Neuronal Mechanisms Mediating Acute Food Deprivation-Induced Reinstatement of Heroin Seeking

Stephanie Tobin

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Dr. M. C. Olmstead — External Examiner

Dr. S. Santosa — External to Program

Dr. W. Brake — Examiner

Dr. C. A. Chapman — Examiner

Dr. U. Shalev — Thesis Supervisor

Approved by

Dr. C. A. Chapman
Chair of Department or Graduate Program Director

2012-08-15

Dr. Brian Lewis
Dean of Faculty

ABSTRACT

Neuronal Mechanisms Mediating Acute Food Deprivation-Induced Reinstatement of Heroin Seeking

Stephanie Tobin, Ph.D.

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One of the most troubling aspects of addiction is the chronic and cyclical nature of this disorder. In particular, the desire to use drugs and subsequent relapse can occur after months or even years of absence. Amongst human drug abusers, exposure to stressful life events and style of stress-coping have been shown to predict relapse. Similarly, in rodents stress can induce the reinstatement of drug seeking, a model of stress-induced relapse. In the following series of experiments the neuronal mechanisms mediating acute 21-48h food deprivation (FD)-induced reinstatement of heroin seeking are investigated. Previously, our laboratory has demonstrated that this form of reinstatement can be attenuated by systemic injection of the dopamine (DA) D1 receptor antagonist SCH 23390. Thus, in chapter 1, the neuronal circuitry mediating the role of DA, acting at the D1 receptor, in acute FD-induced reinstatement was examined. The five experiments described in the chapter reveal a significant attenuation of heroin seeking in rats infused with SCH 23390 into the nucleus accumbens (NAc) shell, dorsal medial prefrontal cortex (dmPFC) or basolateral amygdala (BLA). However, acute FD-induced reinstatement was not affected by infusions of SCH 23390 into the NAc core, or ventral medial prefrontal cortex (vmPFC).

Compulsive drug seeking and the propensity to relapse have been attributed to pathological synaptic plasticity, resulting from drug-induced changes in glutamate synapses. Thus, in chapters 2 and 3 the role of glutamate in acute FD-induced

reinstatement was inspected. In chapter 2, neuroadaptations in the glutamatergic projection from the ventral subiculum (vSub) of the hippocampus to the NAc shell were investigated. The vSub to NAc shell pathway is an important excitatory input, providing spatial and affective information that is modulated by DA release within the NAc. Here, acute 48h FD was shown to block the induction of long-term potentiation (LTP) in both heroin-trained and heroin-naïve rats. In chapter 3, changes in the expression of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamate receptor subunits were investigated in rats trained to self-administer heroin and subsequently tested for acute FD-induced reinstatement of drug seeking. The results of this study revealed a tendency toward a reduction in GluA2 and NR1 expression in the dmPFC and NAc, respectively, of heroin-trained food deprived rats, compared to heroin-trained sated rats, as well as a significant increase in GluA2 expression in the VTA of heroin-trained-sated rats compared to drug-naïve sated rats. Finally, FD increased GluA2 expression, in the BLA, in drug-naïve but not heroin-trained rats. Together, these findings demonstrate an important role for both DA and glutamate in acute FD-induced reinstatement. The current results support existing models that suggest a critical role for DA and glutamate transmission, and the interaction between the two systems, in the reinstatement of drug seeking.

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Mean (+*SEM*) GluA2 (A) and NR1 (B) GluR subunit expression in the VTA for rats trained to self-administer heroin (0.1 mg/kg for 10 days) or drug naïve colony rats. Heroin-trained rats received extinction training and were tested for a reinstatement of drug seeking under sated or 21h food deprivation (FD) conditions. Drug-naïve rats were sated or food deprived for 23h before their brains were collected. GluR subunit expression is presented as percent change from the drug-naïve-sated group. * $p < .05$ relative to drug-naïve sated rats. Blots to the right show a sample band for Naïve-Sated, Naïve-FD, Heroin-Sated and Heroin-FD, in that order.

LIST OF ABBREVIATIONS

6-OHDA: 6-hydroxydopamine

ACTH: Adrenocorticotropic hormone

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

BLA: Basolateral amygdala

BNST: Bed nucleus of the stria terminalis

BSR: Brain stimulation reward

CaMKII: Calcium/calmodulin-dependent protein kinase II

CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione

CPP: Conditioned Place Preference

CRF: Coricotropin-releasing factor

DA: Dopamine

DAT: Dopamine transporter

dmPFC: Dorsal medial prefrontal cortex

DNQX: 6,7-dinitroquinoxaline-2,3-dione

EPSP: Excitatory postsynaptic potential

EPSPs: Evoked synaptic potentials

fEPSPs: Field excitatory postsynaptic potentials

FD: Food deprivation

FR: Fixed ratio

GABA: γ-Aminobutyric acid

GluR: Glutamate receptor

G protein: Guanine nucleotide-binding protein

HPA: Hypothalamic-pituitary-adrenal axis

icv: Intracerbroventricular

iGluR: Ionotropic glutamate receptor

IV: Intravenous

LTD: Long-term depresssion

LTP: Long-term potentiation

MAPK: Mitogen-activated protein kinase

mGluR: Metabotropic glutamate receptor

NMDA: N-methyl-D-aspartate

NAc: Nucleus accumbens

PFC: Prefrontal cortex

PKC: Protein kinase C

PVN: Paraventricular nucleus of the hypothalamus

Ser: Serine

SN: Substantia nigra

TBS-T: Tris-buffered saline + tween

TTX: Tetrodotoxin

vmPFC: Ventral medial prefrontal cortex

VP: Ventral pallidum

vSub: Ventral subiculum

VTA: Ventral tegmental area

INTRODUCTION

One out of every 6 Canadians reports having used an illicit drug other than Cannabis at some point in life (Health Canada, 2005). However, the vast majority of these individuals do not develop drug dependency. It is currently estimated that 200,000 Canadians (roughly 0.08% of the population) are addicted to an illicit drug, or drugs (Ronald, Connoly, & Bédard, 2004). Drug addiction or substance dependence disorder, as it is referred to clinically, is defined as a cluster of symptoms including a preoccupation with the abused drug, tolerance to the effects of the drug, persistent failed efforts to control drug use, withdrawal symptoms during periods of drug abstinence, unintentional drug overuse and continued drug use despite major drug related problems (Diagnostic Statistical Manual (DSM)-IV-TR; American Psychiatric Association, 2000). Implied in this definition is a difficulty in ceasing drug use and a high likelihood of relapse during periods of drug abstinence. Sadly, even in long-time abstinent addicts there is a high probability of relapse. Relapse to drug use can come months or even years after drug use has ceased, at a time when the individual is no longer suffering from physical symptoms associated with drug withdrawal (Jaffe, 1990; O'Brien, & McLellan, 1996).

Understanding the compulsion to seek drugs during periods of abstinence is of the utmost importance in treating addiction and curtailing the negative consequences of this disorder to society and the individual. Within Canada illicit drug use results in approximately 1,695 deaths per year. Moreover, illness associated with illicit drug use is associated with 352,121 days of hospitalization, per year, as well as $1,134.6 million in health care costs. Illicit drug use is also associated with $2,335.5 million in law enforcement costs, and $4,678.6 million in costs associated with lost productivity due to

illness and premature death (Rehm et al., 2006). As mentioned above, a hallmark of addiction is persistent drug use despite negative consequences. Thus, for addicted individuals the personal costs of drug use can be immense. In addition to health risks, potential incarceration, and loss of employment many addicts also lose significant relationships with family and friends and migrate to the periphery of society (Parliament of Canada, 2001).

Amongst human drug abusers stress appears to be a central factor in the initiation, maintenance and relapse to drug seeking during periods of abstinence. Individuals exposed to high levels of stress such as those having experienced sexual and physical abuse begin illicit drug and alcohol use at an earlier age and have an increased risk for dependency (Dembo, Dertke, Borders, Washburn, & Schmeidler, 1988; Widom, Weiler, & Cottler, 1999). Moreover, maladaptive stress coping is predictive of an escalation of drug use, in current drug users, and stress-induced craving in abstinent users (Grüsser, Mörsen, Wölfling, & Flor, 2007; Hser, 2007; Wills, McNamara, Vaccaro, & Hirky, 1996). Since it is highly unlikely that recovering addicts will be able to avoid exposure to stressful live events, elucidating the brain mechanisms that underlie stress-induced relapse is essential for the development of effective treatments for addiction.

Numerous experimental methodologies have been designed to study relapse in human drug abusers. These studies have contributed greatly to our knowledge of addiction; however, studies of this nature can also be limited by ethical and methodological constraints surrounding the study of human drug users. These constraints, as well as ease of pharmacological manipulation, have lead to the popularization of animal models of relapse, most notably the reinstatement procedure (de Wit & Stewart,

1981; Epstein, Preston, Stewart, & Shaham, 2006; Shaham, Shalev, Lu, de Wit, & Stewart, 2003). Using this procedure in rodents, various stressors, including acute 21-48h food deprivation (FD) (Shalev, Highfield, Yap, & Shaham, 2000; Tobin, Newman, Quinn, & Shalev, 2009), have been shown to reinstate extinguished drug-seeking behavior. In this thesis, the mechanisms underlying acute FD-induced reinstatement are investigated in rats with a history of heroin self-administration. The experiments described below were designed to address some of the existing knowledge gaps regarding the mechanisms involved in stress-induced reinstatement.

Studying Relapse in Humans

Estimates of drug relapse vary greatly depending upon the definition of relapse, the time elapsed since treatment or detoxification, and the drug of abuse. For heroin abusers, relapse rates tend to be high. In one study of 242 clients seeking treatment for heroin abuse in one of 23 residential treatment programs, it was found that 60% (145 people) of the sample relapsed before a follow-up interview conducted 94 days (*SD* = 107) after leaving treatment. Of those relapsing, defined as using the drug at least once, 34% of the original 242 relapsed within 3 days and 50% relapsed within 14 days of the cessation of treatment (Gossop, Stewart, Browne, & Marsden, 2002). Further research has suggested that approximately 25% of heroin users remain abstinent 5 years after treatment (Hunt & Bespalec, 1974).

Given the high likelihood of relapse, the empirical study of this phenomenon is of the utmost importance. Conventional studies of relapse have involved interviewing recovering addicts after lapses. Studies of this type are limited in that they are retrospective; thus, individuals may not remember or be aware of the cause of their lapse

at the time of the interview (McKay, Franklin, Patapis, & Lynch, 2006). Despite these limitations, it has been revealed that drug lapses are often preceded by re-exposure to the previously abused or similar drugs, exposure to cues associated with drug use such as drug associated peers, places and paraphernalia, and stressful life events capable of producing a negative mood (Childress et al., 1994; Sinha, 2001). For example, alcohol and illicit drug abusers often cite negative affect and stress as reasons for relapse (Bradley, Phillips, Green, & Gossop, 1989; Litman, Eiser, Rawson, & Oppenheim, 1977; McKay, Rutherford, Alterman, & Cacciola, 1996; Wallace, 1989).

In order to avoid the confounds associated with retrospective self-report, Epstein and colleagues (2009) provided cocaine- and heroin-abusing outpatients with personal digital assistants to gain ecological momentary assessment data. The results of this study showed that cocaine craving was associated with exposure to drug cues and good mood; whereas, heroin craving was associated with negative affect such as feelings of sadness or anger. Within a controlled laboratory setting, person-specific stress-related scripts and exposure to drug-associated cues have been shown to increase drug craving and measures of salivary cortisol (Sinha, 2009). Furthermore, stress-related imagery has been used to induce drug craving in cocaine-dependent subjects within the confines of a magnetic resonance imaging scanner, revealing reduced activity in areas associated with control, and regulation of emotion and distress states, compared to healthy controls. In contrast, cocaine-dependent subjects exhibited stronger brain activation in areas associated with the reward pathway, and the circuitry involved in habitual behavior (Sinha et al., 2005).

Studying Relapse in Animal models: The Reinstatement Procedure

The value of studies of addiction in humans cannot be underestimated. However, there are numerous ethical considerations associated with exposing abstinent drug abusers to states likely to provoke relapse or drug naïve persons to illicit potentially dependency producing drugs. Furthermore, animal models of drug reinforcement and relapse offer distinctive advantages such as experimental control, ease of pharmacological manipulation, the ability to manipulate genetic factors, and the ability to investigate cellular processes. Thus, a combination of animal and human studies is needed to gain a broader understanding of drug relapse.

Several models of relapse exist for rodents. These models typically focus on the recommencement or *reinstatement* of an operant behavior previously associated with drug self-administration (i.e., the reinstatement procedure; de Wit & Stewart, 1981) or the reinstatement of a preference for a place paired with the effects of a drug (the reinstatement of a conditioned place preference (CPP; Mueller & Stewart, 2000). In brief, the reinstatement of CPP involves training a rat to associate the contextual cues of a particular environment with a drug's reinforcing effects to establish a preference for this environment over a distinctively different environment that was never paired with the drug. Consequently, this preference is extinguished by repeated exposure to the apparatus in the absence of the drug. Following extinction, triggers thought to be associated with drug relapse can be used to elicit a reinstatement of the preference for the previously drug-paired environment (Cruz, Leão, Marin, & Planeta, 2010; Lu et al., 2005). Whereas the CPP procedure requires "passive" exposure to drug through experimenter-initiated administration, the reinstatement of a behavior associated with self-administration

involves training an animal to perform an operant task such as lever pressing (de Wit & Stewart, 1981) or runway traversing (Ettenberg, 2009) to receive a drug reinforcer. Once this task is preformed to a criterion level the drug is removed and behavioral extinction of the operant task is learned to criterion.

The popularity of the reinstatement model lies, in part, in its intuitive appeal. A reinstatement of drug seeking behavior or a drug CPP, under abstinent conditions, can be triggered by a re-exposure to the previously administered or related drugs (drug priming), cues associated with previous drug administration, and stress which are similar to factors which have been shown to precipitate relapse in humans (Epstein et al., 2006). With the exception of priming-induced reinstatement, which involves a single drug administration, animals are not re-exposed to the previously self-administered drug prior to reinstatement testing. Thus, both versions of the reinstatement model allow for the investigation of neuronal and environmental mechanisms associated with *drug craving* in the absence of the pharmacological effects of the drug.

The majority of the results obtained from the reinstatement of CPP and the reinstatement of operant drug seeking behavior are in agreement. For instance, stress has been shown to reinstate operant responding associated with cocaine, amphetamine, morphine, and heroin self-administration (Buczek, Lê, Wang, Stewart, & Shaham, 1999; de Wit & Stewart, 1981; LeSage, Burroughs, Dufek, Keyler, & PenteL, 2004; Shaham et al., 1997) as well as the reinstatement of a preference for a place previously associated with morphine, cocaine, or nicotine administration (Der-Avakian et al., 2005; Leão, Cruz, & Planeta, 2009; Sanchez, Bailie, Wu, Li, & Sorg, 2003; Will, Watkins, & Maier, 1998). Despite the similarities between these models it has been suggested that both models

evaluate different aspects of reward and as a result different aspects of relapse. In the operant reinstatement model the performance of a behavior is maintained by the self-administration of the drug. Thus, operant behavior is the result of the primary rewarding properties of drugs; whereas, CPP results from the incentive value of cues which have come to be associated with the passive (experimenter) administration of drug (see Aguilar, Rodríguez-Arias, & Miñarro, 2009). Human drug abusers are rarely exposed to drug passively; thus, the reinstatement of an operant behavior previously associated with drug self-administration may be more representative of the experience of relapse than the reinstatement of a CPP.

Stress and Drug Seeking

Most, if not all, models of addiction postulate that stress plays a significant role in the onset and maintenance of drug use (Brewer, Catalano, Haggerty, Gainey, & Fleming, 1998; Matheny & Weatherman, 1998; Sinha, 2001). For instance, the stress coping model of addiction suggests that drug use during times of stress leads to the removal of an aversive state which enhances mood and serves to reinforce drug use (Khantzian, 1985; Sinha, 2001). Empirical studies of the initiation of drug use suggest a strong link between stressful childhood experiences such as abuse, neglect, and trauma and the use of illicit drugs. In one study of 8613 clients of a primary health care facility in California, a direct relationship between the number of aversive/stressful childhood events and dependency was found. In this study, individuals reporting 5 or more aversive childhood experiences were 7-10 times more likely to report addiction to, or problems with, illicit drugs (Dube et al., 2003). Moreover, amongst recovering drug addicts, particularly those with poor coping skills, stress is a commonly cited reason for relapse. In particular stress coping

styles such as positive thinking, negative thinking, and avoidance have been liked with relapse (Litman, Stapleton, Oppenheim, Peleg, & Jackson, 1984; Miller, Westerberg, Harris, & Tonigan, 1996).

Unfortunately, it is not possible to expose rodents to the types of stressors generally experienced by human drug abusers. Thus, it is important to have a clear understanding of what stress is and how it can be applied in an animal model. A single all encompassing definition of stress is somewhat difficult to arrive at. However, a useful working definition of stress is the process involved in sensing and responding to physical or psychological events or stimuli which are perceived as threatening, harmful, or challenging (Lazarus & Folkman, 1984). In animal models of stress these events are typically pharmacological in nature or involve forced exposure to stimuli that in other circumstances would be avoided (Piazza & Le Moal, 1998). For instance, stressors such as tail pinch, electrical footshock, food restriction, and exposure to a dominant male have been shown to speed the acquisition of self-administration for a variety of drugs (Carroll, 1985; Haney, Maccari, Le Moal, Simon, & Piazza, 1995). Once self-administration behavior has been acquired, stress has been shown to lead to an increase in the rate of responding for drug (Carroll, 1985; Kanarek & Marks-Kaufman, 1988; Miczek & Mutschler, 1996) and an increase in progressive ratio breakpoint, a measure of the motivation to take the drug (Shaham & Stewart, 1994).

The Neuronal Correlates of Stress and Drug Seeking

While discussing the role of stress in the initiation, maintenance, and reinstatement of drug seeking it is important to briefly touch upon the physiological response to stress. In brief, exposure to a stressor causes an activation of the

hypothalamic-pituitary-adrenal axis (HPA) (Dunn & Berridge, 1990; Rivier & Plotsky, 1986) which leads to cascade of events beginning with the release of corticotropin releasing factor (CRF) from the hypothalamus. CRF release triggers the secretion of adrenocorticotropic hormone (ACTH), a pituitary hormone capable of eliciting the production and release of corticosterone (cortisol in humans) from the adrenal cortex. The release of corticosterone into the bloodstream mobilizes a quick response, generally referred to as the 'fight or flight response,' in a threatening situation (Kosten & Ambrosio, 2002). In addition to HPA axis activation, stress also activates extra-hypothalamic CRF pathways, which innervate limbic and forebrain regions such as the hippocampus, amygdala, bed nucleus of the stria terminalis (BNST), ventral subiculum (vSub), ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) (Kosten & Ambrosio, 2002; Sinha, 2001).

Evidence for HPA axis disregulation has been observed in both human and rodents exposed to drugs of abuse. Amongst individuals dependent upon opiates a reduction in ACTH and cortisol has been observed during drug taking (Facchinetti et al., 1985; Ho et al., 1977). In contrast, an increase in CRF, ACTH, cortisol, norepinephrine, and epinephrine is observed during withdrawal (Adinoff et al., 1991; Ehrenreich et al., 1997; Koob, 1997; Kreek & Koob, 1998; Mello & Mendelson, 1997; Vescovi, Coiro, Volpi, & Passeri, 1992) as well as a hyper-responsive HPA axis response during periods of abstinence (Culpepper-Morgan & Kreek, 1997; Schluger et al., 1998). Similarly, in rodents the acute delivery of morphine produces an activation of the HPA axis; however, with chronic drug administration the activation of the HPA axis is suppressed (Kreek & Koob, 1998).

There is ample evidence linking HPA activation to drug-associated behaviors. For example, inhibition of corticosterone synthesis by metyrapone or ardenalectomy has been shown to block the locomotor response to cocaine or morphine (Marinelli et al., 1994) and the stress-induced sensitization to the locomotor activating effects of amphetamine and morphine (Deroche et al., 1992a; Deroche, Piazza, Le Moal, & Simon, 1993; Marinelli, Le Moal, & Piazza, 1996). Similarly, repeated corticosterone administration sensitizes the locomotor response to amphetamine (Deroche, Piazza, Maccari, Le Moal, & Simon, 1992b). Finally, social isolation, thought to represent psychological stress, has been shown to facilitate the locomotor activating effect of morphine in intact but not adrenalectomized rats maintained at a stable level of plasma corticosterone (Deroche, Piazza, Le Moal, & Simon, 1994). These findings are of interest because individual differences in vulnerability to self-administer drug can be correlated with locomotor response to stress or acute drug injection (Deminiere, Piazza, Le Moal, & Simon, 1989; Piazza et al., 1991). Furthermore, in rodents not predisposed to self-administer amphetamine, corticosterone treatment was shown to increase the rewarding aspects of amphetamine and foster the development of self-administration (Piazza et al., 1991).

