarantine the rats were acclimated to the colony room for a period of one week. During quarantine and acclimation, the rats were housed in groups of 5-6 in wire mesh cages (38.0 cm in length × 46.0 cm in width × 20.0 cm in height). Following the quarantine and acclimation periods, the rats were split into non-enriched environment (Non-EE; n=36) and enriched environment (EE; n=23) groups. Rats in the Non-EE group were housed individually in standard wire mesh cages (38.0 cm in length × 21.5 cm in width × 20.0 cm in height) throughout the study (see Figure 5.1, left panel). Rats in the EE group were housed in groups of four in large wire mesh cages (38.0 cm in length × 46.0 cm in width × 20.0 cm in height). After nine days, novel objects (e.g., balls, Polyethylene tubes, paper, etc.) were placed in the cages of the EE rats (see Figure 5.1, right panel). These objects were changed daily for the duration of the experiment. Throughout all behavioral training and experimental testing, all rats (Non-EE and EE) were housed in the same colony room with temperature, humidity, and ventilation controlled automatically.
**Figure 5.1:** Housing conditions. *Left panel.* Non-enriched environment (Non-EE) condition. *Right panel.* Enriched environment (EE) condition.

**Catheter Construction and Implantation**

*Self-administration catheter.* Intra-jugular catheters were custom-made in our laboratory as described by Twining et al. (2009).

*Catheter implantation.* Rats were anesthetized and catheters were implanted into the jugular vein, as described by Twining et al. (2009), nine days after being separated into Non-EE and EE groups. Following surgery, novel objects were introduced into the cages of the rats in the EE group and all rats were allowed at least two days to recover. General maintenance of catheter patency involved daily examination and flushing of catheters with heparinized saline (0.2 ml of 30 IU/ml heparin). Catheter patency was verified, as needed, using 0.2 ml of propofol (Diprivan 1%) administered intravenously.

**Apparatus**

Each rat was trained in one of twelve identical operant chambers (MED Associates, St. Albans, VT) described by Twining et al. (2009). Each chamber measures 30.5 cm in length × 24.0 cm in width × 29.0 cm in height, and is individually housed in a light- and sound-attenuated cubicle. The chambers consist of a clear Plexiglas top, front, and back wall. The side walls are made of aluminum. Grid floors consist of nineteen 4.8-mm stainless steel rods, spaced 1.6 cm apart (center to center). Each chamber is equipped with three retractable sipper spouts that enter through 1.3-cm diameter holes, spaced 16.4 cm apart (center to center). A stimulus light is
located 6.0 cm above each tube. Each chamber also is equipped with a houselight (25 W), a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indianapolis, IN), and a speaker for white noise (75 dB). Cocaine reinforcement is controlled by a lickometer circuit that monitors empty spout licking to operate a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT). A coupling assembly attaches the syringe pump to the catheter assembly on the back of each rat and enters through a 5.0-cm diameter hole in the top of the chamber. This assembly consists of a metal spring attached to a metal spacer with Tygon tubing inserted down the center, protecting passage of the tubing from rat interference. The tubing is attached to a counterbalanced swivel assembly (Instech, Plymouth Meeting, PA) that, in turn, is attached to the syringe pump. Events in the chamber and collection of data are controlled on-line with a Pentium computer that uses programs written in the Medstate notation language (MED Associates).

**Drug Preparation**

Individual 20-ml syringes were prepared for each self-administration chamber prior to each daily session by diluting 2.0 ml of cocaine HCl stock solution (1.24 g cocaine HCl + 150 ml saline) with 18.0 ml of heparinized saline (0.1 ml 1000 IU heparin/60.0 ml saline) for a dose of 0.167 mg/infusion. This relatively low dose was chosen to allow for this initial assessment of the effects of environmental enrichment on the acquisition of cocaine self-administration over trials.
Data Collection

Habituation, self-administration training, and progressive ratio testing were conducted during the light phase of the light/dark cycle.

Habituation Procedure and Spout Training

After nine days of being housed in either the Non-EE condition or the EE condition with social partners only, plus an additional three days with the addition of novel objects, rats were habituated to the operant chambers for 1 h/day for two days prior to the beginning of self-administration training. During this time, each rat was maintained on a water-deprivation regimen in which they received 1-h daily access to water in the operant chamber from the right spout during the habituation session and 25.0 ml of water in the home cage overnight. Thereafter, rats were returned to ad lib access to water for the duration of the study.

Self-Administration Training Procedure

Self-administration training began immediately following the 2-day habituation phase. See Figure 5.2 for a summary of behavioral training and experimental testing. Each rat was
Figure 5.2: Timeline of behavioral training and experimental testing

Trained during daily 65-min sessions for 14 days. Specifically, rats were placed in the operant chambers in darkness. Immediately upon initiation of the 65-min session, the white noise was turned on, the left spout, containing 0.15% saccharin, advanced into the chamber, and the house light was illuminated. Rats were then given 5 minutes to freely consume the saccharin solution. Following the 5-minute saccharin access period, the left spout retracted and the empty center and empty right spouts advanced into the chamber. The cue light above the right empty spout was illuminated. Rats were then allowed to self-administer cocaine (Non-EE: n=27; EE: n=17) or saline (Non-EE: n=9; EE: n=6) for 60 minutes. The right spout was termed the “active” spout, while the left spout was termed the “inactive” spout. A fixed ratio (FR) 10 schedule of reinforcement was implemented initially (Trials 1-10). During this time, completion of 10 licks on the “active” spout was followed by a single intravenous (i.v.) infusion of 0.167 mg cocaine or saline over six seconds. Drug or saline delivery was signaled by offset of the stimulus light, retraction of the “active” spout, and onset of the tone and houselight. The tone and houselight remained on for a 20-sec timeout period, during which time no drug was available. Responding
on the “inactive” spout was without consequence throughout each session. During the final four days of training (Trials 11-14), the reinforcement schedule was increased to an FR20 to better dissociate active and inactive responding. Following each self-administration training session, the rats were returned to their home cages. Saccharin intake, infusion number, goal-directed behavior (i.e., total number of active responses minus total number of inactive responses) and the terminal latency to self-administer the first infusion were measured. Saccharin intake was analyzed across trials, as well as during terminal access (i.e., averaged across the final two days of FR training). All cocaine self-administration data were analyzed during terminal access.

**Progressive Ratio Testing**

In addition to fixed ratio training, a progressive ratio (PR) schedule of reinforcement was implemented to test the impact of environmental enrichment on the rats’ willingness to work for drug. Thus, following the final day of FR20 training, PR testing was conducted. During PR testing, rats were placed in the operant chambers with conditions identical to those of self-administration training (including the initial 5-minute saccharin access period), except the number of active responses required to receive each infusion progressively increased by a multiple of five for up to ten infusions (1, 1+5=6, 6+10=16, 16+15=31, 31+20=51, 51+25=76, 76+30=106, 106+35=141, 141+40=181, 181+45=226). Thereafter, the number of required responses increased by 50 for each successive infusion (226+50=276, 276+50=326, 326+50=376, etc.; see Chapter 2, 3, and Puhl et al., 2009). During this PR session, rats were allowed to self-administer cocaine (0.167 mg/infusion) until a period of 30 min elapsed without
receipt of an infusion. Break point (the highest ratio completed) and goal-directed behavior were measured.

Data Analysis

All data were analyzed with Statistica (StatSoft, Tulsa, OK) using repeated measures analysis of variance (ANOVA) tests. Fisher least significant difference (LSD) post hoc tests were conducted on significant ANOVAs, when appropriate, with $\alpha$ set at 0.05.

RESULTS

Saccharin Intake

A $2 \times 2 \times 14$ repeated measures ANOVA varying housing condition (Non-EE or EE), infusion (saline or cocaine), and trials (1-14) revealed significant main effects of infusion, $F(1, 55)=8.42, p < 0.01$, and trials, $F(13, 715)=32.78, p < 0.01$, indicating, overall, that rats self-administering cocaine drank less saccharin than rats self-administering saline, and that saccharin intake increased across trials (see Figure 5.3). In addition, a significant infusion $\times$ trials
Figure 5.3: Mean (+/- SEM) saccharin intake (licks/5 min) across trials for rats in the saccharin-saline (open symbols) or saccharin-cocaine (closed symbols) condition. Rats housed in the non-enriched environment (Non-EE) condition are shown in the left panel, while rats housed in the enriched environment (EE) condition are shown in the right panel. * denotes statistical significance (p < 0.05).

interaction also was found, F(13, 715)=5.05, p < 0.01. Post hoc tests of this two-way interaction revealed that all rats in the saccharin-cocaine condition suppressed intake of the saccharin cue beginning with trial 8, relative to intake of saline treated controls, ps < 0.05. Housing condition, however, was not significant, F(1, 55)=1.88, p > 0.05, nor did it reliably interact with any other variable (housing condition × drug, F < 1; housing condition × trials. F(13, 715)=1.45, p > 0.05;
housing condition × drug × trials, F < 1). These data suggest that enriched housing had no effect on cocaine-induced suppression of CS intake.

Consistent with previous findings (Grigson and Twining, 2002; Puhl et al., 2009; Twining et al., 2009), however, two sub-populations of rats emerged during self-administration training: low drug-takers (n=38) and high drug-takers (n=6). Those groups were identified by calculating the mean number of cocaine infusions self-administered during the final two days of self-administration training (Trials 13-14 on the FR20 schedule of reinforcement) and then by determining the median value of those means. All rats that had a mean greater than or equal to the median value were defined as high drug-takers. Those that had a mean less than the median value were defined as low drug-takers. Identification of such a small number of high drug-takers, relative to our previous findings, is likely due to the low dose of cocaine employed. Our previous studies used a relatively high dose of 0.33 mg/infusion, twice the dose of the current study. Regardless, it is interesting to note that none of the rats in the EE condition fell into the high drug-taking group.

This observation called for a reanalysis of the saccharin intake data. As such, the intake data were revisited to evaluate the impact of individual differences in drug taking (i.e., low vs. high drug-takers) on intake off the taste cue. A 5 × 14 repeated measures ANOVA varying group (Non-EE Sacc-Sal, Non-EE Sacc-Coc Low, Non-EE Sacc-Coc High, EE Sacc-Sal, EE Sacc-Coc) and trials (1-14) revealed a significant main effect of group, F(4, 54)=2.90, p < 0.05. Post hoc tests indicated that rats in the Non-EE Sacc-Sal group drank more saccharin than rats in the Non-EE Sacc-Coc Low, Non-EE Sacc-Coc High, and EE Sacc-Coc groups (i.e., all rats that received training where the saccharin taste cue predicted access to cocaine; see Figure 5.4a).
Also, a significant main effect of trials, $F(13, 702)=27.02$, $p < 0.01$, was found, indicating that saccharin intake increased across trials. In addition, a significant group $\times$ trials interaction was
found, $F(52, 702) = 2.55, p < 0.01$. Post hoc tests of this two-way interaction indicated that rats in the Non-EE Sacc-Sal group drank more saccharin than rats in the Non-EE Sacc-Coc Low group on trials 7-8, 10, and 12-14 ($p < 0.05$), drank more saccharin than rats in the Non-EE Sacc-Coc High group on trials 7-14 ($p < 0.03$), and drank more saccharin than rats in the EE Sacc-Coc group on trials 6-14 ($p < 0.01$; see Figure 5.4a). A one-way ANOVA conducted on terminal saccharin intake (averaged across trials 13-14) also revealed a significant main effect of group, $F(4, 54) = 2.66, p < 0.05$. Post hoc tests indicated that rats in the Non-EE Sacc-Sal group had higher terminal saccharin intakes than rats in the Non-EE Sacc-Coc High and EE Sacc-Coc groups ($p < 0.02$; see Figure 5.4b). Collectively, these data indicate that Non-EE saline animals drank the most saccharin, Non-EE low drug-takers suppressed intake of saccharin slightly, and Non-EE high drug-takers suppressed intake of saccharin the most (see Figures 5.4a and 5.4b, left panels). Also, similar to high drug-taking rats housed in the Non-EE condition, rats housed in the EE condition suppressed intake of the cocaine-associated saccharin cue (see Figures 5.4a and 5.4b, right panels).
Cocaine/Saline Self-Administration