The Neuronal Circuitry Involved in Stress-Induced Reinstatement of Drug Seeking

Of the utmost importance for the current studies, is the finding that exposure to stress can lead to a reinstatement of previously extinguished drug reinforced behavior. Using the reinstatement model, a return to drug seeking has been shown following footshock (Shaham & Stewart, 1995) and acute 21-48h FD (Shalev et al., 2000; Tobin et al., 2009) stress. Moreover, administration of pharmacological stressors such as CRF (Erb, Petrovic, Yi, & Kayyali, 2006; Erb, Shaham, & Stewart, 1998; Shaham et al.,

1997), or yohimbine, an α_2 noradrenergic receptor agonist (Feltenstein & See, 2006; Shepard, Bossert, Liu, & Shaham, 2004), have also been shown to reinstate drug-seeking behavior. Similarly, using the CPP procedure it was shown that footshock-stress and forced swim-stress reinstate extinguished morphine and cocaine CPP (Lu, Ceng, & Huang, 2000; Redila & Chavkin, 2008.

In contrast to a stress-induced increase in drug intake, CRF and not corticosterone appears to play a critical role in stress-induced reinstatement of extinguished drug seeking (Shalev, Erb, & Shaham, 2010). For instance intracerbroventricular (icv) infusion of α-helical CRF, a nonselective CRF receptor antagonist, has been shown to attenuate footshock-induced reinstatement (Shaham et al., 1997), acute FD-induced reinstatement (Shalev, Finnie, Quinn, Tobin, & Wahi, 2006), and footshock-induced renewal of a morphine CPP (Lu et al., 2005). Furthermore, stress-induced corticosterone release, from the adrenal cortex, is not necessary for footshock- (Erb et al., 1998; Shaham et al., 1997) or acute FD-induced reinstatement (Shalev et al., 2006), suggesting that it is the actions of extra-hypothalamic CRF that are important for stress-induced reinstatement. More specifically, CRF receptors in the BNST (Erb & Stewart, 1999), VTA, and median raphe nucleus are thought to be central in stress-induced reinstatement (see Shalev et al., 2010 for a review of this literature).

Additionally, it is worth noting that acute-FD-induced stress is dissociable from other forms of stress in that it combines both a *pure* stress effect and a disregulation of the homeostatic circuitry involved in the control of energy balance. For instance, leptin, an anorexigenic hormone secreted by peripheral adipose tissue in correlation with the

amount of stored fat, has been shown to block acute FD-induced reinstatement, but has no effect on footshock-induced reinstatement (Shalev, Yap, & Shaham, 2001).

The Role of Dopamine (DA) in Drug Reinforcement

Clearly, the study of the "classical" stress-related pathways in the brain is central to understanding the role of stress in drug relapse. However, studies performed over the last decade have suggested a critical role for the neuronal circuitry implicated in reward and motivation in stress-induced reinstatement of drug seeking. The rewarding property of abused drugs depends, almost exclusively, on the ability of these drugs to elicit an enhanced DAergic response in the NAc (Di Chiara, Bassareo, Fenu, & De Luca, 2004). DA terminals in this region originate from DA cellbodies in the VTA which also project to the mPFC, amygdala, hippocampus, and ventral pallidum (VP) (Pierce & Kumaresan, 2006). Activation of this pathway, known as the mesocorticolimbic DA pathway is critically involved in the detection and prediction of reward (Schultz, 2001; Schultz, Dayan, & Montague, 1997), as well as the motivation to gain reinforcement (Berridge, 1996; Robinson & Berridge, 2003). Recently, however, this view of the mesocorticolimbic DA pathway as a singular contributor to reward and motivation function has been challenged. The nigrostriatal DA pathway, which originates in the substantia nigra (SN) that is anatomically adjacent to the VTA and is typically discussed in the context of motor function, was suggested to be importantly involved in reward and addiction (Wise, 2009).

Psychostimulant drugs such as amphetamine or cocaine act directly on the mesocorticolimbic, and possibly nigrostriatal, pathway through their actions as indirect agonists at DA receptors. Amphetamine acts to increase the release of DA while cocaine

inhibits the actions of the DA transporter (DAT) allowing DA to stay in the synapse longer (Koob, 1992). Opiate drugs such as heroin act on DA neurons in a more indirect manner. These drugs increase DA release by acting on γ-Aminobutyric acid (GABA) terminals, which in turn exert an inhibitory effect on VTA and possibly SN DA neurons (Johnson & North, 1992; Wise, 2009). Removal of this inhibitory tone, by activation of opiate receptors located on inhibitory interneurons, increases NAc DA release. Finally, drug-induced NAc DA release, particularly in the NAc shell, may depend on response contingency. For instance, NAc shell DA release is potentiated in animals trained to self-administer drug, compared to drug naïve animals and animals given non-contingent yoked drug administration, demonstrating a further distinction between the self-administration and CPP models of reinstatement (Lecca, Cacciapaglia, Valentini, Acquas, & Chiara, 2007a; Lecca, Valentini, Cacciapaglia, Acquas, & Di Chiara, 2007b).

Once released, the actions of DA in the brain are mediated by two distinct families of guanine nucleotide-binding proteins (G-protein) coupled receptors: The DA D_1-like family of receptors and the DA D_2-like family of receptors. The DA D_1-like family of receptors includes the DA D_1 and DA D_5 receptors both of which are positively coupled to adenylate cyclase through an excitatory G-protein. Whereas, the D_2-like family of receptors includes the DA D_2, D_3 and D_4 receptor subtypes and are negatively coupled to adenylate cyclase through an inhibitory G-protein (Sokoloff & Schwartz, 1995). The unique role of each of these DA receptor subtypes, in drug reinforcement, can be assessed by pharmacological activation or blockade using agonists or antagonists, respectively, or through genetic manipulations producing a change in the expression of a particular receptor subtype. Studies of this nature have demonstrated that rodents will

self-administer DA D_1-like agonists suggesting that activation of these receptors is reinforcing (Self & Stein, 1992; Self, Belluzzi, Kossuth, & Stein, 1996). Moreover, systemic administration of the DA D_1 or D_2 receptor antagonists, SCH 23390 and spiperone, respectively, leads to a compensatory increase in cocaine self-administration suggesting that antagonism of these receptors causes a reduction in the reinforcing value of cocaine (Corrigall & Coen, 1991). However, when given a choice rats will prefer to self-administer cocaine over a DA D1 or D2 receptor agonist suggesting that these compounds do not fully duplicate the reinforcing effects of cocaine (Manzardo, Del Rio, Stein, & Belluzzi, 2001).

Interestingly, genetic studies of DA receptor knockout mice have revealed significant differences between in the function of DA D1 and DA D2 receptors. For instance DA D2, but not DA D1, receptor knockout mice continue to show cocaine self-administration (Caine et al., 2002; 2007) suggesting a more direct role for DA D1 receptors in the reinforcing effects of cocaine and perhaps heroin.

The Role of the DA in the Reinstatement of Drug Seeking

In addition to its role in the reinforcing effects of drugs, DA has also been shown to play a role in drug craving during abstinent conditions. For instance, in abstinent opiate-dependent individuals, drug craving, triggered by a video of heroin related cues, is associated with an increase in striatal DA release and a reduction in D2 receptor availability compared to age and gender matched participants (Zijlstra, Booij, Van den Brink, & Franken, 2008). The role of the DA D1 receptor in drug craving has been less well studied; however, a role for this receptor is indicated in a reinstatement of drug seeking behavior in rodents, as detailed below.

Activation of the DAergic system is of great importance in priming- and cue-induced reinstatement, in rodents. For instance, the DA agonists amphetamine and cocaine have been shown to trigger the reinstatement of drug-seeking behavior in animals trained to self-administer cocaine or heroin (de Vries, Schoffelmeer, Binnekade, Mulder, & Vanderschuren, 1998; de Wit & Stewart, 1981). Furthermore, systemic pharmacological blocking of DA D1 receptors, using SCH 23390 (Shaham & Stewart, 1996) or intra-NAc blocking of DA D1 or D2 receptors, using SCH 23390 or raclopride, has been shown to attenuate cocaine and heroin priming-induced reinstatement of drug seeking (Anderson, Bari, & Pierce, 2003; Anderson, Schmidt, & Pierce, 2006). Furthermore, blockade of the DA D1 or DA D2 receptor has been shown to attenuate discrete cue- (Cervo, Carnovali, Stark, & Mennini, 2003; Ciccocioppo, Sanna, & Weiss, 2001) and context-induced reinstatement (Crombag, Grimm, & Shaham, 2002). Taken together these studies suggest a crucial role for DA transmission through the DA D1 and D2 receptors, in priming- and cue-induced reinstatement.

However, the involvement of DA in stress-induced reinstatement of drug seeking is less clear. Footshock-induced reinstatement of heroin seeking is attenuated by systemic administration of the non-selective DA antagonist flupenthixol. Yet, systemic treatment with the selective DA D_1 antagonist, SCH 23390, or the selective D_2 antagonist, raclopride, has no effect on footshock-induced reinstatement (Shaham & Stewart, 1996). In contrast, we have recently demonstrated that systemic injection of SCH 23390 causes an attenuation of acute FD-induced reinstatement of heroin seeking. Interestingly, the doses used in our study were 10 fold lower than those shown to have no effect on footshock-induced reinstatement (Tobin et al., 2009).

As mentioned above, leptin attenuates acute FD-induced reinstatement but has no effect on footshock-induced reinstatement. Interestingly, leptin receptors are expressed within the VTA and leptin-responsive VTA DA neurons project to the NAc (Fulton et al., 2006). Furthermore, leptin administration has been shown to reverse chronic food restriction-induced sensitization of lateral hypothalamic brain stimulation reward (BSR) (Fulton, Woodside, & Shizgal, 2000). Thus, leptin appears to play a critical role in nutritionally based changes in motivational state (Shalev et al., 2001). However, in what seems to be a contradicting finding, leptin-deficient mice show a diminished locomotor response to amphetamine and a marked reduction in DA release from NAc shell terminals (Fulton et al., 2006). It is therefore unclear, at this point, whether the attenuation of acute FD-induced reinstatement of heroin seeking, by central leptin injection, was mediated by the actions of leptin on the function of DA neurons.

The Role of Glutamate in Drug Seeking

As discussed above, the DAergic system is critically involved in the acutely rewarding effects of abused drugs as well as in drug seeking under abstinent conditions. However, pharmacotherapies targeting the DA system cannot completely eliminate drug craving and relapse (Cornish & Kalivas, 2000; O'Brien, 2005; Spanagel & Kiefer, 2008). Furthermore, the pervasive nature of addiction as well as the propensity to relapse long after drug administration has ceased (see Bossert, Ghitza, Lu, Epstein, & Shaham, 2005; Jaffe, 1990; O'Brien & McLellan, 1996; Wallace, 1989) suggest that these behaviors might be driven by drug-induced long-term adaptations in the neuronal circuitry underlying reward and motivation (Kalivas, 2004). Thus, a considerable effort has been directed at identifying neuronal circuits that undergo long-term adaptations following

drug exposure (Nestler, 2001).

Glutamate has been identified as a major regulator of the synaptic plasticity involved in learning and memory (Abraham, 2008; Maren & Baudry, 1995). Consequently, it has been suggested that drug-induced pathological changes in glutamaterigc synapses play a critical role in the development of addiction (Kalivas, 2004; 2009; Wolf, 2002). The vast majority of research on the neural mechanisms underlying drug addiction has focused on the DAergic projection from the VTA to the NAc. Importantly, both of these structures also receive extensive glutamatergic input (Cooper, Klipec, Fowler, & Ozkan, 2006a; Gass & Olive, 2008; Geisler, Derst, Veh, & Zahm, 2007; Goto & O'Donnell, 2001; Groenewegen, Zee, & Kortschot, 1987; Omelchenko & Sesack, 2007; Sesack, Carr, Omelchenko, & Pinto, 2003; Yang & Mogenson, 1985). Moreover, drugs of abuse as well as drug-predictive cues have been shown to elicit the release of both DA and glutamate in the VTA (Gass & Olive, 2008; Wise, Wang, & You, 2008). Furthermore, the role of glutamate in the VTA seems to be tied to the added value provided by drug-associated cues, rather than drug reward itself. Thus, cocaine methiodide, an analogue of cocaine that does not cross the blood brain barrier, has no effect in drug naïve animals; but, in rats previously trained to lever press for cocaine it can trigger VTA glutamate release sufficient to reinstate extinguished cocaine-seeking behavior (Wise et al., 2008).

The actions of glutamate are mediated by two distinct classes of glutamate receptors (GluR): ligand gated, ionotropic receptors (iGluR), and G-protein coupled, metabotropic (mGluR), receptors. These classes of receptors are functionally distinct. The iGluRs mediate fast excitatory neurotransmission; whereas, mGluRs are involved in

mediating less rapid synaptic processes (Conn & Pinn, 1997; Gass & Olive, 2008; Guo, Wang, Xiang, & Zhao, 2009). Both iGluR and mGluRs have numerous receptor subtypes. There are three groups of mGluRs (Group 1-3), which include the $mGluR_1$-$mGluR_8$ receptors, and three families of iGluRs: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kinate receptors, all composed of multiple subunits (Dingledine, Borges, Bowie, & Traynelis, 1999; Gass & Olive, 2008). Numerous studies have investigated the role of iGluRs in drug seeking behavior as it pertains to the reinstatement model. For instance, intra-NAc infusion of the AMPA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) blocked the reinstatement of cocaine seeking induced by systemic cocaine injection, NAc DA infusion and NAc AMPA receptor agonist infusion (Cornish & Kalivas, 2000). Similarly, intra-NAc shell infusion of the mGluR2/3 agonist LY379268, which decreases glutamate release, has been shown to attenuate context-induced reinstatement of heroin seeking (Bossert, Gray, Lu, & Shaham, 2006a), and injections of CNQX into the NAc core attenuated both heroin priming- and discrete cue-induced reinstatement of heroin seeking (LaLumiere & Kalivas, 2008). Additionally, systemic LY379268 has also been shown to attenuate cue- and context-induced reinstatement of sucrose seeking (Bossert, Poles, Sheffler-Collins, & Ghitza, 2006b).

Taken together, these studies indicate a critical role for glutamate transmission in the reinstatement of extinguished drug seeking. Moreover, long-term changes in glutamate synapses following drug exposure may be critically involved in the persistent risk for relapse observed in drug addicts. With regard to changes in glutamate synapses following drug exposure, the vast majority of research has focused on changes in the

expression of iGluR subunits (see chapter 3). For example, the incubation of drug seeking behavior, a time-dependent increase in drug seeking behavior, which is thought to represent an intensification in drug craving over time, is associated with an increase in GluA2-lacking AMPA receptors in the NAc (Conrad et al., 2008). Similarly, cue-induced reinstatement of heroin seeking has been associated with a reduction in synaptic membrane AMPA receptor expression in the medial PFC (mPFC), and particularly the internalization of the GluA2 receptor subunit (Van den Oever et al., 2008).

Drug Exposure-Induced Changes in Synaptic Plasticity

Changes in the expression and trafficking of glutamate receptors and subunits are thought to be central to the regulation of synaptic plasticity, the activity-dependent strengthening or weakening of the connection between neurons (Malinow & Malenka, 2002). Long-term potentiation (LTP), which is a sustained increase in the strength of transmission between neurons brought about by high frequency stimulation of the afferent neuron, is a particularity important form of synaptic plasticity, as it is thought to be the cellular correlate of learning and long-term memory (Malenka, 1994; Malinow & Malenka, 2002). The induction of LTP depends heavily on the activation of NMDA and AMPA glutamate receptors. In brief, high frequency stimulation causes a sustained activation of AMPA receptors, which allows sodium to enter the post-synaptic cell causing it to depolarize. Following this depolarization the blockade of NMDA calcium channels by magnesium is removed and calcium enters the cell. As calcium concentration increases, it leads to a cascade of biochemical events, resulting in a more efficient synapse (Malenka, 1994; Malenka & Nicoll, 1999).

I have focused on synaptic plasticity within the glutamateric projection from the vSub to the NAc shell following heroin self-administration and subsequent acute FD-induced reinstatement. There are several reasons for the interest in this pathway. Firstly, both the NAc and vSub have been shown to play a role in the reinforcing aspects of drugs. As discussed above, most, if not all, drug of abuse facilitate the release of NAc DA and glutamate (Di Chiara & Imperato, 1988). Furthermore, inactivation of the NAc, with a mixture of the $GABA_A$ and $GABA_B$ receptor agonists muscimol and baclofen, or targeted blockade of the DA D1 receptor within this region, attenuate reinstatement of drug seeking behavior (Bossert, Poles, Wihbey, Koya, & Shaham, 2007; McFarland & Kalivas, 2001; McFarland, Davidge, Lapish, & Kalivas, 2004). Similarly, activation or transient inactivation of the vSub, a major source of output from the hippocampal formation, has been shown to increase amphetamine-induced locomotor activity (Riegert et al., 2004) and attenuate cocaine-priming induced reinstatement (Cooper et al., 2006a), respectively. Moreover, the vSub is an important regulatory input to the NAc. Activation of the vSub by electrical stimulation or inactivation of this structure with tetrodotoxin (TTX) has been shown to increase or block, respectively, extracellular NAc DA release. Conversely, DA, through activation of post-synaptic DA D1 receptors, increases the drive of NAc neurons by vSub input (Goto & Grace, 2005a).

Importantly, drugs of abuse can modulate synaptic plasticity within the vSub to the NAc pathway. For instance, repeated cocaine treatment, leading to behavioral sensitization, has been shown to disrupt the induction of LTP within the NAc following tetanic stimulation of the vSub (Goto & Grace, 2005b). Furthermore, stress induced by acute (12h) withdrawal from chronic morphine administration has been shown to blunt

the induction of LTP in the vSub-NAc shell pathway, an effect which is absent after 4 days of withdrawal (Dong, Cao, & Xu, 2007). The involvement of this pathway in acute FD-induced reinstatement is currently unknown and the subject of further investigation in the current thesis.

The Relationship between DA, Glutamate, and Stress

Until now, I have discussed the roles of DA and glutamate in the reinstatement of drug seeking separately; however, the interaction between these two neurotransmitter systems is critical for the development of addiction. At the level of the VTA, glutamate acts to increase the activity of DA cellbodies, which in turn results in an increase in NAc DA release (Suaud-Chagny, Chergui, Chouvet, & Gonon, 1992; Westerink, Kwint, & deVries, 1996). Similarly, an interaction between DA and glutamate in the NAc is indicated by the finding that DAergic projections from the VTA and glutamatergic projections from the mPFC and BLA to the NAc can, in some cases, terminate on the same medium spiny neurons (French & Totterdell, 2002; French, Hailstone, & Totterdell, 2003; Sesack & Pickel, 1992). Furthermore, stimulation of regions which provide glutamatergic input to the NAc may presynaptically modulate DA by acting upon glutamatergic receptors in the NAc (Blaha, Yang, Floresco, Barr, & Phillips, 1997; Floresco, Yang, Phillips, & Blaha, 1998). Also, DA can act to modulate NAc glutamate release. For instance, intra-NAc infusion of the DA agonists, amphetamine or quinpirole, cause a dose dependent reduction in extracellular NAc glutamate, an effect that can be prevented by administration of the DA D1 or D2 receptor antagonists SCH 23390 or sulpiride, respectively (Kalivas & Duffy, 1997).

The relationship between DA and glutamate can be shaped by previous drug exposure. For example, repeated cocaine exposure has been shown to increase the behavioral response to intra-NAc infusion of the glutamate receptor agonist, AMPA, but only in rats having developed behavioral sensitization and not in rats that received the same cocaine exposure but have not developed behavioral sensitization (Pierce, Bell, Duffy, & Kalivas, 1996). On a related note, footshock stress, a common trigger for stress-induced reinstatement, has been shown to facilitate the release of CRF in the VTA in both rats trained to self-administer cocaine and control rats trained to self-administer saline. However, a reinstatement of operant behavior and an elevation of VTA glutamate was only observed in cocaine-trained rats (Wang et al., 2005). Wang et al. (2005) also report that VTA infusion of α-helical CRF prevented reinstatement and VTA glutamate and DA release, suggesting that in cocaine-trained animals CRF acquired the ability to modulate VTA glutamate and subsequent DA release. Thus, the relationship between CRF, released by stress, glutamate, and DA appears to result from neuroadaptions occurring over the course of drug use.