Terminal latency to first infusion. Not surprisingly, a one-way ANOVA conducted on the terminal latency to the first infusion (averaged across trials 13-14) revealed a significant main effect of group, $F(4, 37)=6.71, p < 0.01$. Post hoc tests indicated that EE rats in the saccharin-cocaine condition were slower to self-administer their first cocaine infusion than all other groups (ps < 0.02; see Figure 5.5, right panel).
**Figure 5.5:** *Left panel.* Mean (+/- SEM) terminal (trials 13-14) latency (s) to self-administer the first infusion for rats housed in the non-enriched environment (Non-EE) in the saccharin-saline (open bars), saccharin-cocaine low (single-hatched bars) or saccharin-cocaine high (cross-hatched bars) condition. *Right panel.* Mean (+/- SEM) terminal (trials 13-14) latency (s) to self-administer the first infusion for rats housed in the enriched environment (EE) in the saccharin-saline (open bars) or saccharin-cocaine (single-hatched bars) condition. * denotes statistical significance (p < 0.02).

*Terminal number of infusions.* A one-way ANOVA conducted on the terminal (averaged across trials 13-14) number of infusions revealed a significant main effect of group, F(4, 54)=8.42, p < 0.01. Post hoc tests indicated that rats in the Non-EE Sacc-Coc High group self-administered much more cocaine than all other groups (ps < 0.01; see Figure 5.6). Interestingly, Sacc-Coc rats housed in the EE condition behaved like low drug-takers housed in the Non-EE
Figure 5.6: Left panel. Mean (+/- SEM) terminal (trials 13-14) number of infusions self-administered by rats housed in the non-enriched environment (Non-EE) in the saccharin-saline (open bars), saccharin-cocaine low (single-hatched bars) or saccharin-cocaine high (cross-hatched bars) condition. Right panel. Mean (+/- SEM) terminal (trials 13-14) number of infusions self-administered by rats housed in the enriched environment (EE) in the saccharin-saline (open bars) or saccharin-cocaine (single-hatched bars) condition. * denotes statistical significance (p < 0.01).

condition, suggesting that environmental enrichment was capable of protecting these rats from the acquisition of cocaine self-administration.

Progressive Ratio Testing

Terminal break point. Similar results were found during PR testing (see Figure 5.7). A one-way ANOVA conducted on the terminal PR infusions (i.e., infusions self-administered during the final PR test) revealed a significant main effect of group, F(4, 54)=7.80, p < 0.01.
Figure 5.7: Left panel. Mean (+/− SEM) number of infusions self-administered during the final progressive ratio (PR) test by rats housed in the non-enriched environment (Non-EE) in the saccharin-saline (open bars), saccharin-cocaine low (single-hatched bars) or saccharin-cocaine high (cross-hatched bars) condition. Right panel. Mean (+/− SEM) number of infusions self-administered during the final PR test by rats housed in the enriched environment (EE) in the saccharin-saline (open bars) or saccharin-cocaine (single-hatched bars) condition. * denotes statistical significance (p < 0.01).

Post hoc tests indicated that rats in the Non-EE Sacc-Coc High group worked significantly harder to receive infusions (as indicated by significantly higher break points) than all other groups (ps < 0.01; see Figure 5.7). In accordance with the FR data, neither EE rats self-administering saline nor cocaine were willing to work for infusions (see Figure 6, right panel).

Terminal goal-directed behavior. The data illustrating goal-directed behavior towards the cocaine/saline-associated empty spout were also consistent with the infusion data obtained during both FR training and PR testing (see Figure 5.8). A one-way ANOVA conducted on the
Figure 5.8: Goal-directed behavior exhibited during the final progressive ratio (PR) test by rats housed in the non-enriched environment (Non-EE) in the saccharin-saline (open bars), saccharin-cocaine low (single-hatched bars) or saccharin-cocaine high (cross-hatched bars) condition. * denotes statistical significance (p < 0.01).

Right panel. Goal-directed behavior exhibited during the final PR test by rats housed in the enriched environment (EE) in the saccharin-saline (open bars) or saccharin-cocaine (single-hatched bars) condition. terminal goal-directed behavior (i.e., number of inactive responses subtracted from the number of active responses during the final PR test) revealed a significant main effect of group, F(4, 54)=6.21, p < 0.01. Post hoc tests indicated that rats in the Non-EE Sacc-Coc High group exhibited significantly more goal-directed behavior than all other groups (ps < 0.01) and that rats in the EE Sacc-Sal group exhibited significantly less goal-directed behavior than rats in the Non-EE Sacc-Sal and Non-EE Sacc-Coc Low groups (ps < 0.05). As such, rats housed in the EE condition failed to exhibit any goal-directed behavior toward the operant for saline or for cocaine (see Figure 5.8, right panel). A full overview of the results can be found in Table 5.1.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Non-enriched Environment (Non-EE)</th>
<th>Enriched Environment (EE)</th>
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<tbody>
<tr>
<td></td>
<td>Sacc-Sal</td>
<td>Sacc-Coc Low</td>
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<tr>
<td>Saccharin Intake</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>FR Cocaine/Saline Intake</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>FR Latency to Initiate Self-Administration</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>PR Goal-Directed Behavior</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>PR Break Point</td>
<td>↓</td>
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Table 5.1: Behavioral effects of environmental enrichment in adult male rats. ↑ indicates an increase in magnitude of the behavioral parameter shown, while ↓ indicates a decrease in magnitude of the behavioral parameter shown.

DISCUSSION

Consistent with previous findings with male Sprague-Dawley rats (Grigson and Twining, 2002; Piazza et al., 1989; Piazza et al., 2000; Puhl et al., 2009; Twining et al., 2009), individual
differences in responding for cocaine were evident among our subjects. Specifically, we were able to identify two sub-populations of rats based on their terminal cocaine intake: low drug-takers (n=38) and high drug-takers (n=6). Surprisingly, while these differences were clearly evident among rats in the Non-EE condition, not a single rat from the EE condition fell into the high drug-taking group (i.e., all of the rats in the EE condition that had the opportunity to self-administer cocaine had very little motivation to do so). Thus, when examining their behavior, rats housed in the enriched environment performed like low drug-takers housed in the non-enriched condition. They self-administered very few infusions on the FR schedule of reinforcement and when tested on the PR schedule of reinforcement, they failed to work for cocaine and they did not exhibit any goal-directed behavior toward the cocaine-associated operant. These PR data suggest that exposure to an enriched environment for a relatively short period of time in adulthood (and, in this case, during the same period of time as cocaine self-administration training) can decrease the perceived incentive reward value of cocaine and, ultimately, prevent acquisition of drug-taking behavior. To the author’s knowledge, these are the first data of their kind.

In addition to the hypothesis that rats in the EE condition would exhibit reduced acquisition of drug intake compared to rats in the Non-EE condition, we also hypothesized that rats in the EE condition would not devalue a saccharin cue that had been paired with the opportunity to self-administer cocaine. Specifically, we hypothesized that housing in the EE condition would reduce cocaine-induced devaluation of the saccharin cue because, at the very least, cocaine itself would lose some of its incentive value. It certainly appears that cocaine lost some of its incentive value for rats housed in the EE condition, however, EE rats in the saccharin-cocaine condition continued to consume less of the cocaine-associated saccharin cue
than did EE rats in the saccharin-saline condition. This pattern of data may suggest that housing in the EE condition may have blunted the perceived incentive value of both the drug and the sweet, with relative differences in reward remaining, otherwise, intact. The rats, then, may continue to avoid the lesser valued taste cue. This conclusion is consistent with the trend of reduced saccharin intake in the EE rats and with other studies indicating that environmental enrichment causes a decrease in sucrose consumption (Brenes and Fornaguera, 2008), attenuates cue-induced reinstatement of sucrose seeking (Grimm et al., 2008), and decreases responding for non-drug rewards, such as novel environmental stimuli (Cain et al., 2006). An alternative explanation could be that, although rats in the EE condition self-administered very low levels of drug, they still experienced potent drug cravings or withdrawal symptoms induced by exposure to the drug-predicting saccharin cue. While possible, this seems unlikely given previous data suggesting that cue-induced craving and withdrawal (as evidenced by aversive taste reactivity behavior) leads to very short latencies to self-administer the drug in question (Wheeler et al., 2008). Data from the current study show that rats housed in the EE condition, in fact, had very long latencies to self-administer compared to rats housed in the Non-EE condition. Nonetheless, environmental enrichment, in and of itself, mediates some kind of devaluation of other immediately present natural rewards.

The composition of one’s surrounding environment and the value (i.e., negative or positive) of the stimuli that make up that environment have very powerful effects on drug-taking and drug-seeking behaviors. In fact, stress and environmental enrichment have been shown to exert opposite effects on such behaviors. Generally, stress has been shown to increase responding for drugs of abuse, including cocaine, amphetamine, and heroin (see Goeders, 2002, Lu et al., 2003, and Marinelli and Piazza, 2002 for a review), while environmental enrichment
during adolescence has been shown to decrease responding (Bardo et al., 2001; El Rawas et al., 2008; Green et al., 2002; Green et al., 2003). In addition, exposure to stress is a potent inducer of reinstatement and relapse in animals and humans, respectively (see Shaham et al., 2000 and Stewart, 2000 for a review). In contrast, environmental enrichment has been shown to be protective against the development of drug-seeking and drug-taking behaviors (Bardo et al., 2001; El Rawas et al., 2008; Green et al., 2002; Green et al., 2003; Solinas et al., 2008a) and recent evidence shows that the availability of a novel object alternative decreases cocaine seeking in a conditioned place preference paradigm (Reichel and Bevins, 2010).

Insight into the differential effects of stress and environmental enrichment may be found in the underlying changes in neuroanatomy and neurochemistry instituted by each. Exposure to stressors (e.g., footshock, social isolation, food deprivation/restriction) results in the activation of the hypothalamic-pituitary-adrenal (HPA) axis, stimulating the production of corticotrophin-releasing hormone (CRH) by the hypothalamus, which, in turn, stimulates the production of glucocorticoid hormones (cortisol in humans, corticosterone in rodents; see Papadimitriou and Priftis, 2009 for a review). Corticosterone has been shown to have site-specific actions modifying dopamine transmission in the nucleus accumbens (see Marinelli and Piazza, 2002 for a review), a prominent structure in the mesocorticolimbic dopamine system, which is heavily involved in the processing of rewards. In addition, corticosterone and CRH have been shown to be critical for the acquisition and maintenance of cocaine, amphetamine, and heroin self-administration (see Goeders, 2002, Lu et al., 2003, and Marinelli and Piazza, 2002 for a review) and, as discussed previously, corticosterone has been shown to play a permissive role in the food deprivation-induced reinstatement of cocaine seeking (Shalev et al., 2003). Also, CRH and norepinephrine signaling in the bed nucleus of the stria terminalis and central nucleus of the
Environmental enrichment has been shown to have profoundly different effects on some of the same neural substrates. In mice, environmental enrichment has several effects on gene expression in the striatum. It causes a reduction in cocaine-induced expression of the immediate early gene zif-268 in the nucleus accumbens (Solinas et al., 2008b) and also affects levels of several genes involved in synaptic plasticity, such as protein kinase C λ (PKCλ) and mitogen-activated protein kinase kinase kinase 12 (MAP3K12; Thiriet et al., 2008). In addition, environmental enrichment results in higher baseline levels of ΔFosB in the striatum, but abolishes the increase in ΔFos B stimulated by repeated cocaine self-administration (Solinas et al., 2008b). Mice housed in an enriched environment are resistant to the locomotor effects of cocaine and express a lower number of DAT proteins in the striatum compared to mice housed in a standard environment (Bezard et al., 2003). In rats, environmental enrichment causes a decrease in baseline levels of corticosterone (Belz et al., 2003; Welberg et al., 2006) and adrenocorticotropic hormone (ACTH; Belz et al., 2003), a decrease in the expression of DAT proteins in the medial prefrontal cortex (Zhu et al., 2005), and an increase in the binding of serotonin in the forebrain (Hellemans et al., 2005). Finally, environmental enrichment also causes an increase in cortical weight and thickness (Bennett et al., 1969; Diamond et al., 1972), an increase in neuronal densities (Turner and Greenough, 1985), stimulates dendritic growth and branching (Wallace et al., 1992; Volkmar and Greenough, 1972), and stimulates the maintenance of a greater number of synaptic connections in the visual cortex (Briones et al., 2004).