The effect of chronic heroin exposure on VTA glutamate release has been less well studied. However, systemic or intra-VTA administration of dizocilpine, a non-competitive NMDA receptor antagonist, leads to an increase in the rate of heroin self-administration and a rightward shift in the dose response curve, suggesting that glutamate receptors in the VTA are involved in mediating the reinforcing effects of opiates (Xi & Stein, 2002).

Rationale of the Current Studies

The following thesis employed the reinstatement procedure to elucidate the neuronal mechanisms mediating acute FD stress-induced reinstatement of heroin seeking. To that end, three approaches have been taken. In chapter 1 the involvement of the DA D1 receptor, in particular regions of the mesolimbic DA system, in acute FD-induced reinstatement was investigated by local intracranial microinjections, of the D1 receptor antagonist SCH 23390. This approach has allowed us to identify areas where DA function is critical for the expression of acute FD-induced reinstatement and highlight differences between this circuitry and the circuitry mediating other forms of reinstatement. The goal in chapters 2 and 3 was to investigate how acute FD may act to modulate synaptic plasticity as a potential mechanism underlying reinstatement of drug seeking. In chapter 2, I present the investigation of acute FD- and heroin exposure-associated changes in the induction of LTP, a model of learning and memory, in the pathway from the vSub to the NAc shell. This pathway has been shown to be involved in synaptic changes relating to both cocaine and morphine use, and may play a role in the perversion of normal learning apparent in heroin addiction. Finally, in the study presented in chapter 3, the technique of Western immunoblotting was used to measure the expression of GluA1, GluA2, and NR1 glutamate receptor subunits in rats that have been trained to self-administer heroin and subsequently exposed to an acute 21h FD-induced reinstatement test. Adaptations in glutamatergic synapses, and more specifically, changes in the expression of iGluR subunits, have been shown to underlie drug seeking during periods of abstinence. Collectively these studies will help to elucidate the neuronal

mechanisms involved in stress-induced reinstatement which may aid in the development of targeted interventions to prevent stress-induced relapse in human drug abusers.

GENERAL METHODOLOGY

Subjects

A total of 327 (chapter 1: $n = 222$, chapter 2: $n = 42$, chapter 3: $n = 63$) male Long Evans rats (Charles River, St. Constant, QC) were tested in squads of 10 over a period of several years. Prior to surgery rats were pair-housed in shoebox cages for approximately 1 week to acclimate them to daily handling as well as facility light (chapters 1 & 3: reverse 12:12 h light-dark cycle; lights off at 9:30 a.m.; chapter 2: normal 12:12h light-dark cycle; lights on at 8:30 am) and temperature (21 °C) conditions. Once the animals reached a bodyweight of approximately 350 g intravenous (IV) catheterization or a combination of IV catheterization and intracranial cannulation or electrode implantation was preformed. Following surgery, rats were singly housed for a 36-48h recovery period prior to being placed in operant chambers where they remained for the duration of experimentation (except for drug-naïve rats, see chapters 2 and 3). With the exception of acute FD-induced reinstatement tests, all rats had *ad libitum* access to food and water. Procedural approval was granted by the Concordia University Animal Care Committee and animals were treated in accordance with the Canadian Council on Animal Care.

Surgery

To allow for drug self-administration and to minimize differences between drug-naïve control animals and those administering heroin all rats were implanted with IV catheters. Catheters were constructed from Silastic tubing (Internal Diameter: 0.51 mm, Outer Diameter: 0.94 mm; Dow Corning, Midland, MI, USA) cut to a length of 12 cm.

During surgery animals in chapters 1 and 2 also received bilateral guide cannulae aimed 2 mm above the NAc shell, NAc core, vmPFC, dmPFC or BLA (for specific details refer to chapter 1) or were implanted with a bipolar stimulating electrode into the vSub and a recording electrode into the NAc shell (for specific details refer to chapter 2).

Rats were anesthetized with a mixture of xylazine and ketamine (10 + 100 mg/kg, i.p.) or with isoflurane (5-1.5%, see chapter 3) and given saline (2 ml, SC) to prevent dehydration. Once fully anesthetized, as confirmed by the absence of a reflex following a pinch to the hind paw, two incisions were made: one on the skull and one on the neck (approximately 1 cm above the ventral side of the shoulder blade). The jugular vein was isolated, cleaned of all surrounding tissue, and a small incision was made. Through this incision the bottom 3 cm of the catheter was inserted and secured in place with three silk sutures. The remaining portion of catheter was threaded subcutaneously to the skull, where it was attached to a modified 22-guage cannula (Plastics One Industries, Roanoke, VA). Depending on the experiment, guide cannulae (chapter 1) or electrodes (chapter 2) were inserted through burr holes drilled on the skull. Once in place, all cannulae or electrodes were mounted to the skull using jewelers' screws and dental cement.

Following surgery rat were given penicillin (450,000 IU/rat) to prevent infection, and the analgesic buprenorphine (10 μg/kg; Schering-Plough Ltd., Welwyn Garden City Hertfordshire, UK) to relieve pain. Catheters were also flushed, on a daily basis, throughout drug training with heparin and gentamicin (7.5 IU + 12.0 μg/rat/day) to maintain patency.

Apparatus

Following approximately 48h recovery all non-drug-naïve animals were moved from their home cages, in the animal care facility, and placed in operant chambers (Coulbourn Instruments, Allentown, PA, USA; 29.0 cm x 29.0 cm x 25.5 cm) where they were individually housed for the duration of experimentation. Each chamber consisted of two Plexiglas walls, one front and one back, and two metal panel sidewalls. Two retractable levers, an 'active' drug-paired and an 'inactive' non-drug paired, were located on the right side wall approximately 11 cm above the floor. A cuelight was located above each lever, a tone emitter (2.9 KHz tone module, Coulbourn Instruments, Allentown, PA, USA) was located above the active lever and a houselight was positioned in the top center of the left sidewall. The floor of the operant chamber was composed of stainless steel rods to allow for the collection of waste into a removable tray filled with Betachip bedding. Each chamber was enclosed in a sound attenuating wooden compartment equipped with a fan for ventilation and external noise masking.

Responding on the active lever was paired with the delivery of 0.13 ml of heroin solution, inactivation of the houselight, activation of the tone module and illumination of a cuelight above the active lever. Drug infusions, active lever presses, and inactive lever presses were recorded using Graphic State Software and a personal computer connected to the operant chambers via linc boxes (Coulbourn Instruments, Allentown, PA, USA). To allow for drug infusions rats were attached to a liquid swivel (Instech Swivel Assembly, Med Associates, St. Albans, VT, USA) and pump (Harvard Apparatus, St. Laurent, QC, Canada) via Tygon tubing (Internal Diameter: 0.50 mm, Outer Diameter:

1.52 mm; Saint-Gobain Performance Plastics, Granville, NY, USA), shielded by a metal spring.

Drugs

Heroin (diacetylmorphine HCL; National Institute for Drug Addiction, Baltimore, Maryland, USA) was dissolved in sterile saline to produce a stock solution of 5 mg/ml. This stock was further diluted with physiological saline and stored in 20 ml heroin syringes resulting in a concentration of 0.10 mg/kg/infusion.

General Procedure

The experiments to be described in the following chapters employ the same basic procedure (specific details and exceptions will be described in each chapter). In all chapters, except chapter 1, experimental animals consisted of heroin-trained and heroin-naive control rats. Heroin-naive rats remained in the animal care facility and were handled in an equivalent manner to heroin-trained rats, but were never exposed to the operant training chambers and never received heroin. Prior to the commencement of training, all heroin-trained animals were given one day of habituation, which consisted of placing the animals in their respective operant chambers for a period of approximately 24h. During this time levers were not extended and no discrete drug-associates cues were present. Following habituation rats were trained to self-administer heroin (0.10 mg/kg/infusion) for a minimum of 10 days. The nature of the training procedure differed across experiments. However, in all experiments the first 5 days of training consisted of three 3h sessions, separated by a 3h interval. In the experiments described in chapters 1 and 3 the first session began shortly after the onset of the dark phase (approximately 10:00 a.m.); whereas, in experiment 2 the first session began during the light phase and

started at approximately 1:00 p.m. The beginning of the first session was marked by the insertion of both levers, the turning on of a houselight and the activation of the cuelight/tone complex located above the active drug-paired lever. This complex remained on for 30s or until the first active lever response. Responding on the active lever resulted in a 5s drug infusion (0.10 mg/kg/infusion; volume 0.13 ml) and a 20s timeout period during which the houselight remained off, the cuelight/tone complex remained on and no further drug was available. At the end of each session the active lever was retracted but the inactive lever remained extended in order to increase the discrimination between the active and inactive lever.

Following self-administration training, the drug was removed and all animals entered a period of extinction. Extinction training followed the same procedure as self-administration training except that active lever responding did not result in the delivery of heroin. In addition, unless noted otherwise, extinction training consisted of one 3h session per day. After a minimum of 4 extinction sessions, drug seeking was considered extinguished if responding on the previously active lever fell at or below an extinction criterion of 15 or 20 responses (per 3-h extinction session). At this point the animal was subject to reinstatement testing in a counterbalanced order while sated or following acute 21 or 48h FD. In the experiments described in chapter 1, rats were intracranially infused with the DA D1 receptor antagonist SCH 23390, approximately 5-10 min prior to the commencement of reinstatement testing. In the experiments described in chapters 2 and 3, rats received no special treatment prior to the test. However, in these experiments the reinstatement test period was reduced to a single 2h session. The electrophysiological

recording or the decapitation and removal of the brain for later analysis was preformed immediately after this test (see specific details in each chapter).

Histology

In order to verify cannulae and electrode placements rats were first overdosed with pentobarbital sodium (Euthanyl, 72-120 mg/rat, ip). Once unconscious, rats with guide cannulae (chapter 1) were infused with 0.5 ul of Evans Blue dye. The injector extended 2 mm below the tip of the guide cannulae. Following euthanasia, confirmed by the lack of a pulse, rats were decapitated and their brains were rapidly removed. Rats used in the experiments described in chapter 2 were overdosed with Euthanyl and transcardially perfused with phosphate buffered saline and 4% paraformaldehyde. In chapter 1, brains were soaked in 4% paraformaldehyde for approximately 48h; whereas, in chapter 3 brains were transferred from paraformaldehyde to a 30% sucrose+paraformaldehyde solution after 4h. Brains remained in sucrose+paraformaldehyde solution until sinking. Following fixation, brains were frozen with dry ice and stored at -80° C. Finally, brains were sliced using a cryostat and sections of 30 µm were placed on gel coated slides. Brain slices were treated with cresyl violet and coverslipped for placement verification. Slides were inspected and placement was determined by the most ventral portion of dye, in the case of guide cannulae, or the most ventral portion of tract, in the case of electrodes. Rats used in the experiments described in chapter 3 were rapidly decapitated and their brains were flash-frozen for 5s in isopenthane and stored at -80° C. Brains were later sliced on the cryostat and bilateral tissue punches (0.8-1.6 mm^2) were collected from the BLA, dmPFC, NAc and VTA (see

chapter 3 for specific details on the preparation of tissue punches and Western immunoblotting).

CHAPTER 1

THE ROLE OF THE DOPAMINE D1 RECEPTOR IN ACUTE FOOD DEPRIVATION-INDUCED REINSTATEMENT OF HEROIN SEEKING

ABSTRACT

The neurotransmitter DA has been heavily implicated in both the learning and rewarding aspects of drug use and the transition from controlled to compulsive drug seeking. Moreover, a role for DA has been firmly established with regard to both drug-induced (priming) and drug cue-induced reinstatement of extinguished drug-seeking behavior, two variations of an animal model of relapse. Until recently, stress-induced reinstatement, in rodents, has been studied almost exclusively by using inescapable footshock stress. In this form of reinstatement DA is thought to play a somewhat limited role. However, our laboratory has recently demonstrated that systemic administration of the DA D1 receptor antagonist, SCH 23390, will attenuate acute FD-induced reinstatement of heroin seeking. In contrast, systemic injection of SCH 23390 has no effect on footshock-induced reinstatement. Thus, the current study was designed to elucidate the neuronal regions critical to the effect of SCH 23390 on acute FD-induced reinstatement of heroin seeking. In brief, rats were trained to self-administer heroin (0.1 mg/kg/infusion) over a minimum of 10 days. Following training, heroin was removed leading to an extinction of responding. Next, rats were tested twice, under extinction conditions, for reinstatement: once following acute 21-48h FD and once under sated conditions. Prior to testing, SCH 23390 was administered into the NAc shell (0.0, 0.3, 0.6 ug/side), NAc core (0.0, 0.3, 0.6 ug/side), BLA (0.0, 1.0, 2.0 ug/side), dmPFC; 0.0, 0.2, 2.0 ug/side or vmPFC; 0.0, 2.0 ug/side. An attenuation of drug seeking behavior was seen in rats pre-treated with SCH 23390, at the highest dose, in the NAc shell, dmPFC, or BLA suggesting that acute FD-induced reinstatement is dependent upon activation of DA D1 receptors in these regions, but not, the NAc core or vmPFC.

INTRODUCTION

Drug addiction is a devastating disease characterized, in part, by recurrent cycles of abstinence and relapse. Understanding this cyclicity, and more specifically, factors mediating the transition from a drug-free state to an active state of drug seeking is of particular importance in developing effective strategies to treat addiction. In humans, the study of relapse poses several ethical and practical concerns. Fortunately, relapse can be modeled, in animals, using the reinstatement procedure; a procedure in which animals have been found to return to extinguished drug seeking, under abstinent conditions, following a variety of manipulations. Studies employing the reinstatement procedure have consistently demonstrated a role for the neurotransmitter DA in drug seeking. Interestingly, DA has been heavily implicated in the acute rewarding effects of abused drugs as well as the learning of associations between drug effects and stimuli predicting drug availability or consumption (Spanagel & Weiss, 1999; Wise, 2004; 2008). With regard to the reinstatement of drug seeking, DA is critically involved in both drug priming- and cue-induced reinstatement (for examples see Bossert et al., 2005; de Wit & Stewart, 1983; Shalev, Grimm, & Shaham, 2002). However, the role of DA in stress-induced reinstatement has yet to be fully elucidated.

Historically, research focusing on the effect of stress on the reinstatement of drug seeking behavior, has tended to utilize inescapable footshock stress to trigger drug-seeking. These studies have revealed an attenuation of drug seeking following systemic administration of the mixed DA D1/D2 receptor antagonist flupenthixol, but not the selective DA D1 or DA D2 receptor antagonists SCH 23390 or raclopride, respectively (Shaham & Stewart, 1996). In contrast, systemic injection of SCH 23390, at a dose

substantially lower than that used by Shaham and Stewart (1996), has been shown to attenuate reinstatement in rats exposed to acute 48h FD stress (Tobin et al., 2009). This finding suggests that the role of DA in stress-induced reinstatement may be dependent upon the nature of the stressor used. Thus, while the neuronal circuitry involved in footshock-induced reinstatement has been thoroughly investigated, it cannot be assumed to be the same as the neuronal circuitry underlying acute FD-induced reinstatement. Here, the role of the DA D1 receptor in acute FD-induced reinstatement is studied by selectively blocking this receptor in the NAc shell, NAc core, BLA, dmPFC and vmPFC.

The NAc and the Reinstatement of Drug Seeking

The NAc is a central component of the mesolimbic DA pathway and receives a large DAergic input from the VTA. Mesolimbic DA, particularly DA release in the NAc, has been consistently linked to the attribution of reward value. For instance, microdialysis studies, measuring extracellular DA release in the NAc, have shown an increase in the release of DA following the presentation of rewarding stimuli such as specific foods, exposure to drugs of abuse, and following BSR (Di Chiara et al., 2004; Hernandez & Hoebel, 1988; Spanagel, Herz, & Shippenberg, 1990; Wise, Leone, Rivest & Leeb, 1995). The majority of early dialysis studies do not distinguish between DA release in the NAc shell and NAc core. However, the dissociation between these structures is crucial since the NAc is a functionally heterogeneous structure. Anatomically, the NAc is poised to act as an interface between the limbic system, involved in regulating the desire to use drugs, and the motor system, involved in directing the behavioral output necessary to procure drugs. This interface is mediated by the NAc shell and NAc core, respectively (Groenewegen & Trimble, 2007; Mogenson, Jones, & Yim, 1980). Recently, it has been

demonstrated that conditioned stimuli associated with food increase extracellular DA release in the NAc core, but not the NAc shell; whereas, conditioned stimuli associated with morphine increase NAc shell, but not NAc core, DA release (Bassareo, Musio, & Di Chiara, 2011). Furthermore, rats have been shown to self-administer both DA agonists and psychostimulant drugs directly into the NAc shell, but not the NAc core (Carlezon & Wise, 1996; Ikemoto, Glazier, Murphy, & McBride, 1997; McKinzie, Rodd-Henricks, Dagon, Murphy, & McBride, 1999). In contrast, transient inactivation of the NAc core, but not NAc shell, with a combination of the $GABA_A$ and $GABA_B$ agonists muscimol and baclofen, respectively, has been shown to abolish both cue- and priming-induced reinstatement of cocaine seeking (Fuchs, Evans, Parker, & See, 2004; McFarland et al., 2004). Whereas, transient inactivation of either the NAc shell or core has been shown to block footshock-induced reinstatement of cocaine seeking (McFarland et al., 2004). Similar to the findings with cocaine-trained animals, transient inhibition of the NAc core, but not shell, has been shown to attenuate cue-induced reinstatement of heroin seeking. In contrast, heroin priming-induced reinstatement of heroin seeking is attenuated by transient inhibition of the NAc shell or NAc core (Rogers, Ghee, & See, 2008). Collectively, these studies suggest that the neuronal circuitry mediating reinstatement may differ as a function of the reinstatement trigger and as a function of previous drug history.

The BLA and Reinstatement of Drug Seeking

The BLA is thought to be critically involved in the regulation of associative conditioning processes involved in learning the relationship between a stimulus and a reward. Such learning is central to the maintenance and reinstatement of drug seeking, as

it underlies the attribution of salience to cues paired with drug self-administration (Grimm & See, 2000; Kantak, Black, Valencia, Green-Jordan, & Eichenbaum, 2002; Meil & See, 1997). The role of the BLA in addiction has been most extensively investigated with regard to psychostimulant drugs. Studies of this nature have shown that lesioning or pharmacological inactivation of the BLA, but not the central amygdala, interferes with the acquisition of a cocaine or amphetamine CPP, and reduces responding for cocaine on a second order schedule of reinforcement (Fuchs & See, 2002; Hiroi & White, 1991). Both CPP and second order schedules of reinforcement are paradigms in which behavior is maintained largely by drug associated cues (Bossert et al., 2005; de Wit & Stewart, 1983; Shalev et al., 2002; Whitelaw, Robbins, & Everitt, 1996). In contrast, self-administration of cocaine on a fixed ratio (FR) schedule of reinforcement, is not effected by lesions of the BLA, suggesting that the BLA is not involved in the primary reinforcing aspects of cocaine (Meil & See, 1997). Furthermore, lesions of the BLA have been shown to have no effect on heroin self-administration under a continuous or second order schedule of reinforcement, suggesting that the BLA is not critically involved in maintaining heroin seeking (Alderson, Robbins, & Everitt, 2000).

Despite these findings, the BLA appears to be crucially involved in the reacquisition of behaviors previously associated with cocaine and heroin self-administration. For instance, cocaine seeking can be triggered, in abstinent animals, by electrical or pharmacological stimulation of the BLA (Hayes, Vorel, Spector, Liu, & Gardner, 2003; Ledford, Fuchs, & See, 2003). Similarly, lesioning or transient inhibition of the BLA has been shown to block cue- and context-induced reinstatement, but has no effect on cocaine priming- or footshock-induced reinstatement of cocaine seeking (Fuchs

et al., 2005; McFarland et al., 2004; Meil & See, 1997). In contrast to findings with psychostimulant drugs, inactivation of the BLA with TTX has been shown to abolish both cue- and drug priming-induced reinstatement of heroin seeking. Similarly, inactivation of the BLA blocks the facilitation of CPP following a single reconditioning exposure to heroin in the previously heroin-paired context (Rizos, Ovari, & Leri, 2005). Thus, the BLA may play a dissociable role in reinstatement depending upon the drug type (Fuchs & See, 2002).

The mPFC and Reinstatement of Drug Seeking

The mPFC is a central component in the regulation of the mesolimbic pathway. Pyramidal neurons in the mPFC send glutamatergic input to VTA DA neurons, which in turn project to the mPFC (Carr & Sesack, 2000; Floresco, West, Ash, Moore, & Grace, 2003). Furthermore, DA within the mPFC, acts to regulate glutamatergic output to subcortical regions involved in regulating drug seeking, such as the NAc (Gulley & Stanis, 2010; Kalivas, Pierce, Cornish, & Sorg, 1998; Robinson & Berridge, 1993). This network of anatomical connections to and from the mPFC has been shown to regulate drug seeking. For instances, rats will self-administer cocaine, a DA agonist, directly into the mPFC, an effect which is blocked by a 6-hydroxydopamine (6-OHDA)-induced selective lesions of DA neurons, and is restored by DA infusion (Goeders & Smith, 1986). Furthermore, administration of cocaine into the mPFC will reinstate cocaine seeking in rats previously trained to self-administer cocaine, suggesting that DA transmission in the mPFC is critical for drug seeking (Park et al., 2002).

In rodents, the mPFC can be subdivided into two functionally distinct regions: the dmPFC consisting of the cingulate and prelimbic cortices and the vmPFC consisting of

the infralimbic cortex and the dorsal peduncular nucleus. These regions have been proposed to play unique roles in drug seeking and extinction, respectively. For instance, discriminative stimuli previously paired with cocaine produce both a reinstatement of drug seeking behavior and induce Fos expression in the prelimbic and cingulate regions of the dmPFC; both of which are reversed by a systemic injection of the DA D1 receptor antagonist SCH 23390 (Ciccocioppo et al., 2001). Furthermore, targeted blockade of the DA D1 or D2 receptor at the border of the prelimbic and cingulate cortex, has also been shown to significantly reduce reinstatement of drug seeking following the administration of a cocaine prime (Sun & Rebec, 2005; but see Capriles, Rodaros, Sorge, & Stewart, 2003 for contradicting findings). Similarly, infusion of SCH 23390 in the prelimbic cortex attenuates heroin priming-induced reinstatement of drug seeking (See, 2009), as well as footshock-induced reinstatement of cocaine seeking (Capriles et al., 2003). Moreover, transient inactivation of the prelimbic cortex with TTX or a combination of muscimol and baclofen, blocks cue-, footshock-, and cocaine-priming-induced reinstatement of cocaine seeking (Capriles et al., 2003; McFarland et al., 2004; McLaughlin & See, 2003).

In contrast, inactivation of the vmPFC, with muscimol and baclofen, immediately following extinction training or prior to reinstatement testing, impairs extinction learning and reinstates extinguished cocaine seeking, respectively. Thus, it has been suggested that the vmPFC is an inhibitory structure which facilitates the extinction of drug seeking (Peters, LaLumiere, & Kalivas, 2008; LaLumiere, Niehoff, & Kalivas, 2010). However, the inhibitory role of the vmPFC in drug seeking may not be consistent across drug classes. Recently, Bossert et al. (2011), demonstrated that inactivation of the infralimbic

cortex decreases context-induced reinstatement of heroin seeking; yet, inactivation of the infralimbic cortex has no effect on cue-induced reinstatement of cocaine seeking (McLaughlin & See, 2003).

The Current Studies

Here, the role of DA transmission in acute FD-induced reinstatement was investigated in an effort to elucidate the neuronal circuitry underling this form of reinstatement. More specifically, the role of the DA D1 receptor in the NAc shell (Experiment 1), NAc core (Experiment 2), BLA (Experiment 3), dmPFC (Experiment 4) and vmPFC (Experiment 5) was investigated by localized administration of the D1 antagonist SCH 23390. It was hypothesized that blockade of DA D1 receptors in the NAc shell and dmPFC would attenuate drug-seeking behavior. As discussed above, these structures have been extensively investigated for their role in addiction and more specifically the reinstatement of drug seeking under extinction conditions. The role of DA D1 receptors in the NAc core, BLA, and vmPFC in acute FD-induced reinstatement is less clear. Inactivation studies suggest that the NAc core, but not the BLA, plays a critical role in stress-induced reinstatement of psychostimulant drugs (McFarland et al., 2004); whereas, the vmPFC has been shown to play an inhibitory role with regard to cocaine, but not heroin seeking. Furthermore, the role of DA transmission in these areas, as it relates to stress-induced reinstatement, has not been extensively investigated. Additionally, the vast majority of studies have investigated stress-induced reinstatement of cocaine seeking and relatively few studies exist focusing on the neural circuits involved in the reinstatement of heroin seeking. Finally, there are no studies addressing the role of DA, in the regions investigated here, in acute FD-induced reinstatement.

METHODS

Subjects

A total of 222 (Experiment 1: *n* = 70; Experiment 2: *n* = 40; Experiment 3: *n* = 45; Experiment 4: *n* = 47; Experiment 5: *n* = 20) male Long Evans rats (Charles River, St. Constant, QC) were tested in squads of 10 or less. As described previously, rats were pair-housed prior to surgery and given time to acclimate to daily handling and facility conditions. Once the animals reached a bodyweight of approximately 350 g, IV catheterization and intracranial cannulation surgeries were preformed. Following surgery, rats were given a recovery period and placed into the operant chambers for heroin self-administration.

Surgery

In brief, rats were implanted with IV catheters into the right jugular vein (see general methodology) and bilateral guide cannulae (22 gauge) were aimed 2 mm above the NAc shell (AP: +1.7, ML: +/- 3.7; 20°, DV: -5.8 from destination skull); NAc core (AP: +1.7, ML: +/- 2.5, 6° angel; DV: -5.5); BLA (AP: -2.5, ML: +/- 5.0, DV: -6.6); dmPFC (AP: +3.0, ML: +/- 1.5, 10°, DV: -1.7); and vmPFC (AP: +3.0, ML: +/- 1.3, 10°, DV: -3.0). Coordinates were based upon previous research and the atlas of Paxinos and Watson (2005). After the last reinstatement test rats were injected with a fatal dose of Euthanyl and infused with Evans Blue dye (0.3 ul/side) 2 mm below the guide cannulae to verify the location of drug delivery. Subsequently rat brains were removed, soaked in 4% paraformaldehyde for approximately 48h, frozen with dry ice, and stored at -80 °C. For histology brains were sliced on a cryostat, mounted and stained for later placement verification.

Drugs & Microinjection Procedure

Heroin was diluted in saline to produce a stock solution (5 mg/ml). Stock heroin was further diluted with saline in 20 ml syringes to produce a final concentration of 0.1 mg/kg/infusion. The DA D1 receptor antagonist SCH 23390 was diluted in sterile saline to produce the following region specific doses: NAc (shell and core): 0.0, 0.3 and 0.6 μg/side; BLA 0.0, 1.0 and 2.0 μg/side; mPFC (dmPFC and vmPFC): 0.0, 0.2 and 2.0 or 0.0 and 2.0 μg/side. These doses were chosen to reflect the range of doses used with the existing literature (for examples see: Anderson et al., 2003; Berglind, Case, Parker, Fuchs, & See, 2006; Bossert et al., 2007; Nair et al., 2011; See, 2009).

Microinjections were given 5-10 min prior to the start of reinstatement testing. Intracranial infusions were made using a syringe pump (Harvard Apparatus, Holliston, MA, USA) connected to a 10 ul Hamilton syringe. This syringe was attached via polyethylene-20 tubing to a 28-gauge injector, which was cut to extend 2 mm below of the guide cannulae. Injections for all regions of interest were made over 1 min and the injectors were left in place for 1 min following the infusion. For mock injections (see below) the syringe pump was run for 1 min and short injectors, not extending beyond the guide cannulae, were inserted into the guide cannulae but were not attached to the infusion pump.

Experimental Procedure

The following five experiments employed the procedure described previously in the general methodology section; however, there are several exceptions, which are noted below. Depending upon the experiment, animals were given one day of autoshaping before starting self-administration training. Autoshaping consisted of a single 1h session

during which the levers were not extended and heroin delivery was not contingent upon a response. During this session animals received 6 heroin infusions, each paired with the same cues as during training. Small methodological differences between experiments reflect procedural tweaking over the course of the project, meant to optimize the testing conditions.

Training & Reinstatement Testing

Experiment 1 & 2: Administration of the DA D1 receptor antagonist, SCH 23390, into the NAc shell or core

Animals were trained to self-administer heroin on an FR-1 schedule of reinforcement over a minimum of 10 days (days 1-5: three 3h sessions; days 6-10 one 3h session). Animals that demonstrated substantially lower active lever responding, relative to the others in their cohort, or showed an inability to distinguish between the active and inactive lever were given extra self-administration training sessions. Following training, rats were given a minimum of four 3h extinction sessions during which heroin syringes were removed but all the cues associated with heroin self-administration remained.

Beginning on the third day of extinction and continuing throughout extinction training, rats received mock intracranial infusions. Upon reaching a criterion of 15 or fewer responses on the previously active lever, heroin seeking was considered extinguished and reinstatement testing commenced. Under extinction conditions, rats received two 3h reinstatement test sessions separated by a minimum of one extinction session and until the extinction criterion was met again. Prior to testing, rats were given a 48h ‘off’ period during which time they were food deprived or sated in a counterbalanced order. For the ‘off’ period animals remained in the operant chamber but no extinction

session was run. Approximately 5-10 min before the start of each reinstatement test rats were infused with one of three SCH 23390 (0.0, 0.3 and 0.6 μg/side) doses into the NAc shell or core. For FD tests, food hoppers were removed from the operant chamber and returned at the end of the session.

Experiment 3-5: Administration of the DA D1 Receptor Antagonist, SCH 23390, into the BLA, vmPFC or dmPFC

Animals were trained to self-administer heroin on an FR-1 schedule of reinforcement for 5 days and then moved to an FR-3 schedule for the last 5 days of training. Animals that had difficulty in maintaining responding following the change in reinforcement schedule were given extra training days and in some cases returned to the FR-1 schedule until responding improved. As in self-administration training, the first day of extinction consisted of three 3h sessions; however, all subsequent extinction days were reduced to one 3h session. For reinstatement testing rats were assigned to one of two or three SCH 23390 dose groups. (BLA: 0.0, 1.0 or 2.0 μg/side; dmPFC: 0.0, 0.2 or 2.0 μg/side; vmPFC: 0.0 or 2.0 μg/side) and given an infusion into the BLA, dmPFC, or vmPFC 5-10 min prior to reinstatement testing. Each animal received two counterbalanced tests, one under sated conditions and one following 21h FD.

Statistical Analysis

The effect of DA D1 receptor antagonism in the NAc, BLA or mPFC on acute FD-induced reinstatement of heroin seeking was preformed using SPSS Software v. 19.0.0 for Mac (IBM Inc., Armonk, New York, USA). A series of mixed factorial ANOVAs were preformed to investigate active and inactive lever responding following infusion of SCH 23390 into each region of interest. *Deprivation state* (baseline, FD,

sated) was used as a within subjects factor and *antagonist dose* (vehicle, low, high or vehicle, high) was used as a between subjects factor for all ANOVAs. Following significant ANOVA results, pair-wise comparisons were conducted using Sidak corrected t-tests to control for multiple comparisons. All analyses were evaluated for significance at $\alpha = .05$.

RESULTS

Experiments 1 & 2: Administration of the DA D1 receptor antagonist, SCH 23390, into the NAc shell or NAc core

Fifty animals were eliminated from the analysis of this data set. The reasons for these eliminations included: catheter failure or a failure to train ($n = 15$), sickness or death ($n = 6$), improper cannulae placements ($n_{both\ incorrect} = 10$; $n_{one\ correct} = 10$; $n_{one\ shell\ \&\ one\ core} = 7$; $n_{cannulae\ through\ ventricle} = 2$). A failure to train was considered apparent if rats responded an average of 15 times, or less, during the last 5 training sessions (3h sessions) or an average of 20 times, or less, across all 10 training sessions. Of the remaining 60 rats, half had placements localized in the NAc shell ($n = 30$) and half had placements localized in the NAc core ($n = 30$) (see *Figure 1*).

Training data did not differ between rats with placements located in the NAc shell or NAc core, across the 10 days of training, in terms of the number of heroin infusions, $F(1, 58) = 2.61$, $p = .11$, $\eta_p^2 = .04$, active lever responses, $F(1,58) = 2.76$, $p = .10$, $\eta_p^2 = .05$, or inactive lever responses, $F(1, 58) = 0.12$, $p = .73$, $\eta_p^2 = .01$, thus, the training data for these experiments were combined. As seen in *Figure 2*, rats showed a clear preference

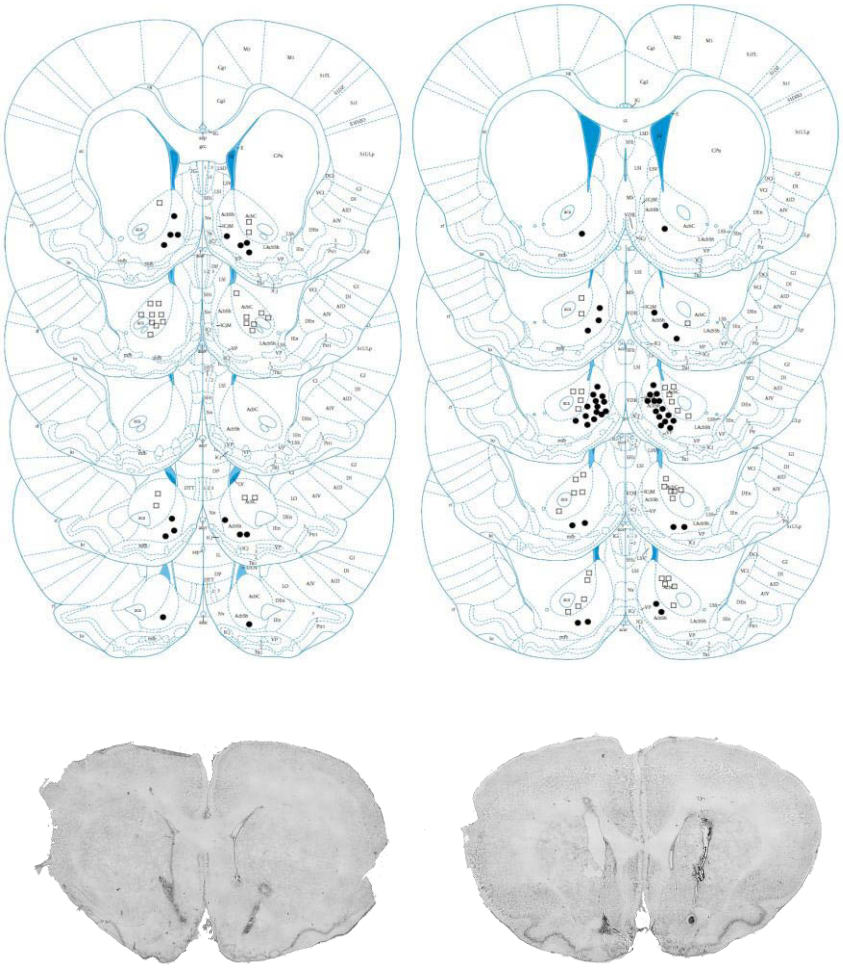

Figure 1.

Anatomical position of microinjector tips targeting the NAc. Top: closed circles indicate NAc shell placements (n = 30) and open squares indicate the NAc core (n = 30) placements. Images correspond to Figures 12-21 (2.76-1.44 mm from bregma) from the atlas of Paxinos & Watson (2005). Figures are arranged from top to bottom with Figure 12 being the bottom left and Figure 21 being the top right. Bottom: sample photomicrographs from the NAc shell (left) and core (right) placement.

for the 'active' drug-paired lever over the 'inactive' non-drug paired lever throughout training. Following training, rats received a mean (+/-*SEM*) of 6.83 (+/- 0.36) days of extinction training prior to their first reinstatement test and an additional 4.12 (+/- 0.34) extinction days prior to their second reinstatement test. The rate of extinction did not differ with respect to guide cannulae placement for either of the reinstatement tests, F_{test1} (1,58) = 0.21, p = .65, η_p^2 = .00; F_{test2} (1,58) = 2.70, p = .11, η_p^2 = .04.

Rats with guide cannulae targeted at the NAc shell or NAc core differed substantially in terms of how the administration of the DA D1 receptor antagonist SCH 23390 affected acute FD-induced reinstatement. Thus, these two placement groups are discussed separately for the remainder of the analyses. Prior to reinstatement testing rats were assigned to one of three antagonist dose groups (vehicle: n_{shell} = 7, n_{core} = 9; low: n_{shell} = 14, n_{core} = 8; high: n_{shell} = 9, n_{core} = 13). For rats with guide cannulae correctly targeting the NAc shell there were no significant differences between the antagonist dose groups in any of the training variables: infusions (M_{Veh} =18.69, *SEM* = 3.86; M_{Low} = 18.47, *SE* = 1.96; M_{High} = 17.42, *SEM* = 2.18), F(2,27) = 0.07, p = .94, η_p^2 = .01, active lever responses (M_{Veh} = 77.14, *SEM* = 27.96; M_{Low} = 56.17, *SEM* = 10.81; M_{High} = 41.04, *SEM* = 13.16), F(2,27) = 1.04, p = .37, η_p^2 = .07, or inactive lever responses (M_{Veh} = 6.06, *SEM* = 2.91; M_{Low} = 7.67, *SEM* = 2.61; M_{High} = 3.33, *SEM* = 0.71), F(2, 27) = .86, p = .44, η_p^2 = .06, during the last 5 days of training. For rat with guide cannulae correctly targeting the NAc core there were also no significant differences observed in the number of infusions (M_{Veh} =13.02, *SEM* = 1.65; M_{Low} = 18.68, *SEM* = 2.23; M_{High} = 18.05, *SEM* = 3.34), F(2,27) = 1.08, p = .35, η_p^2 = .07, active lever responses (M_{Veh} = 36.54, *SEM* =

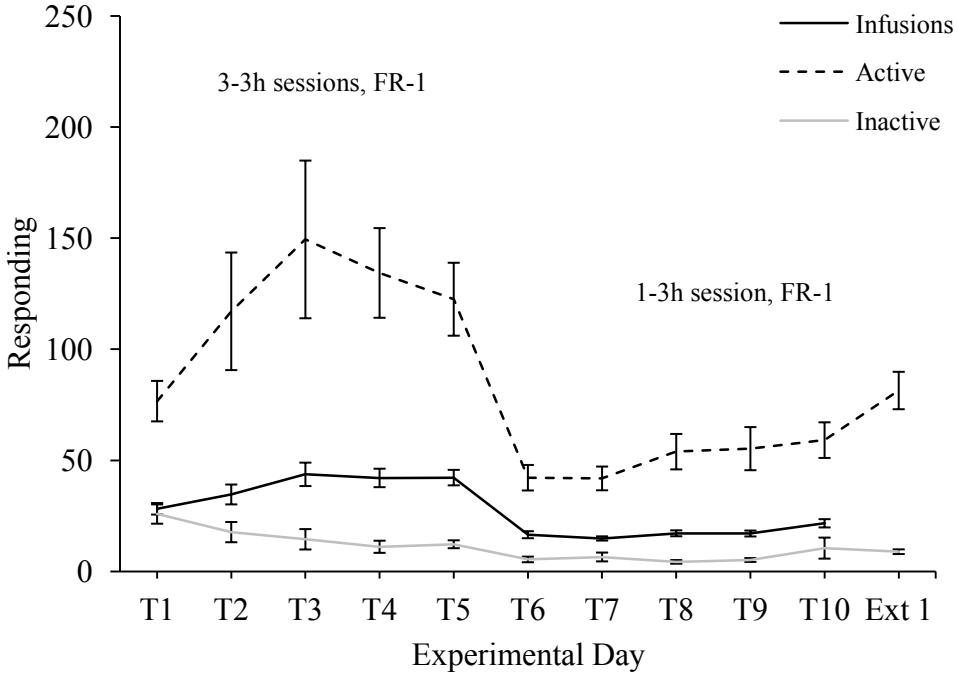

Figure 2.

Heroin self-administration for rats with guide cannulae located in the NAc. Data shown are the mean (+/- *SEM*) numbers of active lever presses, inactive lever presses and heroin infusions (0.1 mg/kg/infusion) for the 60 rats with confirmed placement in the NAc for the ten training days (T1-10; days 1-5: three 3h sessions, FR-1; days 6-10: one 3h session, FR-1) and the first extinction day (Ext 1). A significant difference existed between active and inactive lever responding on all training days and during extinction ($p < .05$).