It is important to note that all of the above mentioned molecular and anatomical studies, with the exception of Zhu et al. (2005), were conducted in rats that had been reared in an

amygdala mediate stress-induced reinstatement of drug-seeking and drug-taking behaviors (Leri et al., 2002).
enriched environment throughout adolescence. The current study, then, suggests that environmental enrichment experienced only during adulthood has the ability to mediate some of these same effects in the adult brain, a presumably less dynamic milieu with a decreased capacity for changes in plasticity. Not only does environmental enrichment appear to be a stimulus powerful enough to prevent the acquisition of drug-taking and drug-seeking behaviors, but it also is capable of reducing the intake of a palatable natural reward, such as saccharin. It is possible that environmental enrichment may alter the perceived hedonic value of rewarding stimuli by acting in the same regions of the brain (namely the mesocorticolimbic dopamine system) as natural rewards and drugs of abuse. It is also important to note that the Non-EE rats in the current study were not exposed to any sort or deprivation state or deficient circumstances; they were simply housed in standard conditions, with food and water freely available. Because the “non-enriched” environment is consistent with standard housing conditions, these data have implications for much of the self-administration data obtained in adult rats. Of course, we must consider the likelihood that “standard” housing conditions are, in fact, impoverished for rats. These results also must bring into question the settings most commonly used for human rehabilitation from drug abuse (e.g., correctional facilities and rehabilitation clinics). The make-up of such environments may be acting counterproductively to the goals of treatment. This is a critical point, given data suggesting that nearly 70% of those in prison are incarcerated due to drug-related crimes (National Institute on Drug Abuse, 2006). With such an alarming percentage, a little enrichment may go a long way and would be relatively inexpensive compared to the costs of years of incarceration. Support for such an idea is provided by data showing improved abstinence in non-incarcerated humans “working” (i.e., maintaining abstinence) to earn tokens for natural rewards (Higgins et al., 1993).
Chapter 6
General Discussion

SUMMARY

The experiments discussed in this thesis present three novel behavioral factors that influence and interact with the acquisition and expression of drug seeking and drug taking. Chapters 2 and 3 demonstrated the facilitative effects of sleep deprivation on cocaine-seeking and cocaine-taking. Acute sleep deprivation enhanced cocaine-induced reinstatement and also increased the speed with which rats self-administered cocaine, as well as the goal-directed nature of their responding. Interestingly, these behaviors were augmented in rats that otherwise exhibited very little motivation to seek and take drug (i.e., low drug-takers). Likewise, chronic sleep restriction increased the rate, efficiency, and amount of cocaine self-administration on both FR and PR schedules of reinforcement. Chapter 4 showed that a simple history of exposure to a diet high in fat tended to increase responding for cocaine. In addition, animals with a history of bingeing on fat exhibited more robust “addiction-like” behaviors for cocaine than non-bingeing animals (i.e., they persisted in responding for cocaine even when it was signaled that drug was no longer available, they worked harder for cocaine on a PR schedule of reinforcement, and they exhibited more goal-directed responding toward the drug-associated operant during SNA periods, as well as PR testing). These data suggest that loss of control over one consummatory behavior serves as the gateway for loss of control over another. Finally, Chapter 5 highlighted the protective influence that environmental enrichment has over the acquisition of cocaine-seeking and cocaine-taking in adult rats. In addition, while environmental enrichment appeared
to have no effect on the cocaine-induced suppression of saccharin intake, it seems that enrichment itself reduced intake of the otherwise palatable natural reward.

**NATURAL REWARDS: A BLESSING AND A CURSE**

As discussed in Chapter 1, the behavioral effects of all three of the factors presented in this thesis share a common neural substrate with the perception of the hedonic value of natural rewards and, unfortunately, drugs of abuse: the mesocorticolimbic DA system. This overlap in circuitry involved in the processing of these stimuli is not surprising when considering that sleep, fatty foods, and environmental enrichment are all very rewarding in and of themselves, especially when an organism’s circumstances produce a state of need for a particular stimulus (e.g., sleep deprivation, hunger, impoverished or unstimulating environment). Exposure to a facilitative stimulus (e.g., sleep deprivation or a high-fat diet), then, can prime the system so that it responds more robustly to the presentation of subsequent stimuli (e.g., drugs of abuse). Conversely, exposure to a protective stimulus (e.g., an enriched environment) can blunt the system so that it responds less robustly (or not at all) to the presentation of subsequent stimuli (e.g., sugar or drugs of abuse). As such, the mesocorticolimbic DA system can be likened to a revolving door. If one person moves through the revolving door quickly, anyone following is forced to move through quickly as well. Likewise, if the door is spinning too quickly, the doorman can slow it down so that the next person can enter at a normal pace, or even lock it in position so that it can no longer be used.

Therefore, in order to prevent maladaptive functioning of the circuit, modulation of the need for a particular stimulus is critical. For example, the motivation for drug is enhanced when
it occurs in conjunction with the presence of the increased need for sleep due to sleep deprivation so that both need states (the need for drug and the need for sleep) can be satisfied in a relatively short timeframe (see Chapters 2 and 3). In addition, moderation of exposure to certain stimuli is also important. For example, when intake of fat escalates out of control, the sensitivity to other highly rewarding behaviors, such as drug use is magnified (see Chapter 4). The hope is that exposure to protective stimuli, such as an enriched environment, is enough to counteract the negative effects of facilitative stimuli (see Chapter 5). However, one must consider the quantity and length of time of exposure to such facilitative stimuli, as there is certainly a point of no return beyond which attempted protective manipulations to the system will be without effect.