6.66; M_{Low} = 51.40, *SEM* = 14.12; M_{High} = 45.55, *SEM* = 15.78), *F(*2,27) = .25, *p* = .78, η_p^2 = .02, or inactive lever responses (M_{Veh} = 3.51, *SEM* = 0.80; M_{Low} = 15.50, *SEM* = 11.63; M_{High} = 3.62, *SEM* = 0.84), *F(*2, 27) = 1.46, *p* = .25, η_p^2 = .01, between antagonist dose groups for the last 5 days of training.

Separate mixed factorial ANOVAs were preformed to evaluate the effect of SCH 23390, infused into the NAc shell or NAc core, on acute FD-induced reinstatement of active or inactive lever responding. Analysis of the number of active lever responses in rats with guide canulae aimed at the NAc shell, revealed a significant effect of *deprivation state*, *F(*2, 54) = 16.96, *p* < .001, η_p^2 = .39, *antagonist dose, F(*2, 27) = 3.33, *p* = .05, η_p^2 = .19 and *deprivation state* X *antagonist dose* interaction, *F(*4, 54) = 3.53, *p* = .01, η_p^2 = .20 (see *Figure 3A*). Follow-up analyses revealed a significant increase in active lever responding in the FD condition (*M* = 35.03; *SEM* = 5.86) compared to both the baseline (*M* = 10.48; *SEM* = 0.73) and sated (*M* = 10.95; *SEM* = 1.50) conditions (*p*s < .01). A one-way ANOVA assessing active lever responding on the deprivation test day revealed a significant effect of *antagonist dose*, *F(*2, 27) = 3.68, *p* = .04, η_p^2 = .21. Pair-wise comparisons revealed a significant reduction in responding in the high antagonist dose group relative to the vehicle group (*p* < .05). Next, separate one-way repeated measures ANOVAs were conducted for each antagonist dose group. These ANOVAs revealed a significant *deprivation state* effect in the vehicle, *F(*2,12) =17.00; *p* < .001, η_p^2 = .74 and low, *F(*2,26) = 6.57; *p* = .01, η_p^2 = .33, dose groups but not in the high antagonist dose group, *F(*2,16) = .60, *p* = .56, η_p^2 = .07. For rats in the vehicle group significantly more active lever responding was observed on the FD test day than on the BL or sated days (*p*s <. 05). A tendency toward increased active lever responding on the

FD test day relative to the BL (p = .08) and sated (p = .06) tests was also observed in the low antagonist dose group. Analysis of the number of responses on the inactive revealed no significant effect of *antagonist dose*, $F(2,54) = 2.35$, p = .11, $\eta_p^2 = .08$, *deprivation state*, $F(2,27) = 2.36$, p = .11, $\eta_p^2 = .15$, or *deprivation state* X *antagonist dose* interaction, $F(4,54) = 0.78$, $p = .54$, $\eta_p^2 = .06$.

For the NAc core, a significant effect of *deprivation state* on active lever responding, $F(2, 54) = 19.99$, $p < .001$, $\eta_p^2 = .43$, was observed. Further analyses revealed a significant increase in responding during the acute FD test (M = 36.46, SEM = 5.80) relative to the baseline (M = 10.87, SEM = 0.47) and sated (M = 10.84, SEM = 1.97) conditions (ps < .001). However, there was no significant effect of *antagonist dose,* $F(2,27) = 0.15$, $p = .86$, $\eta_p^2 = .01$, and no significant *deprivation state X antagonist dose* interaction, $F(4,54) = 0.47$, $p = .76$, $\eta_p^2 = .03$ (see *Figure 3B*). Acute FD also significantly increased responding on the inactive lever responding, $F(2, 54) = 10.09$, $p < .001$, $\eta_p^2 = .27$. Further analysis of this effect showed that acute FD (M = 6.85, SEM = 1.30) caused a significant increase in inactive lever responding relative to both the baseline (M = 3.43, SEM = 0.58) and sated (M = 1.92, SEM = 0.34) conditions, ps < .05. Moreover, baseline inactive lever responding was also shown to be greater than responding on the sated test day, $p < .05$. Non-specific responding on the inactive lever is not an unexpected consequence of acute FD and the re-exposure to drug associated cues. However, it is clear that rats were able to differentiate between the levers, and preferred to respond on the previously active, drug-associated lever.

In interpreting the effect of SCH 23390 on drug seeking behavior it is often difficult to disentangle the effects of this antagonist on motivational and motor systems

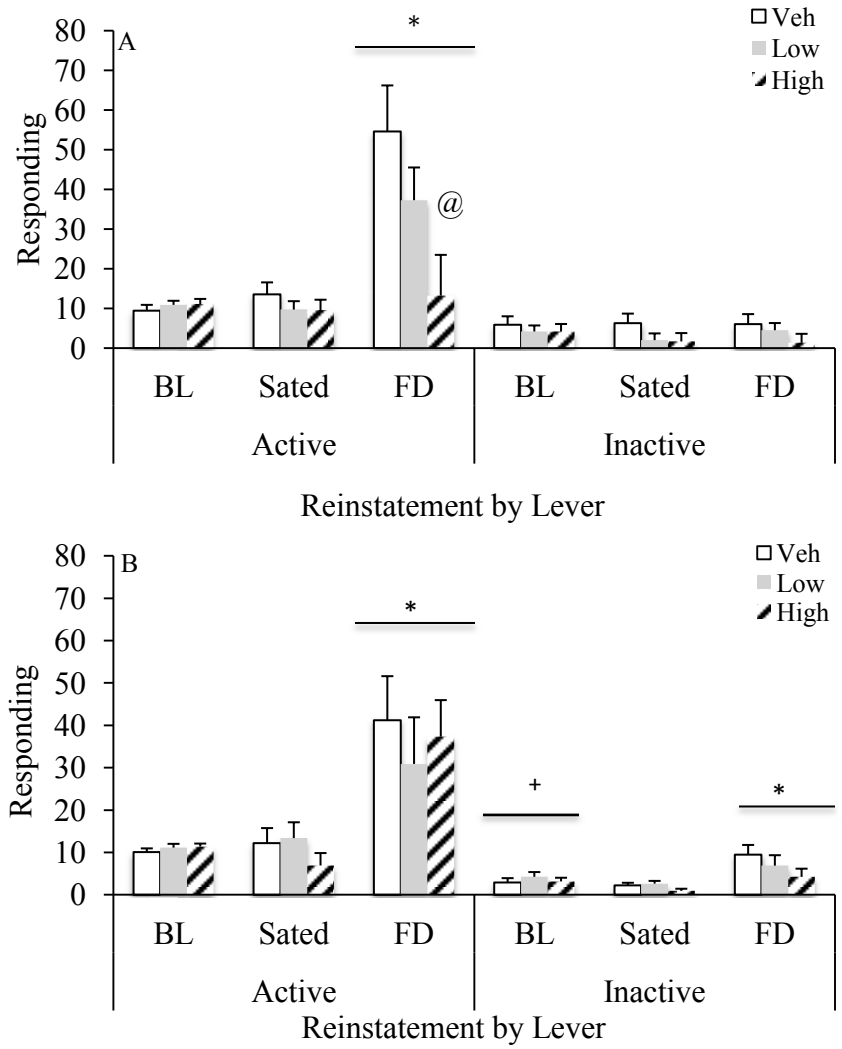

Figure 3.

The effect of intra-NAc SCH 23390 infusion on acute FD-induced reinstatement of heroin seeking. Data are mean (+*SEM*) number of active (left) and inactive (right) lever responses made during baseline (BL), following 48h unlimited access to food (Sated) and following 48h FD for rats with placements into the NAc shell (A) or NAc core (B). Different groups of rats were used for each dose of SCH 23390 (Shell: Veh (0.0 µg), n = 7; Low (0.3 µg), n = 14; High (0.6 µg), n = 9; Core: Veh, n = 9; Low, n = 8; High, n = 13). * $p \leq .01$ when compared to BL and sated conditions, @ $p < .05$ when compared to the vehicle group, under FD, + $p < .05$ when compared to the sated condition.

(Crombag et al., 2002). Thus, to ensure that the effect of intra-NAc shell SCH 23390 on acute FD-induced reinstatement was not due to a non-specific inhibition of motor activity, an ANOVA was preformed comparing the number of photocell beam breaks in the operant training chamber for rats in the vehicle (n = 4), low (n = 10) and high (n = 6) antagonist dose groups. The number of rats within each dose group differ from those presented above because of missing photocell data. Additionally, one rat was eliminated due to an excessive number of photocell beam breaks during the baseline period (9665 beam breaks). A mixed factorial ANOVA revealed no significant effect of *deprivation state*, $F(2, 34) = 1.66$, $p = .21$, $\eta_p^2 = .09$, *deprivation state* X *antagonist dose* interaction, $F(4, 34) = 0.53$, $p = .71$, $\eta_p^2 = .06$. However, a significant antagonist dose effect was observed, $F(2, 17) = 4.20$, $p = .03$, $\eta_p^2 = .31$; however, somewhat contrary to expectations, this effect seems to be accounted for by a tendency toward an increase in motor activity in the high antagonist dose group (M = 1171.53; SEM = 130.92) relative to the vehicle (M = 683.33; SEM = 160.34, $p = .09$) and significantly more beam breaks relative to the low antagonist dose groups (M = 734.05; SEM = 101.41, $p = .05$), respectively (see *Figure 4*). Despite the lack of a significant interaction, a one-way ANOVA was preformed to assess the effect of antagonist dose only on the FD-test day. This analysis is justified for two reasons: 1. Locomotor behavior was not considered when assigning the rats to antagonist dose group, thus there is a large amount of variability in this measure which may mask an actual effect; 2. The FD-test day is the most critical day to assess the effect of the antagonist on motor behavior. This ANOVA revealed a significant effect of *antagonist dose*, $F(2, 17) = 4.30$, $p = .03$, $\eta_p^2 = .34$. Again,

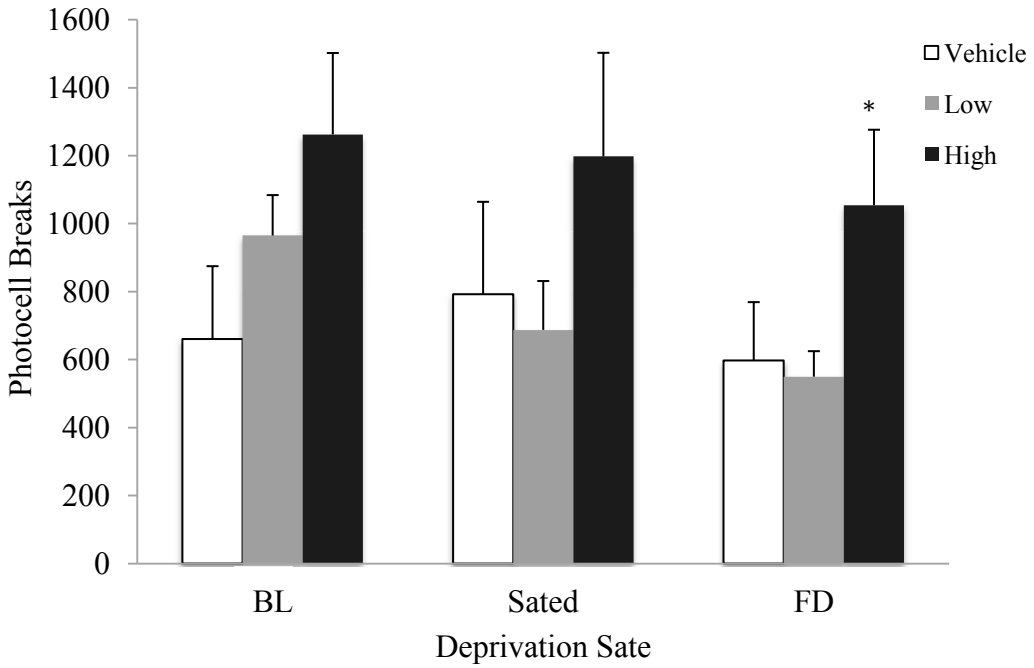

Figure 4.

Number of photocell breaks for rats with confirmed placements in the NAc shell during reinstatement. Data shown are mean (+*SEM*) number of photocell breaks on baseline (BL), sated and food deprived (FD) days for rats in the vehicle ($n = 5$), low ($n = 10$) and high ($n = 5$) SCH 23390 dose groups. * Indicates a significant difference relative to low antagonist dose ($p < .05$).

contrary to expectations, pair-wise comparisons showed that animals in the low antagonist group moved significantly less than those in high antagonist group, $p < .05$.

Experiment 3: Administration of the DA D1 receptor antagonist, SCH 23390, into the BLA

Of the initial 45 rats, 2 died before the completion of the experiment. Additionally, 2 rats were removed due to a failure to train, 2 rats were removed because the guide cannulae location could not be verified, and 16 rats were excluded due to injector tip placement. Finally, 1 rat was treated as an outlier based upon responding on the acute FD test day (reinstatement day active lever responding = 165; z-score = 4.27) and the data for this rat were not considered. Thus, 24 rats (see *Figure 5*) were considered in the following analyses of the effect of intra-BLA SCH 23390. Of the 13 rats excluded due to injector tip location, 4 had only one injector tip located within the BLA, whereas the remaining 9 had both cannulae located near, but outside of the BLA. These 9 rats were treated as anatomical controls. As seen in *Figure 6*, rats with guide cannulae targeting the BLA were able to distinguish between the active and inactive lever. On the last day of training the mean +/- SEM number of infusions, active, and inactive lever responding were 28.87 +/- 3.78, 165.11 +/- 39.64, and 12.42 +/- 2.77, respectively.

For tests of reinstatement, rats were assigned to one of three antagonist dose groups ($n_{Veh} = 10$; $n_{Low} = 7$, $n_{High} = 7$) such that there were no significant differences between the groups in terms of the average number of infusions ($M_{Veh} = 20.32$, $SEM = 2.09$; $M_{Low} = 37.63$, $SEM = 10.64$; $M_{High} = 47.46$, $SEM = 19.68$), $F(2,21) = 1.58$, p = .23, $\eta_p^2 = .13$, active ($M_{Veh} = 94.12$, $SEM = 10.90$; $M_{Low} = 256.48$, $SEM = 96.94$; $M_{High} = 80.71$, $SEM = 30.51$), $F(2,21) = 1.33$, $p = .29$, $\eta_p^2 = .11$, and inactive ($M_{Veh} = 6.07$,

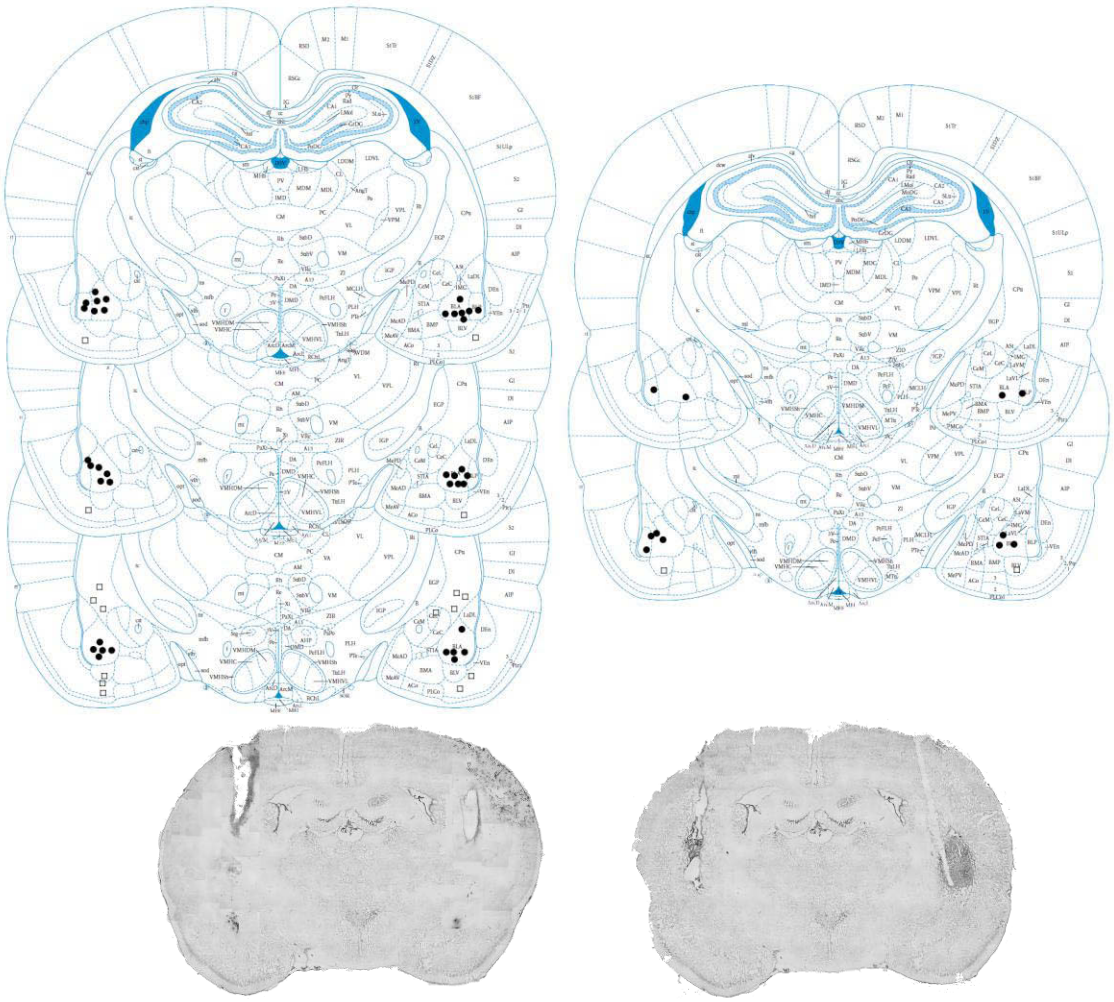

Figure 5.

Anatomical position of microinjector tips for rats with guide cannulae targeting the BLA. Top: confirmed BLA placements (n = 24) are indicated by closed circles; anatomical control placements (n = 8) are indicated by squares. Images correspond to Figures 52-56 (-2.28 to -2.76 mm from bregma) from the atlas of Paxinos & Watson (2005). Figures are arranged from top to bottom with Figure 52 being the bottom left and Figure 56 being the top right. Bottom: photomicrographs for the BLA (left) and control rats (right).

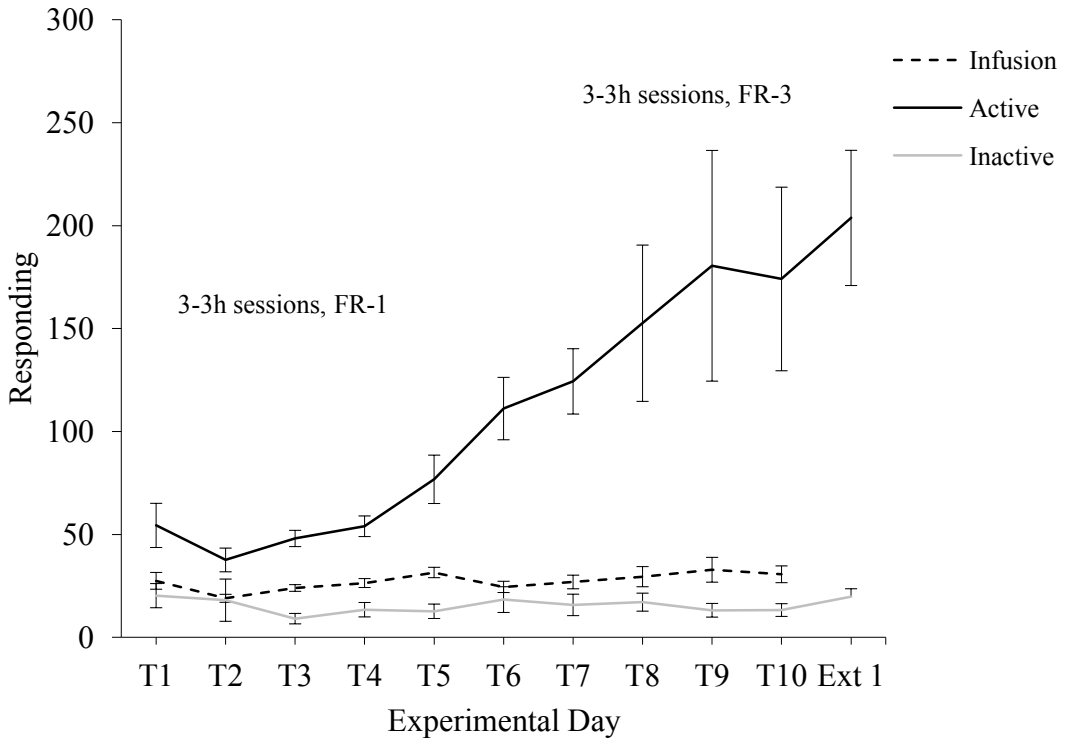

Figure 6.