**INDIVIDUAL DIFFERENCES IN GENETIC VARIABILITY AND EXPERIENCE**

Differences in genetic variability and experience also govern an individual’s susceptibility to the development of dysfunction within the mesocorticolimbic DA system. The data presented in Chapters 2 and 5, as well as the scientific literature, clearly demonstrate that individual differences in the propensity to develop drug-seeking and drug-taking behaviors are present in outbred populations of rats (Deroche-Gamonet et al., 2004; Grigson and Twining, 2002; Piazza et al., 1989; Piazza et al., 2000; Puhl et al., 2009). In fact, certain strains of mice and rats have been shown to be more sensitive to the rewarding effects of drugs of abuse than others (Horan et al., 1997; Schlussman et al., 2008; Xi and Kruzich, 2007; Zhang et al., 2002). These differences can be likened to the variation seen in human drug-taking behavior. For example, certain individuals may use substances of abuse casually or recreationally for months, or even years, and never develop compulsive drug-seeking and drug-taking habits. However,
others may use a substance of abuse a single time and immediately become chronic, compulsive addicts. Recently, a single nucleotide polymorphism (SNP) on the *OPRM1* gene, which encodes the µ opioid receptor, was linked to the striatal DA response to alcohol. Transgenic mice expressing the mutation exhibited a DA response to alcohol that was four times greater than that seen in controls (Ramchandani et al., 2010). Likewise, neuroimaging studies confirmed that only humans expressing the specific *OPRM1* genotype exhibited striatal DA responses to alcohol (Ramchandani et al., 2010). The same genetic variation also has been shown to increase the risk of heroin addiction in humans (Levran et al., 2008).

There also is evidence for the role of genetic variability in the vulnerability to the behavioral effects of sleep deprivation, as well as overlap between genes that contribute to the regulation of circadian rhythms and those involved in substance abuse and fat intake. For instance, functional neuroimaging studies have demonstrated that differences in frontal and parietal cortices may contribute to individual differences in the vulnerability to the cognitive effects of sleep deprivation (Goel et al., 2009). In addition, studies using a transgenic mouse model have shown that the *Per2* clock gene is involved in regulating diurnal patterns of alcohol consumption. Specifically, mice with a mutant form of the gene display 24-h sensitivity to the behavioral effects of alcohol, as opposed to an increased sensitivity for a short period before the light cycle that is exhibited by controls (Perreau-Lenz et al., 2009; Spanagel et al., 2005). Also, expression and activity of the enzyme monoamine oxidase A (MAOA), a major degradation enzyme of the catecholamines, including DA, fluctuate based upon control of *Per2*. Transgenic mice with mutant forms of the *Per2* gene exhibit decreased expression and activity of MAOA in the striatum (Hampp et al., 2008). Presumably, then, the effects of DA could be prolonged during periods of the day when MAOA activity is low, possibly enhancing the perceived
incentive reward of stimuli (e.g., natural rewards or drugs of abuse) presented during those same time periods. The lengths of those periods could vary from individual to individual as a function of genetic makeup. Furthermore, a SNP on the PER2 gene has been linked to excessive alcohol consumption in humans (Spanagel et al., 2005). It is plausible, then, that disruption of normal circadian rhythms could have direct effects on the perceived incentive reward value of both natural rewards and drugs of abuse via neurochemical perturbation of the mesocorticolimbic DA system. Finally, preference for fat has also been shown to differ across inbred and outbred strains of mice (Dym et al., 2010).

While genetic variation plays a role in the sensitivity to both natural rewards and drugs of abuse, experience also is important. Chronic exposure to, or absence of, certain stimuli (e.g., drugs of abuse, sleep, a high-fat diet, an enriched environment) can shift the baseline behavioral and physiological responses to subsequent exposures or absences. For instance, the development of tolerance to drugs of abuse is one of the hallmark signs used to diagnose substance dependence (American Psychiatric Association, 2000), and, as such, addicts typically must continuously increase the dose of drug they are using in order to reach the same “high” they are accustomed to experiencing. In addition, individuals vary greatly in their susceptibility to fatigue, partly due to the fact the each individual establishes a baseline level of sleep that they are accustomed to obtaining. While receiving only 5-6 hours of sleep on a given night would have noticeable cognitive effects on an individual used to sleeping 8 hours per night, an individual used to receiving only 6 hours would not experience the same effects. Also, individuals who maintain diets high in fat or sugar do not experience the same physiological sensation after eating fat- and sugar-rich foods that an individual who maintains a leaner diet would experience.
Likewise, what may be enriching for some may not be enriching for others. Substance abuse occurs in individuals with high economic means, not just poverty-stricken populations. Presumably, wealthy addicts have access to luxuries and amenities that most individuals do not, yet they still use drugs of abuse.

THEORETICAL CONSIDERATIONS

The studies discussed in this thesis were designed specifically to investigate the effects of sleep deprivation, exposure to a high-fat diet, and environmental enrichment on the acquisition of drug-seeking and drug-taking behaviors. As such, the most likely neural substrate underlying the interaction between these factors is the mesocorticolimbic DA system. However, it is also possible that the effects, as well as the individual differences in vulnerability and behavior, that have been described are mediated more globally via the HPA axis. In fact, CRF signaling in the central nucleus of the amygdala, which is known to mediate stress-induced relapse (Erb et al., 2001), as well as the withdrawal syndromes of essentially all drugs of abuse (Koob, 2008), also has been implicated in behavioral responses involved in compulsive sugar seeking following the discontinuation of intermittent access. Cottone and colleagues (2009) posited that the negative affect associated with sucrose withdrawal resulting from the discontinuation of a restricted access diet is mediated by the central CRF system. Likewise, if sleep deprivation is not affecting drug-seeking and drug-taking behaviors directly via the mesocorticolimbic DA system, an alternative neural substrate for the effects described in Chapters 2 and 3 is the HPA axis, as sleep deprivation is a potent stressor that has been shown to cause an increase in CORT (España and Scammell, 2004). Accordingly, many of the protective effects of environmental enrichment
have been attributed to changes in the HPA axis. Enriched rats exhibit lower baseline levels of CORT (Belz et al., 2003; Welberg et al., 2006) and ACTH (Belz et al., 2003), as well as lower levels of ACTH release induced by chronic stress (Welberg et al., 2006).

In addition to the interactions between these factors and drug-seeking and drug-taking behaviors, there also is evidence that they interact with one another. The influence of orexin on sleep, feeding, and reward is well documented. As described in Chapter 1, orexin has been implicated in reward signaling in the mesocorticolimbic DA system (Fadel and Deutch, 2002; Harris et al., 2005; Marcus et al., 2001; Trivedi et al., 1998) and also has been shown to reinstate previously extinguished drug-seeking behavior (Boutrel et al., 2005; Harris et al., 2005). In addition, orexin plays a role in modulating the sleep and waking systems of the brain (España and Scammell, 2004). Also, orexin is heavily involved in the control of food intake (see Sakurai, 2006 for a review). As such, orexin serves as a neuromodulator, possibly helping to synchronize reward-directed behaviors, such as feeding, with the sleep/wake cycle (Bingham et al., 2006; Tsujino and Sakurai, 2009). Evolutionarily, the salience of natural rewards should be the greatest during periods of increased activity when the animal is awake to actively seek and obtain them. In addition, acute sleep deprivation due to stress has been shown to decrease the appetite-inhibiting hormone leptin, increase the appetite-stimulating hormone ghrelin, and increase the subjective experience of hunger (Spiegel et al., 2004a, 2004b). However, acute sleep deprivation under less stressful circumstances has the opposite effect, increasing leptin levels (Pejovic et al., 2010). Also, BDNF, which has been shown to mediate some of the neuroprotective effects of environmental enrichment, has been implicated in the processing of the hedonic value of food. Specifically, depletion of BDNF results in overeating and obesity (Rios et al., 2001), as well as increased intake of highly palatable foods, especially when access
to those foods is restricted (Cordeira et al., 2010). Conversely, intake of highly palatable foods causes a decrease in BDNF expression in the VTA (Cordeira et al., 2010). In addition, mutant mice lacking BDNF in the mesocorticolimbic DA system exhibit a marked decrease in the DA response in the NAc and striatum that is stimulated by intake of highly palatable foods (Cordeira et al., 2010). Given that one of the neuroprotective effects of environmental enrichment is that it stimulates the expression of BDNF in the hippocampus (Zhu et al., 2006) and striatum (Bezard et al., 2003), it stands to reason that enrichment could have similar effects on compulsive fat intake as it does on drug seeking and drug taking.

Finally, it is important to keep in mind that the behavioral manipulations discussed in this thesis may fall somewhere along a spectrum of behaviors, rather than being absolute measures. For example, several studies have examined the effects of environmental impoverishment versus enrichment. Part of the power of the results discussed in Chapter 5 is derived from the fact that the non-enriched group was simply housed in standard conditions used in essentially all animal studies around the world, not “impoverished” conditions. Given the robust differences seen between the enriched group and the non-enriched controls, those results have very important implications for all behavioral studies using rodent models, especially those investigating the intake of natural rewards and drug addiction. Interpretation of the data presented in this thesis regarding drug-seeking and drug-taking behaviors, the effects of sleep deprivation, and exposure to a high-fat diet, then, should be conducted under the assumption that similar spectra exist for drug intake, sleep, and fat consumption. While most behavioral studies on drugs of abuse focus on high drug-takers, the most interesting results are often obtained from subjects that maintain low levels of drug intake, as was the case with the study described in Chapter 2. Clearly, low-drug takers possess motivation to seek and take drug, but small amounts of drug are able to quell
their need. Any number of different degrees of drug seeking and drug taking may exist between the high and low drug-taking extremes. Likewise, similar degrees of behavior are undoubtedly present in the realm of fat and sugar intake. Also, severity of sleep deprivation can occur to many degrees, which may have similar or drastically different effects.

WHY DO WE BECOME ADDICTED?

What, then, is the purpose of this system that can seemingly be influenced and manipulated with such ease to the point that it mediates maladaptive behaviors that, in many cases, pose direct danger to the organism? The answer may lie in the fundamental and evolutionarily important notion of attachment. It is the job of the mesocorticolimbic reward system to rank the salience and value of environmental stimuli so that some can be engaged, while others can be ignored. As such, the importance of certain stimuli outweighs that of others, and attachment to the valued stimuli develops. There are two periods of time in life when interpersonal attachment is critical for survival of the individual or the species as a whole: shortly after birth, when offspring become attached to their mother, and during the search for a mate. Interestingly, the same OPRM1 polymorphism discussed above, in relation to its role in the susceptibility of individuals to alcohol and heroin addiction, also has been shown to mediate maternal attachment in infant primates. Subjects carrying the SNP exhibited greater vocalization during periods of prolonged maternal separation compared to non-carriers of the SNP (Barr et al., 2008). Also, the amount of time spent with the mother upon reunion increased as a function of repeated separation, while interaction with other social partners decreased relative the behavior of non-carriers (Barr et al., 2008). In addition, imaging studies have identified a role
for the mesocorticolimbic reward system in mediating romantic attraction in humans. Individuals that reported being “in love” exhibited greater activation of the VTA when shown an image of their loved one compared to activation after being shown a neutral control image (Aron et al., 2005). It seems, then, that while the mesocorticolimbic reward system plays a crucial role in mediating attachment behaviors that are of evolutionary importance to the organism, problems arise when genetics or experience predispose dysfunction of the neural substrates involved. Alterations due to genetic variability or neuroanatomical and neurochemical changes caused by overexposure to sugar, fat, or drugs of abuse set the system awry, mediating the development of compulsive behaviors born of dysfunctional attachment.