Heroin self-administration for rats with guide cannulae located in the BLA. Data shown are the mean (+/- *SEM*) numbers of active lever presses, inactive lever presses and heroin infusions (0.1 mg/kg/infusion) for the 24 rats with confirmed placement in the BLA for the ten training days (T1-10; days 1-5: three 3h sessions, FR-1; days 6-10: three 3h sessions, FR-3) and the first extinction day (Ext 1). A significant difference existed between active and inactive lever responding on all training days and during extinction ($p < .05$).

SEM = 1.91; M_{Low} = 27.70, *SEM* = 10.47; M_{High} = 17.91, *SEM* = 6.79), *F*(2,21) = 1.33, *p* = .29, η_p^2 = .11, across the last five days of training. Following training, rats received an average of 9.50 (*SEM* = 0.80) days of extinction training before the first reinstatement test and 2.54 (*SEM* = 0.54) days of extinction training before the second reinstatement test.

A repeated measures ANOVA preformed to evaluate the effect of SCH 23390 infusion into the BLA on acute FD-induced reinstatement revealed a significant effect of *deprivation state*, *F*(2, 42) = 15.69, *p* < .001, η_p^2 = .43, *antagonist dose, F*(2, 21) = 4.96, *p* = .02, η_p^2 = .32 and *deprivation state* X *antagonist dose* interaction, *F*(4, 42) = 3.21, *p* = .02, η_p^2 = .23 (see *Figure 7*). Follow-up pair-wise comparisons revealed a significant increase in active lever responding in the FD condition (*M* = 24.10; *SEM* = 2.38) compared to both the baseline (*M* = 11.27; *SEM* = 1.70) and sated (*M* = 11.50; *SEM* = 1.70) conditions (*p*s < .001). A one-way ANOVA of active lever responding on the FD test day revealed a significant effect of *antagonist dose*, *F*(2, 24) = 4.91, *p* = .02, η_p^2 = .32. Further analyses revealed a significant and a tendency toward significant reduction in responding in the high (*p* < .05) and low (*p* = .06) antagonist dose groups, respectively, relative to the vehicle group. However, there was no difference in responding between the low and high antagonist groups. Separate one-way repeated measures ANOVAs investigating the effect of *deprivation state* for each antagonist dose revealed a significant deprivation effect in the vehicle, *F*(2,18) =17.77, *p* < .001, η_p^2 = .66, and high, *F*(2,12) = 8.81; *p* = .004, η_p^2 = .59, but not low, *F*(2,12) = 0.93; *p* = .42, η_p^2 = .13, antagonist dose groups. Further analyses revealed significantly more responding on the active lever on the FD test day than on the BL or sated days for rats in the vehicle (*p* <. 05), but not low

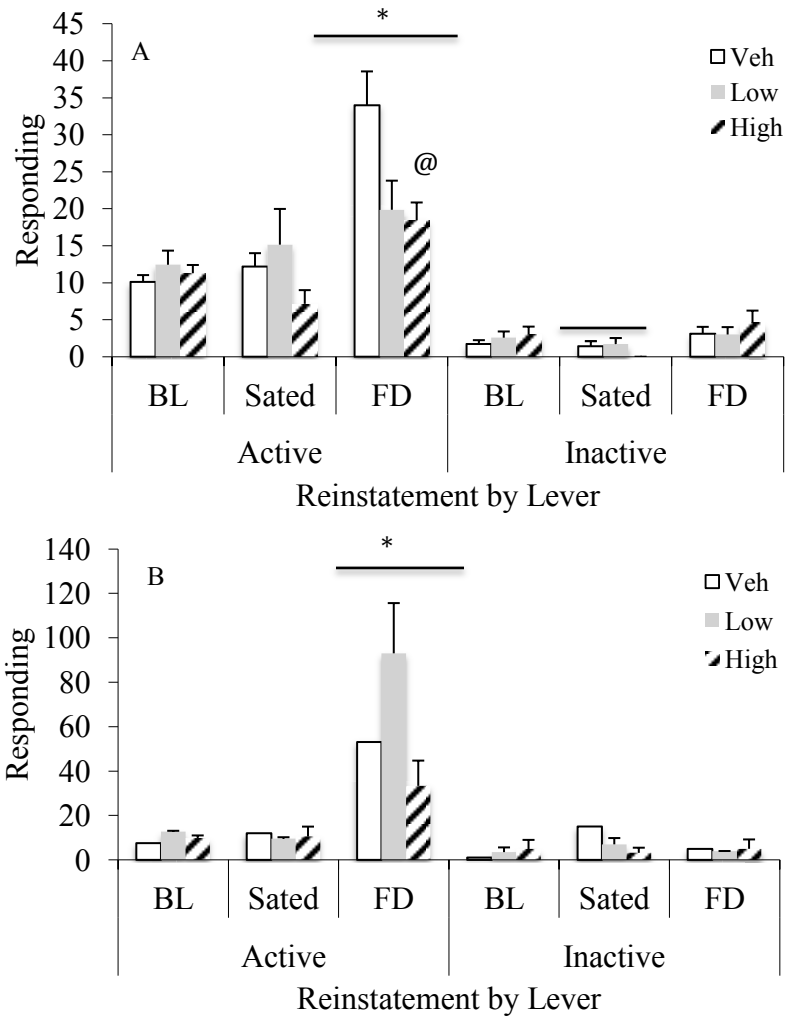

Figure 7.

The effect of intra- BLA SCH 23390 infusion on acute FD-induced reinstatement of heroin seeking. Data are mean (+*SEM*) number of active (left) and inactive (right) lever responses made during baseline (BL), following 21h FD or sated condition for rats with confirmed placements in the BLA (A) and anatomical control rats (B). Different groups of rats were used for each dose of SCH 23390 (BLA: Veh (0.0 µg), $n = 10$; Low (1.0 µg), $n = 7$; High (2.0 µg), $n = 7$; Control: Veh, $n = 1$; Low µg, $n = 2$; High, $n = 6$). * $p < .05$ compared to BL and sated conditions, @ $p < .05$ compared to Veh group under FD conditions, + $p < .05$ relative to the BL and FD conditions.

groups. For the high antagonist dose group there was a significant increase in active lever responding on the FD test relative to the sated test ($p <$. 05). For the inactive lever, a significant effect of *deprivation state* was also observed, $F(2, 42) = 5.31$, $p < .01$, $\eta_p^2 = .20$. This effect was accounted for by significantly less responding in the sated condition ($M = 1.40$; $SEM = 0.38$) relative to the baseline ($M = 2.42$; $SEM = 0.46$) and food deprived condition ($M = 3.56$; $SEM = 0.70$), ps < .05. No significant effect of *antagonist dose*, $F(2, 21) = 0.27$, $p = .78$, $\eta_p^2 = .03$, or *antagonist dose* X *deprivation state* was observed, $F(4, 42) = 1.00$, $p = .42$, $\eta_p^2 = .09$.

For the 9 rats with injector tips located outside, but within approximately 1 mm, of the BLA there was 1 rat from the vehicle group, 2 rat from the low antagonist group and 6 rats from the high antagonist group. As seen in *Figure 7*, there was a significant effect of *deprivation state*, $F(2,16) = 10.92$, $p < .01$, $\eta_p^2 = .57$, with rats responding more on the active lever on the FD test ($M = 48.78$; $SEM = 11.24$) than on the baseline day ($M = 10.22$; $SEM = 0.88$) or sated conditions ($M = 10.44$; $SEM = 2.66$), ps < .05. Due to the small number of subjects from the low and high antagonist dose groups, the effect of antagonist dose and antagonist dose by deprivation state interaction was not investigated. Rather, a planned comparison was preformed to compare responding on the FD test day for anatomical control rats administered with the high antagonist ($M = 33.33$; $SEM = 10.38$) and rats with confirmed placements in the BLA administered with the vehicle ($M = 34.00$; $SEM = 4.56$). The results of this comparison showed no significant difference between the rats, $t(14) = -0.07$, $p = .95$. Additionally, no significant effect of *deprivation state* was observed for inactive lever responding for anatomical control rats, $p > .05$.

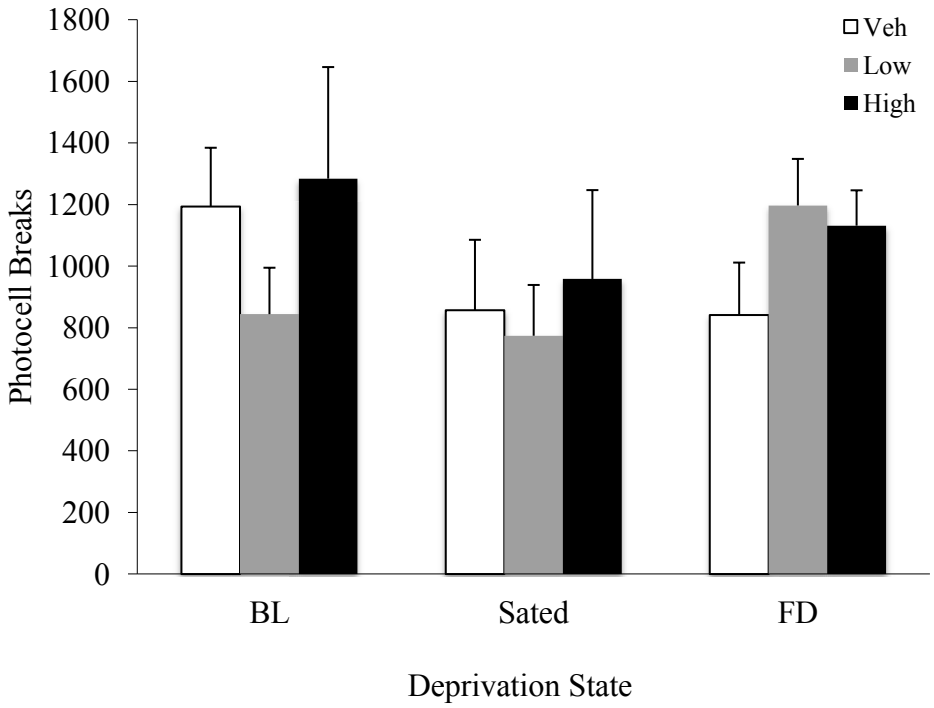

Figure 8.

Number of photocell breaks for rats with confirmed placements in the BLA during the reinstatement test. Data shown are the mean (+ *SEM*) number of photocell breaks on baseline (BL), sated and food deprived (FD) days for rats in the vehicle (n = 6), low (n = 6) and high (n = 5) SCH 23390 dose groups.

As previously discussed, the inhibition of drug seeking behavior observed in the current experiment may be the result of motor suppression. To investigate this possibility an ANOVA was preformed comparing the number of moves for a subset of rats from the vehicle (n = 6), low (n = 6) and high (n = 5) antagonist dose groups on each test day. The discrepancy in subject numbers is due to missing photocell data. The results of this ANOVA showed no significant effect of *deprivation state*, *antagonist dose* or *antagonist dose* X *deprivation state* interaction on photocell beam breaks, ps > .05.

Experiments 4 & 5: Administration of the DA D1 receptor antagonist, SCH 23390, into the dmPFC and vmPFC

Of the 67 rats originally placed in the operant chambers 45 were included in the following analyses. Two rats were removed during training due to sickness, two rats were eliminated due to catheter failure, three rats were eliminated due to a failure to train and the others were eliminated following inspection of injector tip placement (see *Figure* 9). As expected, training data did not differ between rats with placements located in the dmPFC and vmPFC, across the 10 training days, in terms of the number of heroin infusions, $F(1, 43) = .98$, $p = .33$, $\eta_p^2 = .02$, number of active lever responses, $F(1,43) = .33$, $p = .57$, $\eta_p^2 = .01$, or number of inactive lever response, $F(1, 43) = .48$, $p = .49$, $\eta_p^2 = .01$. Thus, training data for experiments 4 and 5 were combined. As can be seen in *Figure 10*, rats showed a clear preference for the 'active' drug-paired lever over the 'inactive' non-drug paired lever throughout training. Rats received on average (+/- *SEM*) 8.00 (+/- 0.67) days of extinction training prior to their first reinstatement test and an additional

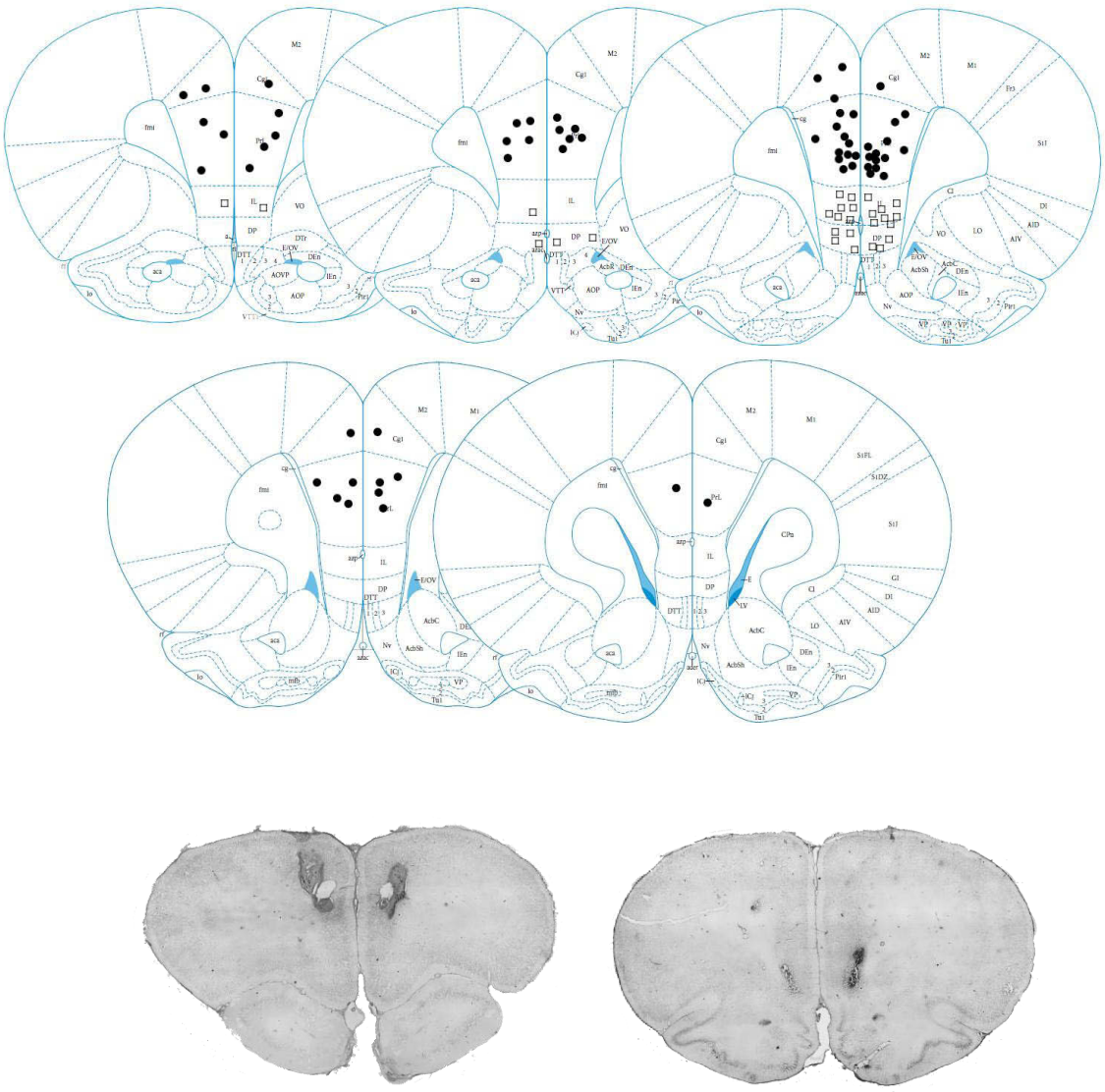

Figure 9.

Anatomical position of microinjector tips targeting the mPFC. Top: approximate anatomical position of microinjector tips for rats with guide cannulae targeting the dmPFC (*n* = 31; closed circles) and the vmPFC (*n* = 14; open squares). Images correspond to Figures *9-13* (3.72 -2.52 mm from bregma) from the atlas of Paxinos & Watson (2005). Figures are arranged from lowest to highest from top left to bottom right. Bottom: sample photomicrographs of a dmPFC (right) and vmPFC (left) placements.

2.51 (+/- 0.33) extinction sessions prior to their second reinstatement test. There was no difference in the rate of extinction observed across experiments, *p*s > .05.

Rats did, however, differ in their reinstatement of heroin seeking following SCH 23390 infusions, depending on the region. Thus, the data for rats with guide cannulae targeting the dmPFC and vmPFC are discussed separately. Prior to tests of reinstatement, rats with guide cannulae targeting the dmPFC were assigned to one of three antagonist dose groups (vehicle: $n = 11$; low: $n = 10$; high: $n = 10$) in such a way that there were no differences between the groups in terms number of infusions received ($M_{Veh} = 27.11$, $SEM = 3.94$; $M_{Low} = 23.94$, $SEM = 4.14$; $M_{High} = 31.10$, $SEM = 4.14$), $F(2, 28) = .75$, $p = .48$, $\eta_p^2 = .05$, active lever responses, ($M_{Veh} = 143.20$ $SEM = 30.95$; $M_{Low} = 121.94$, $SE = 32.46$; $M_{High} = 158.62$, $SE = 32.46$), $F(2, 28) = .32$, $p = .73$, $\eta_p^2 = .02$, in active lever responding, ($M_{Veh} = 18.14$,$SEM = 3.04$; $M_{Low} = 16.42$, $SEM = 3.19$; $M_{High} = 7.96$, $SE = 3.19$), $F(2, 28) = 3.00$, $p = .07$, $\eta_p^2 = .18$, during the last 5 days of training. Rats with guide cannulae targeting the vmPFC were assigned to either a vehicle ($n = 8$) or high ($n = 6$) antagonist dose group. No significant differences between number of infusions, ($M_{veh} = 23.35$, $SE = 4.67$; $M_{High} = 20.30$, $SEM = 5.39$, $F(1, 12) = 0.17$, $p = .69$, $\eta_p^2 = .01$, number of active lever responses, ($M_{Veh} = 133.00$, $SEM = 29.45$; $M_{High} = 100.30$, $SE = 34.11$, $F(1, 12) = 0.53$, $p = .48$, $\eta_p^2 = .04$, or number of inactive lever responses, ($M_{Veh} = 15.75$, $SEM = 7.09$; $M_{High} = 23.00$, $SEM = 8.18$, $F(1, 12) = 0.44$, $p = .56$, $\eta_p^2 = .04$, were observed across the last 5 days of training, $p < .05$.

For rats with guide cannulae targeting the dmPFC, a repeated measures ANOVA, investigating active lever responding during the reinstatement testing revealed a significant effect of *deprivation state*, $F(2, 56) = 24.25$, $p < .001$, $\eta_p^2 = .46$, *antagonist*

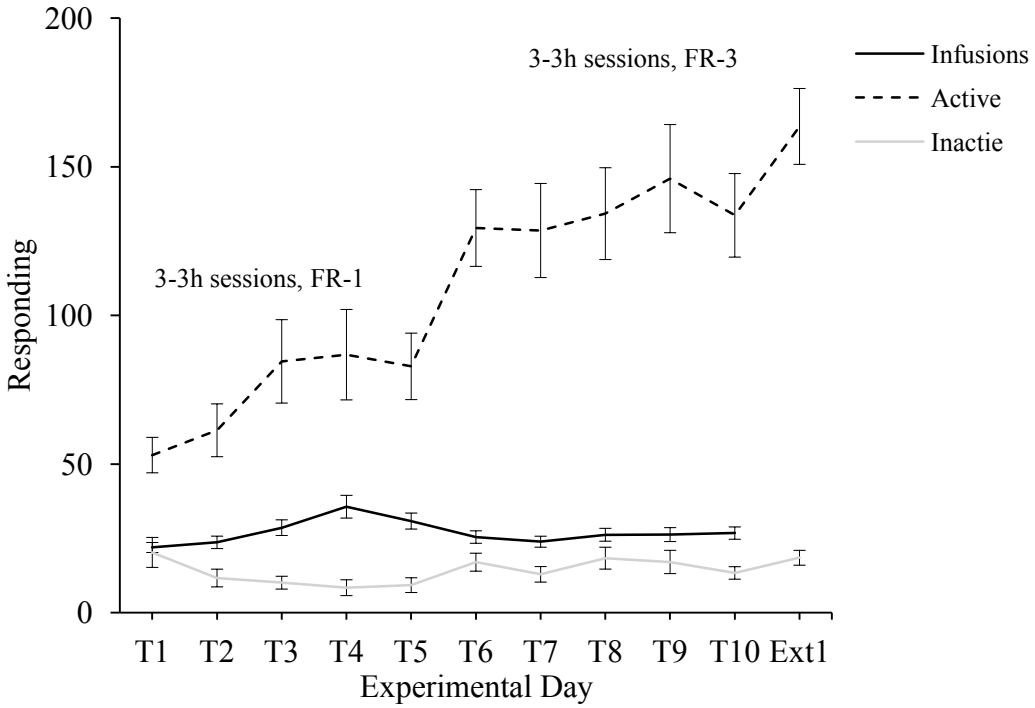

Figure 10.

Heroin self-administration for rats with guide cannulae located in the mPFC. Data shown are the mean (+/- *SEM*) numbers of active lever presses, inactive lever presses and heroin infusions (0.1 mg/kg/infusion) for the 45 rats with confirmed placement in the mPFC for the ten training days (T1-10; days 1-5: three 3h sessions, FR-1; days 6-10: three 3h sessions, FR-3) and the first extinction day (Ext 1). A significant difference existed between active and inactive lever responding on all training days and during extinction ($p < .05$).

dose, $F(2,28) = 4.18$, $p = .03$, $\eta_p^2 = .23$ and *deprivation state* X *antagonist dose* interaction, $F(4,56) = 3.91$, $p < .01$, $\eta_p^2 = .22$ (see *Figure 11A*).

Follow-up pair-wise comparisons revealed a significant increase in active lever responding in the FD condition ($M = 47.92$; $SEM = 6.73$) compared to both the baseline ($M = 13.16$; $SEM = 0.79$) and sated ($M = 12.76$; $SEM = 1.70$) conditions, ps $< .001$. A one-way ANOVA assessing active lever responding on the FD test day revealed a significant effect of *antagonist dose*, $F(2, 28) = 4.21$, $p = .03$, $\eta_p^2 = .23$. Pair-wise comparisons revealed a significant reduction in responding in the high antagonist dose group relative to the vehicle antagonist dose group ($p < .05$). Separate one-way repeated measures ANOVAs were conducted for each antagonist dose across deprivation conditions. These ANOVAs revealed a significant *deprivation state* effect in the vehicle, $F(2,20) = 8.83$; $p = .01$, $\eta_p^2 = .47$ and low dose, $F(2,18) = 20.71$; $p = .01$, $\eta_p^2 = .70$, groups; but not in the high antagonist dose group, $p > .05$. Moreover, for both the vehicle and low-dose groups, follow-up analyses revealed significantly more responding on the active lever on the FD test day than on the BL or sated days, ps $< .05$. A mixed factorial ANOVA investigating responding on the inactive lever showed no significant effect of *deprivation state, antagonist dose or deprivation state* X *antagonist dose* ($ps > .05$).

As expected, *deprivation state* had a significant effect on active lever responding in rats with guide cannulae targeting the vmPFC, $F(2, 24) = 8.82$, $p < .001$, $\eta_p^2 = .42$ (see *Figure 11B*). This effect is accounted for by significantly more responding on the FD test ($M = 44.25$; $SEM = 10.40$) than the baseline ($M = 10.20$; $SEM = 0.95$; $p < .05$) and a tendency toward significantly more responding on the FD test than the sated test ($M = 14.56$; $SEM = 3.51$, $p = .06$). Unlike rats with guide cannulae targeting the dmPFC, there

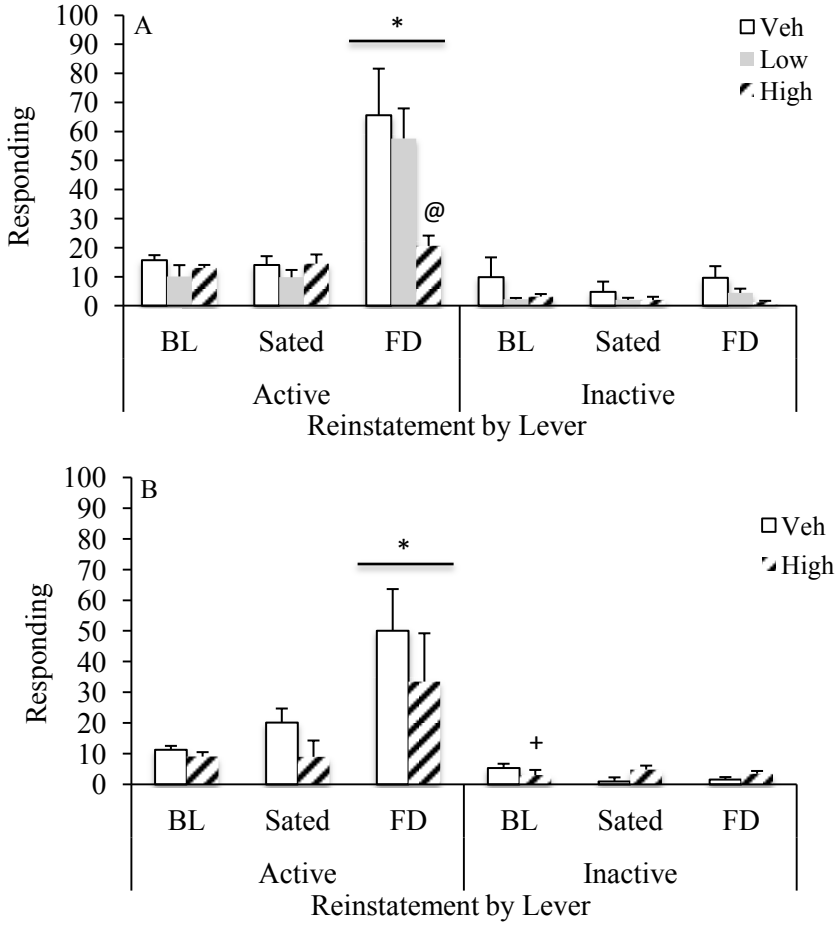

Figure 11.

The effect of intra-PFC SCH 23390 infusion on acute FD-induced reinstatement of heroin seeking. Data are mean (+*SEM*) number of active (left) and inactive (right) lever responses made during baseline (BL), following a 21h sated period, and following 21h of FD for rats with placements in the dmPFC (A) or vmPFC (B). Different groups of rats were used for each dose of SCH 23390 (dmPFC: Veh (0.0 µg), $n = 11$; Low (0.2 µg), $n = 9$; High (2.0 µg), $n = 11$; vmPFC: Veh (0.0 µg), $n = 8$; High (2.0 µg), $n = 6$). * $p < .05$ when compared to BL and sated conditions, @ $p < .01$ compared to the Veh and Low antagonist groups on the FD test day, + $p < .05$ relative to responding in the vehicle group under sated and FD conditions.

was no effect of *antagonist dose*, $F(1, 12) = 1.18$, $p = .30$, $\eta_p^2 = .09$, or *deprivation state* X *antagonist dose* interaction, $F(2, 24) = .18$, $p = .84$, $\eta_p^2 = .02$, for active lever responding.

With regard to inactive lever responding there was no significant effect of *deprivation state*, $F(2, 24) = 1.38$, $p = .27$, $\eta_p^2 = .10$, or *antagonist dose*, $F(1, 12) = .83$, $p = .38$, $\eta_p^2 = .06$; however, a significant *deprivation state* X *antagonist dose* interaction was observed, $F(2, 24) = 4.47$, $p = .02$, $\eta_p^2 = .27$. Analysis of this interaction revealed significantly more responding by the vehicle group on the baseline day ($M = 5.31$; $SE = 1.43$) relative to the sated ($M = 1.00$; $SEM = 1.13$) and FD test ($M = 1.63$; $SEM = 0.77$) days, $ps < .05$.

As previously discussed, the inhibition of drug seeking behavior observed in the current experiment may be the result of motor suppression. To investigate this possibility an ANOVA was preformed comparing the number of moves for a subset of rats with cannulae aimed at the dmPFC from the vehicle ($n = 6$), low ($n = 6$), and high ($n = 5$) antagonist dose groups on each test day. As mentioned above, the discrepancy in subject numbers is the result of missing photocell data. Additionally, one rat was eliminated due to an excessive number of photocell beam breaks the day prior to the stated test (6993 responses). The results of this ANOVA showed no significant effect of *deprivation state*, *antagonist dose* or *antagonist dose* X *deprivation state interaction* on photocell beam breaks, $ps > .05$ (see *Figure 12*).

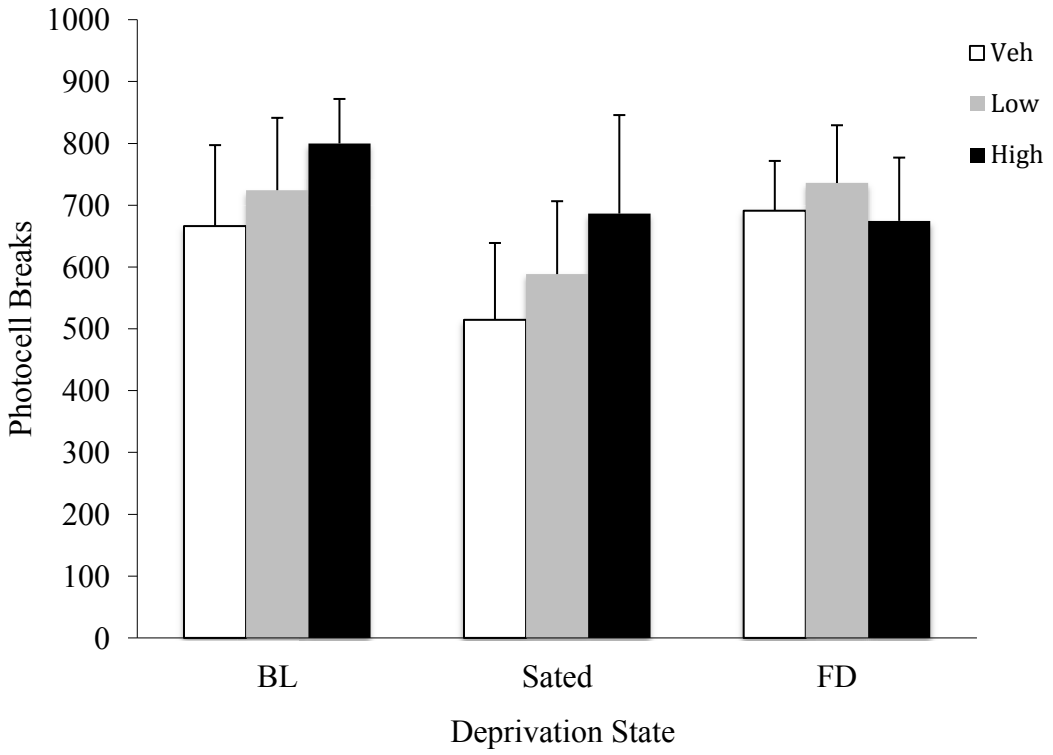

Figure 12.

Number of photocell breaks for rats with confirmed placements in the dmPFC during reinstatement tests. Data shown are the mean (+ *SEM*) number of photocell breaks on baseline (BL), sated and food deprived (FD) days for rats in the vehicle (n = 5), low (n = 10) and high (n = 5) SCH 23390 dose groups.

DISCUSSION

The results of the current series of experiments expand upon previous data from our laboratory demonstrating an attenuation of acute FD-induced reinstatement following systemic injection of the DA D1 receptor antagonist SCH 23390 (Tobin et al., 2009). The current findings suggest a critical role for DA transmission, acting on the D1 receptor in the NAc shell, dmPFC and BLA, in the reinstatement of heroin seeking following acute 21-48h FD. Interestingly, however, administration of SCH 23390 into the NAc core or vmPFC had no effect on drug seeking, suggesting a lack of involvement for DA D1 receptors in these regions in acute FD-induced reinstatement.

Prior to discussing the relevance of the current findings, it is important to address potential methodological caveats. One such caveat relates to the role of DA in the initiation of movement. Thus, in the current series of experiments treatment with SCH 23390 may have reduced responding on the reinstatement test because of a non-specific inhibition of motor performance rather than a change in the motivation to seek heroin (Crombag et al., 2002). It is worth noting here that intra-BLA administration of the high SCH 23390 dose appeared to result in a reduction in active and inactive lever responding during the sated as well as FD test day. Also, a decrease in inactive lever responding, following antagonist administration, was observed in some experiments (see results). However, antagonist doses were drawn from previous research in which motor effects were not reported. Furthermore, as described above, photocells were placed in the center of the operant chambers and the number of beam breaks observed, a reflection of general locomotion, was not found to differ as a function of SCH 23390 dose. Finally, inactive lever responding is generally low in reinstatement studies and as such may not be an

appropriate measure of a non-specific motor impairment (Crombag et al., 2002; Shalev, et al., 2002).

A second potential caveat involves the length of the reinstatement test as it relates to the effective time for SCH 23390 treatment. The half-life of SCH 233390, when injected systemically, is approximately 30 min (Bourne, 2001); whereas, in the current studies we used a 3h reinstatement test. Thus, test session length may have "diluted" the effect of SCH 23390. This possibility seems rather unlikely. Three hours is a standard test-session length within the reinstatement literature, and has been used previously in studies in which SCH 23390 was infused locally and shown to block drug seeking (for example see Bossert et al., 2007). Furthermore, as reported here, SCH 23390 significantly attenuated acute FD-induced reinstatement when injected into the NAc shell, dmPFC, or BLA, under the same test conditions.

Finally, Caine et al. (1995) have suggested that due to the lipophilic nature of SCH 23390, intracranial infusion of this drug can be problematic and result in the spread of the drug away from the site of infusion. Thus, the effects observed here may be related to the actions of SCH 23390 in regions other than those targeted. This possibility seems unlikely given that no effect on acute FD-induced reinstatement was observed when SCH 23390 was infused into the NAc core, vmPFC, or near the BLA.

The Role of NAc DA D1-like Receptors in Acute FD-induced Reinstatement of Heroin Seeking

As expected, antagonism of the DA D1 receptor in the NAc shell resulted in an attenuation of acute FD-induced reinstatement. In contrast, administration of SCH 23390 into the NAc core had no effect. These findings coincide with the previous demonstration

that blockade of the DA D1 and D2 receptor in the NAc core with the non-selective DA antagonist fluphenazine had no effect on footshock-induced reinstatement of cocaine seeking (McFarland et al., 2004). Moreover, SCH 23390 administered into the NAc shell, but not NAc core, has been shown to attenuate cocaine priming- (Anderson et al., 2003), and context-induced reinstatement of heroin seeking (Bossert et al., 2007). Thus, the lack of involvement for DA D1 receptors in the NAc core in acute FD-induced reinstatement is not unexpected.

Previously, McFarland et al. (2004) have suggested that the NAc core forms a central component in the circuitry underlying all forms of reinstatement. In support of this suggestion, transient inactivation of the NAc core, with muscimol and baclofen, has been shown to attenuated priming-, cue-, and footshock-induced reinstatement of cocaine seeking (Fuchs et al., 2004; McFarland et al., 2004; McFarland & Kalivas, 2001). Importantly, McFarland et al. (2004) have also demonstrated that footshock stress leads to an increase in extracellular glutamate, but not DA, in the NAc core during cocaine-seeking reinstatement test. Thus, these researchers conclude that it is the glutamatergic input from the dmPFC cortex to the NAc core, and not DA transmission in the core, that is critical for footshock stress-induced reinstatement of drug seeking. The current results lend support to their conclusion, and extend it to the effects of acute FD stress on the reinstatement of heroin seeking.

In contrast, SCH 23390 administration into the NAc core, but not shell, has been shown to block discrete-cue-induced reinstatement of heroin seeking (Bossert et al., 2007). In addition, transient inactivation of either the NAc core or shell has been shown to block both cocaine priming- and footshock stress-induced reinstatement of cocaine

seeking (McFarland et al., 2004; McFarland & Kalivas, 2001). These findings suggest a dissociation between the role of the NAc shell and NAc core which may be based upon the nature of the reinstatement trigger. Furthermore, the disparate role of the NAc core in the above studies may be attributable to the nature of NAc core manipulations. Here a role for the NAc shell, and not core, in the reinstatement of drug seeking was demonstrated by selectively blocking DA D1 receptors with SCH 23390, a procedure that is qualitatively different from complete inactivation. This dissociation was clearly demonstrated by Yun et al. (2004) who report a difference in discriminative stimulus-induced reinstatement of sucrose seeking following intra-NAc infusion of SCH 23390, TTX or a combination of AMPA and NMDA receptor antagonists (CNQX and AP-5). In rats treated with SCH 23390, a substantial reduction in responding to the discriminative stimulus was observed; whereas, general inactivation via glutamate receptor antagonism or TTX infusion was shown to increase the rate of non-cued responding on the active lever and total inactive lever responses, suggesting disinhibition of non-relevant behaviors.

To the best of my knowledge there have been no published studies investigating NAc shell DA following acute FD-induced reinstatement. However, it has been shown that 7-10 days of restricted feeding (rats restricted to 70-80% of body weight) results in a decrease in extracellular NAc DA, as measured by microdialyisis (Pothos, Creese, & Hoebel, 1995). The authors suggest that this decrease in DA release may signify a starvation-induced, “withdrawal-like”, shift in motivational state leading to the performance of behaviors that will result in the increased release of DA (Pothos et al., 1995). Unfortunately, there is no information in this study about the placement of the

probes within the NAc, limiting the ability to generalize these findings to the current data. Moreover, acute FD stress may differ from chronic food restriction with regard to NAc DA. Exposure to acute stressors such as restraint, footshock, and tail pinch have been shown to increase NAc DA (Abercrombie, Keefe, DiFrischia, & Zigmond, 2006; Imperato, Mele, & Scrocco, 1992) in the NAc shell but not the NAc core (Kalivas & Duffy, 1995).

Although the current FD manipulation is considered an "acute" manipulation compared to chronic food restriction, it is nevertheless different from other acute stressors, as it is prolonged and might allow for some neuronal and physiological adaptations to occur. It is therefore unclear, as yet, how 21-48h FD stress affects NAc DA release during the reinstatement test. Nevertheless, my findings suggest that NAc shell DA, acting through the DA D1 receptor, is involved in mediating the expression of acute FD-induced reinstatement.

Here, the role of the DA D1 receptor was investigated specifically; however, these findings do not preclude a role for other DA receptors, particularly DA D2-like receptors in acute FD-induced reinstatement. For instance, systemic administration of the DA D1 or DA D2 receptor antagonists, SCH 23390 or raclopride, respectively, has been shown to attenuate cocaine seeking behavior, in a renewal paradigm (Crombag et al., 2002) . Furthermore, a cooperative effect of DA D1 and D2 receptors, in the NAc shell, is suggested by reports that rats will self-administer a mixture of SKF 38393, a DA D1 receptor agonist, and quinpirole, a DA D2 receptor agonist, into the NAc shell, but will not administer these agonists separately at equimolar concentrations (Ikemoto et al., 1997). Similarly, co-administration of DA D1 and DA D2 receptor agonists, into the NAc

shell, has been shown to reinstate extinguished cocaine seeking at doses which had no effect on behavior when administered alone (Schmidt & Pierce, 2006). Furthermore, antagonism of the DA D1 or DA D2 receptors prior to the administration of a DA D2 or D1 receptor agonist, respectively, has been shown to block the reinstatement of cocaine seeking (Bachtell, Whisler, Karanian & Self, 2005; Schmidt & Pierce, 2006). Finally, administration of the DA D3 receptor antagonist, SB-277011A into the NAc, at the border of the shell and core, has been shown to block footshock-induced reinstatement of cocaine seeking (Xi et al., 2004). However, it is important to note that we have previously shown no significant effect for systemic administration of DA D2 or D3 receptor antagonists on acute FD-induced reinstatement of heroin seeking (Tobin et al., 2009).

The Role of mPFC DA D1-like Receptors in Acute FD-induced Reinstatement of Heroin Seeking

A role for DA D1 receptors in the dmPFC, in acute FD-induced reinstatement of heroin seeking, is not surprising given that this region has been shown to be involved in stress-induced reinstatement and other forms of drug seeking behavior. Thus, reversible inactivation studies, using muscimol and baclofen, have revealed a critical role for the dmPFC cortex in priming-, cue-, and footshock-induced reinstatement of heroin and cocaine seeking (McFarland et al., 2004; Rogers et al., 2008). These findings also agree with those of Park el al. (2002) who reported that injections of a DA agonist (cocaine) into the mPFC DA can reinstate cocaine seeking, and that this effect can be blocked by administration of the glutamate AMPA receptor antagonist CNQX into the NAc shell. It is unfortunate, though, that Park et al. (2002) did not dissociate between injections of

cocaine into dmPFC and vmPFC. Recently, however, SCH 23390 infusion, into the prelimbic cortex has been shown to block priming- and cue-induced reinstatement of heroin seeking (See, 2009; Zavala, Weber, Rice, Alleweireldt & Neisewander, 2003).