**IMPLICATIONS FOR THE TREATMENT OF ADDICTION IN HUMANS**

Collectively, the data presented in this thesis have important implications for the treatment of addiction in humans. Most importantly, it is critical that clinicians be aware of mitigating factors that may be interacting with the motivation to seek and take drug, including stress, sleep problems, diet, and living conditions. Also, individual differences in genetic variability and experience may cause some addicts to be more vulnerable to certain factors and, likewise, may provide novel avenues for relapse prevention. The full elucidation of the neural mechanisms involved in mediating the effects of sleep deprivation and compulsive fat and sugar intake is needed so that novel targets for pharmaceutical intervention can be discovered and utilized in conjunction with the treatment of addiction. Sleep and eating disorders are complex diseases with difficulties of treatment that are equal to those of drug addiction. An alcoholic can potentially abstain from drinking alcohol, by force or by choice. However, a binge eater cannot
just stop eating. Until the neuroanatomy and neurochemistry of these diseases is better understood, relapse prevention in the face of a gambit of factors that can facilitate drug seeking and drug taking will be no easy task. Finally, while it is true that drugs of abuse devalue natural rewards, the converse is also true. However, it is important to keep in mind the common underlying neural circuitry that is involved in the processing of natural rewards and drugs of abuse. Providing natural reward alternatives to drugs of abuse without taking into account a patient’s genetic makeup and experience could possibly result in the replacement of one addiction with another that could have equally harmful health consequences.

FUTURE DIRECTIONS

While the work presented in this thesis provides a number of animal models that can be used to investigate the effects of sleep deprivation, exposure to a high-fat diet, and environmental enrichment on drug-seeking and drug-taking behaviors, there are a number of future studies that must be conducted in order to fully understand the interactions between these factors and drug addiction. First, cocaine was the only drug used in all of the current studies. It is reasonable to assume that similar effects would be seen with amphetamine, due to the similarity in mechanism of action of the two drugs, however, it remains to be seen whether these effects are the same for other CNS stimulants, such as nicotine, and CNS depressants, such as alcohol or opiates. Also, the data presented in Chapter 4 suggest that exposure to a high-fat diet and fat bingeing can predispose an individual to “addiction-like” behaviors towards a drug of abuse, but it would be interesting to see if the relationship works in reverse (i.e., if development of drug-seeking and drug-taking behaviors can predispose an individual to the compulsive intake of natural rewards,
such as fat or sugar). Furthermore, it has been demonstrated that environmental enrichment can attenuate reinstatement of drug seeking induced by drug, drug-associated cues, and stress (Chauvet et al., 2009; Stairs et al., 2006). However, no work has been done to investigate whether enrichment can be used as a treatment in adults to directly decrease the perceived incentive reward value of drugs of abuse (i.e., reduce the willingness to work for drug on a PR schedule of reinforcement). Fourth, there is a growing body of evidence suggesting that chronic sleep deprivation is becoming an epidemic in the adolescent population (National Sleep Foundation, 2006). Likewise, adolescence is typically the time when individuals are introduced to substances of abuse. As such, it will be important to investigate the effects of facilitative and protective factors on the presumably more plastic environment of the adolescent brain. Finally, the current studies are very informative, but have the drawback of examining behavioral measures alone. As discussed previously, many of the same structures and neurochemicals that are involved in the regulation and modulation of sleep also are critical for reward processing, as well as addiction and relapse. Likewise, those same neural substrates are altered upon excessive exposure to fat and sugar, and also can be changed by environmental enrichment. Thus, the mesocorticolimbic reward system is the logical place to begin the search for possible underlying mechanisms that may mediate the changes in behavior described in this thesis. For example, microdialysis and high-performance liquid chromatography (HPLC) techniques could be utilized to measure the drug-induced changes in levels of DA and DA metabolites (e.g., 3,4-dihydroxyphenyl-acetic acid (DOPAC) and homovanillic acid (HVA)) in the NAc following exposure to sleep deprivation, a high-fat diet, or environmental enrichment. In addition, the expression of DA receptors and DAT in the NAc could be examined. Also as discussed previously, it is possible that the effects that have been described in this thesis relate more
generally to differences in stress reactivity. Therefore, drug-induced changes in circulating levels of CORT could be measured upon exposure to sleep deprivation, a high-fat diet, or environmental enrichment. Lesion studies involving the ablation of the bed nucleus of the stria terminalis (BNST), a structure known to mediate stress-induced relapse, prior to self-administration training and exposure to sleep deprivation, a high-fat diet, or environmental enrichment also could be informative.
References


Corwin, R. L. (2004) Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. Appetite 42(2), 139-142.


amphetamine is predicted by individual differences in sucrose feeding in rats. *Psychopharmacology* 148(1), 52-58.


Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addiction Biology* **12(1)**, 6-16.


National Sleep Foundation, http://www.sleepfoundation.org

National Women’s Health Information Center, http://www.womenshealth.gov


Stewart, J. (2000) Pathways to relapse: The neurobiology of drug- and stress-induced relapse to...


Influence of differential housing on emotional behavior and neuropeptide levels in mice. 
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GRANT SUPPORT

Title: The Effects of Sleep Deprivation on Cocaine Self-Administration and Relapse
Principal Investigator: Matthew D. Puhl
Agency: NIH/NIDA
Type: F31 DA023315-01A1 Period: January 1, 2008 – December 31, 2010
Research Objective: Identify the mechanisms by which sleep deprivation augments cocaine seeking and taking.

Title: The Effects of Sleep Deprivation on Addiction and Relapse
Principal Investigator: Patricia S. Grigson
Agency: The Pennsylvania State University College of Medicine/ Pennsylvania Department of Health Tobacco Settlement Fund
Type: Internal grant
Research Objective: Identify the mechanisms by which sleep deprivation augments drug seeking and taking.

PUBLICATIONS


Puhl, M. D., Blum, J. S., Acosta-Torres, S., and Grigson, P. S. (submitted) Environmental enrichment protects against the acquisition of cocaine taking and seeking in adult male rats, but does not attenuate avoidance of a drug-associated saccharin cue.


Puhl, M. D., Boisvert, M., Fang, J., and Grigson, P. S. (in preparation) Chronic sleep restriction enhances the acquisition of cocaine self-administration on both fixed ratio and progressive ratio schedules of reinforcement.