More importantly, stress-induced reinstatement is also associated with DA transmission in the mPFC. For instance, exposure to a brief footshock stress (10 min) has been shown to result in a prolonged (> 60 min) increase in DA in the prelimbic cortex (McFarland et al., 2004). The same study also demonstrated complete blockade of footshock-induced reinstatement of cocaine seeking following intra-dmPFC injection of the mixed DA receptor antagonist, fluphenazine. These results are consistent with the findings of Capriles et al. (2003), who have reported that injections of SCH 23390, but not the DA D2 receptor antagonist, raclopride, into the prelimbic cortex attenuates footshock stress-induced reinstatement of cocaine seeking.

These studies, and my findings, strongly support the hypothesis that the dmPFC cortex is an important component of a common pathway by which all reinstatement and relapse triggers act to promote drug seeking (Peters, Kalivas, & Quirk, 2009), and that DA transmission in the dmPFC is critically involved in this effect (Bossert et al., 2005; McFarland et al., 2004).

The Role of BLA DA D1-like Receptors in Acute FD-induced Reinstatement of Heroin Seeking

The data presented here suggest a role for DA D1 receptors in the BLA in acute FD-induced reinstatement. The BLA, like the NAc and mPFC, receives a stress responsive DAergic projection from the VTA (Inglis & Moghaddam, 1999; Stevenson & Gratton, 2003). In addition to mediating the behavioral response to stress, the BLA is also

critically involved in the attribution of salience to environmental stimuli (Davis, 1992; Pierce & Kalivas, 1997; Stevenson & Gratton, 2003). Thus, transient inactivation or excitotoxic lesions of the BLA have been shown to interfere with cue-induced reinstatement of cocaine and heroin seeking (Fuchs & See, 2002; Meil & See, 1997; Rogers et al., 2008). However, intra-BLA infusion of the DA agonists amphetamine or cocaine does not lead to the development of a CPP (O'Dell, Sussman, Meyer, Neiswander, 1999), nor does lesioning of the BLA affect ongoing cocaine self-administration (Meil & See, 1997). Thus, the BLA is thought to be involved in the conditioned incentive properties, but not the primary reinforcing aspects, of drug use (Meil & See, 1997).

In contrast, transient inactivation of the BLA fails to attenuate footshock stress-induced reinstatement of cocaine seeking (McFarland et al., 2004). Yet, here acute FD-induced reinstatement is attenuated by intra-BLA infusion of SCH 23390. Thus, it appears that the role of the BLA in stress-induced reinstatement is dependent on the nature of the stressor. This conclusion, however, needs to be considered with some caution, since the current study and that of McFarland et al. (2004) differ in several ways. In McFarland et al. (2004) rats were trained to self-administer cocaine; whereas, in the current study they were trained to self-administer heroin. Previously, inactivation of the BLA has been shown to attenuate heroin, but not cocaine priming-induced of drug seeking (Fuchs & See, 2002; McFarland & Kalivas, 2001; Rogers et al., 2008). Furthermore, as discussed above and elsewhere (Bossert et al., 2007), transient inhibition of a discrete brain area may result in different behavioral outcomes compared to specifically targeting a particular neurotransmitter system. Thus, it is unclear what effect

SCH 23390 infusion, into the BLA, would have on footshock-induced reinstatement of cocaine, or other drug, seeking.

Conclusion

The findings of the current study expand upon previous findings demonstrating an attenuation of acute FD-induced reinstatement following a systemic injection of the DA D1 receptor antagonist SCH 23390 (Tobin et al., 2009). Here a critical role for DA transmission in the NAc shell, dmPFC, and BLA, in acute FD-induced reinstatement of heroin seeking is demonstrated. The neuronal circuitry by which these brain areas interact has yet to be elucidated, but nevertheless, some speculation on possible neural circuitry underling acute FD-induce reinstatement is offered in the general discussion of this thesis.

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CHAPTER 2

A STUDY OF LONG-TERM ADAPTATIONS IN THE VENTRAL SUBICULAR-NUCLEUS ACCUMBENS SHELL PATHWAY FOLLOWING HEROIN SELF-ADMINISTRATION AND ACUTE FOOD DEPRIVATION-INDUCED REINSTATEMENT

ABSTRACT

Long-term exposure to drugs of abuse, such as heroin, can result in the development of drug addiction. Importantly, extensive experience with drugs is associated with an enduring vulnerability to relapse during times of abstinence. This enduring vulnerability is thought to be the result of maladaptive changes in neuronal pathways that occur as a result of chronic drug use. In particular, drugs of abuse have been shown to exert a profound effect on the mesolimbic DA pathway, including the NAc, a major target for VTA DA neurons. Furthermore, stress, a common trigger for drug relapse in humans, has been shown to induce synaptic adaptations in the hippocampus of opiate-dependent rodents. In the current experiment I focus on changes in synaptic plasticity in the pathway from the vSub to the NAc shell. The vSub is a primary output region for the hippocampal formation and sends an excitatory glutamatergic projection to the NAc shell. Adaptations in the vSub-NAc shell pathway have been implicated in opiate withdrawal in passively drug-exposed animals. However, opiate-induced changes in the vSub-NAc shell pathway, and potential modulation of this pathway by stress, following heroin self-administrating have not been explored. Here, male Long Evans rats were implanted with a stimulating electrode into the vSub and a recording electrode into the NAc shell, and then trained to self-administer heroin (0.10 mg/kg/infusion) for a minimum of 10 days. Following training, rats received at least 4 days of extinction training during which time heroin was unavailable. Under extinction conditions, rats were subjected to 48h of FD or given unrestricted access to food and tested for the reinstatement of drug seeking. Field excitatory postsynaptic potentials (fEPSPs) were recorded following self-administration training, extinction training, and

the reinstatement test. In addition, immediately following the 2h reinstatement test, LTP was induced using high-frequency stimulation (200 Hz). In the heroin-trained rats, acute FD resulted in a significant reinstatement of heroin seeking. No differences in fEPSPs size were observed, relative to a drug-naïve control group, over the course of drug training or extinction. However, LTP was blocked in FD rats compared to sated rats, regardless of drug history. Finally, in heroin-trained rats, a significant inverse correlation was observed between LTP magnitude and heroin seeking during the reinstatement test. The current results suggest that FD can alter plasticity in the vSub-NAc shell pathway, and that plasticity in this pathway is associated with acute FD-induced reinstatement of heroin seeking.

INTRODUCTION

Most, if not all drugs of abuse directly, or as in the case of opiates, indirectly act to stimulate the release of DA within the mesolimbic pathway (Bear & Malenka, 1994; Di Chiara & Imperato, 1988; Koob, Sanna, & Bloom, 1998; Nestler, 2001). This property is shared with non-drug rewards; but, unlike natural rewards, drug-induced DA release does not habituate over time. This lack of habituation leads to an abnormal strengthening of the relationship between the effects of a drug and drug associated cues (Di Chiara, 2002; Hansel & Linden, 2001). With repeated drug use, long-lasting adaptations occur in the mesocorticolimbic DA pathway and other reward relevant regions. Such adaptations are critical for the development of drug addiction, a condition characterized by compulsive drug seeking and relapse following periods of drug abstinence (Koob & Volkow, 2010; Martin, Grimwood, & Morris, 2000; Robinson & Berridge, 2000; Wise, 2004).

Of particular importance for the current study is the role of the NAc in the reinstatement of drug-seeking behavior, a model of drug relapse. The NAc serves as a critical integrator of motivational input from the limbic system and the motor control circuitry required to guide behavioral output associated with the procurement of drugs (Kalivas, 2009; Kauer & Malenka, 2007; Mogenson et al., 1980; Nicola, Surmeier, & Malenka, 2000; Zahm, 1999). In addition to modulatory DAergic input, from the VTA, the NAc also receives excitatory glutamatergic input from a variety of sources including the vSub, a primary output region for the hippocampal formation that provides spatial and contextual information (Cooper, et al., 2006a; Goto & O'Donnell, 2001; Groenewegen et al., 1987; Saal, Dong, & Bonci, 2003; Yang & Mogenson, 1985).

Previous research has shown that exposure to drugs of abuse can modulate synaptic transmission at excitatory NAc inputs, including the vSub input to the NAc (Dong et al., 2007; Goto & Grace, 2005b; Thomas, Beurrier, Bonci, & Malenka, 2001). Understanding these changes is of crucial importance in understanding specific synaptic plasticity mechanisms that may underlie a return to drug seeking under abstinent conditions. The vast majority of studies addressing specific changes in synaptic plasticity over the course of drug use have tended to focus on psychostimulant-induced effects (see exceptions below). Thus, it is unclear what, if any, neuroadaptations occur within the vSub-NAc shell pathway following heroin self-administration and subsequent exposure to stress. The current study is designed to investigate changes within this pathway in rodents trained to self-administer heroin and subsequently tested for acute FD-induced reinstatement of heroin seeking, a model of stress-induced relapse.

Understanding Synaptic Plasticity

Synaptic plasticity refers to the ability of synapses to undergo activity dependent changes in synaptic strength. The vast majority of work on synaptic strength in the mammalian brain has focused on LTP and long-term depression (LTD) at excitatory synapses; however, both can also occur at inhibitory synapses (Kauer & Malenka, 2007). LTP and LTD refer to a sustained increase or decrease, respectively, in the strength of connection between neurons brought about by coincident activity of pre- and post-synaptic neuronal elements resulting in the facilitation or inhibition of chemical transmission that can last for weeks, or months *in vivo* (Bear & Malenka, 1994; Madison, Malenka, & Nicoll, 1991; Sesack & Grace, 2010).

LTP is a particularity important model of activity-dependent synaptic plasticity as it is considered to be a cellular correlate of long-term memory and learning in general (Bliss & Collingridge, 1993; Kauer & Malenka, 2007; Malenka, 1994; Malinow & Malenka, 2002; Martin, Chen, Hopf, Bowers, & Bonci, 2006; Thomas et al., 2001). The mechanisms mediating the induction of LTP have been studied extensively and numerous hypotheses, as well as differing forms of LTP, have been proposed (Baudry & Lynch, 2001; Thomas et al., 2001). In brief, the most widely studied form of LTP relies upon glutamaterigic receptors, particularly the NMDA receptor (Kauer & Malenka, 2007; Martin et al., 2006). Under normal conditions, stimulation of the post-synaptic neuron causes the activation of AMPA receptors. Such activation allows sodium ions to enter the neuron, which produces a short-term depolarization known as an excitatory postsynaptic potential (EPSP). With repeated high-frequency stimulation, as is the case for a tetanic stimulation, each successive EPSP contributes to the depolarization produced by the previous EPSP, a process known as EPSP summation (Cornish, Duffy, & Kalivas, 1999; Madison et al., 1991; Wang, Xu, Wu, Duan, & Poo, 2003). Given sufficient depolarization of the neuron, the blockade of NMDA calcium channels by magnesium ions is removed allowing an influx of sodium and calcium ions into the cell. As calcium concentration in the cytosol increases, protein kinase enzymes such as calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) become activated (Baudry & Lynch, 2001; Blaha et al., 1997; Floresco et al., 2003; Kauer & Malenka, 2007; Sweet & Neill, 1999; Sweatt, 2001; Malenka, 1994). These protein kinases are critically involved in the phosphorylation of existing AMPA receptors and the insertion of new AMPA receptors

into the post-synaptic membrane (Esteban et al., 2003; Lee et al., 2003; Legault & Wise, 2001). Such changes ensure that future stimulation of the synapse leads to a greater post-synaptic response. Furthermore, late potentiation of the postsynaptic response is associated with changes in protein transcription and gene expression; the end result of which is a long lasting change in the efficiency of a synapse, or LTP (Black, Green-Jordan, Eichenbaum, & Kantak, 2004; Kauer & Malenka, 2007).

In contrast, an increase in NMDA receptor-dependent calcium influx, which does not reach the threshold for the induction of LTP can result in a short-term potentiation (STP) or a LTD. Unlike LTP, STP is not long lasting and electrophysiological effects return to baseline levels over a period of 5-20 min (Malenka & Nicoll, 1999; Vorel, Liu, Hayes, Spector & Gardner, 2001). LTD, on the other hand, refers to a long-lasting change in synaptic plasticity, which may depend upon similar mechanisms to LTP (Bear & Malenka, 1994; Dong et al., 2007). LTD, particularly within the cerebral cortex, has been implicated in motor learning (Goto & Grace, 2005a; Hansel & Linden, 2001) and may also serve as one of several processes that weaken specific synapses in order to amplify those that have been strengthened by LTP (Martin et al., 2000; Yang & Mogenson, 1984).

Synaptic Plasticity Within the Mesolimbic DA pathway

As alluded to previously, drug abuse can be viewed as a form of aberrant learning and as such, drugs of abuse have been shown to modify synaptic strength within the mesolimbic DA pathway. In particular, synaptic plasticity within the VTA has been implicated in the early behavioral response to drugs of abuse (Cooper, Moore, Staff, & Spruston, 2003; Kauer & Malenka, 2007). For instance, Saal et al. (2003) has shown an

increase, *in vitro*, in the ratio of AMPA receptor mediated synaptic currents to NMDA receptor mediated synaptic currents, indicating an increase in excitatory synaptic transmission on DA neurons in the VTA, 24h after a single *in vivo* administration of cocaine, amphetamine, morphine, nicotine or ethanol. Similarly, Ungless et al. (2001) has shown that a single *in vivo* cocaine exposure induces LTP of AMPA receptor mediated currents at excitatory synapses onto VTA DA neurons, *in vitro*, 24h and 5 days, but not 10 days, after the drug exposure, indicating that cocaine and possibly other drugs or stress-induced synaptic potentiation in the VTA is transient in nature (Ungless et al., 2001). Finally, vivo cocaine exposure resulted in a blockade of LTP and an enhancement of LTD, at the these synapses, indicating that LTP and cocaine-induced synaptic potentiation share similar mechanisms (Sandi & Pinelo-Nava, 2007; Ungless et al., 2001).

Changes in synaptic plasticity which result in stronger excitatory synapses on VTA DA neurons may lead to changes in the release of DA downstream of the VTA (de Quervain, Roozendaal, & McGaugh, 1998; Kauer & Malenka, 2007). One region of particular importance is the NAc. The majority of neurons within the NAc are GABAergic medium spiny neurons. In addition to modulation by VTA DAergic input these neurons also receive excitatory glutamatergic input from a number of cortical and subcortical regions such as the PFC, entorhinal cortex, amygdala, and vSub, as well as inhibitory GABAergic input from regions such as the VP and VTA (Conboy & Sandi, 2010; Sesack & Grace, 2010). Moreover, the VP and VTA constitute the major efferent targets of NAc neurons. Thus, changes in the efficiency of synapses onto medium spiny

neurons in the NAc are likely to feedback into further changes within the mesolimbic DA system (Herman et al., 1995; Kauer & Malenka, 2007; Thomas et al., 2001).

In contrast to findings in the VTA, a single administration of cocaine has been shown to have no effect on the ratio of AMPA to NMDA receptor mediated excitatory postsynaptic currents in the NAc *in vivo*. However, 5 days of chronic cocaine administration followed by a withdrawal period and a challenge dose of cocaine causes a significant reduction in the ratio of AMPA to NMDA receptor mediated excitatory postsynaptic currents at PFC afferents synapsing onto NAc shell medium spiny neurons. Furthermore, a significant reduction in the induction of LTD was observed in cocaine-treated animals suggesting that this reduction in synaptic plasticity was due to mechanisms shared with LTD (Herman et al., 1995; 1998; Thomas et al., 2001). Similarly, cocaine self-administration has been shown to inhibit the induction of LTD in both the NAc shell and core following 1-day of abstinence, but only in the NAc core following 21 days of abstinence. In contrast, LTD was inducible in yoked rats receiving equivalent amounts of cocaine, rats self-administering food, and drug-naïve rats, suggesting that cocaine self-administration may produce long-lasting neuroadaptations in the NAc, particularly in the NAc core, which are distinct from those induced by the basic pharmacological effects of the drug or from operant responding for non-drug reward (Herman et al., 1995; 1998; Martin et al., 2006). Importantly, persistent adaptations in glutamate receptors resulting from changes in synaptic plasticity may contribute to drug seeking behavior under abstinent conditions. For instance, Cornish et al. (1999) have demonstrated that AMPA receptor activation in the rat NAc will reinstate extinguished cocaine seeking; whereas, the AMPA/kainate receptor antagonist CNQX, but not the DA

receptor antagonist fluphenazine, blocks cocaine priming-induced reinstatement of cocaine seeking (Cornish & Kalivas, 2000).

Synaptic Plasticity in the vSub-NAc Shell Pathway in Response to Drug Exposure

As previously mentioned, the NAc shell receives glutamatergic input from the vSub. Moreover, stimulation of the vSub, with NMDA, has been shown to increase the number of spontaneously firing DA cells within the VTA, an effect which is abolished by intra-NAc infusion of the glutamate receptor antagonist kynurenic acid or intra-VP infusion of the $GABA_A$ and $GABA_B$ agonists baclophen and musimol, suggesting that the vSub activates VTA DA neurons through a vSub-NAc shell-VP-VTA pathway (for a detailed description of this pathway see Floresco et al., 2003; Nettles et al., 2000). Additionally, electrical stimulation of the vSub causes an increase in DA oxidation currents, a measure used to assess DA terminal efflux, in the NAc (Blaha et al., 1997; Herman et al., 1998). Moreover, the reinforcing effects of vSub self-stimulation are enhanced following NAc amphetamine infusion suggesting a relationship between NAc DA tone and the influence of the vSub on motivated behavior (Sweet & Neill, 1999). Additionally, intra-NAc infusion of glutamate receptor antagonists block the increase in DA efflux observed in the NAc following vSub stimulation, suggesting that the vSub input to the NAc shell may act upon glutamate receptors to presynaptically modulate DA release (Blaha et al., 1997; Canteras & Swanson, 1992; Cullinan, Herman, & Watson, 1993). Furthermore, inactivation of the vSub with TTX blocks extracellular NAc DA release (Legault & Wise, 2001; Liu & Liang, 2009). Finally, inhibition of the vSub with lidocaine attenuates cocaine priming- and cue-induced reinstatement of drug seeking (Foy, Stanton, Levine, & Thompson, 1987; Shors, Seib, Levine, & Thompson, 1989; Sun

& Rebec, 2003; also see Black et al., 2004 for a discussion of the importance of drug training history) whereas, stimulation of the vSub causes a reinstatement of cocaine-seeking behavior under extinction conditions (Taepavarapruk & Phillips, 2003; Vorel et al., 2001).

Despite the observed role for the vSub in drug seeking behavior there is a paucity of research investigating the relationship between this structure and synaptic plasticity within the NAc shell as it relates to animal models of addiction. Thus, there appear to be no studies investigating LTP, in the vSub-NAc shell pathway, within the context of the reinstatement model. There are, however, several studies that suggest the potential importance of this pathway in neuroplastic changes induced by drugs of abuse. For instance, Dong et al. (2007) has shown an impairment in the induction of LTP within the vSub-NAc shell following 12 days of experimenter administered morphine and 2, but not 4, days of drug withdrawal. Furthermore, Goto and Grace (2005b) showed that synaptic plasticity at vSub inputs in the NAc was disrupted in rats sensitized to experimenter administered cocaine. Moreover, these researchers suggest that phasic DA release, within the NAc, facilitates vSub inputs to the NAc, via DA D1 receptors, shifting the balance of NAc input away from PFC input. Furthermore, mesolimbic DA transmission has been shown to exert a modulatory influence over the vSub input to the NAc. For instance, electrical trains delivered to the VTA prior to stimulating the vSub, reduce the excitation of NAc DA by vSub stimulation (Saal et al., 2003; Yang & Mogenson, 1984).

The Role of the vSub in Stress Responsivity

Since LTP is thought to represent a cellular correlate of learning and memory, it is important to briefly touch on the effect of stress on learning. Studies of this nature have

shown that stress can impair or facilitate learning and memory depending upon the procedure (Kim & Diamond, 2002; Kourrich, Rothwell, Klug, & Thomas, 2007; Rothwell, Kourrich, & Thomas, 2011; Sandi & Pinelo-Nava, 2007). For instance, de Quervain et al. (1998) have shown that exposure to footshock stress 30 min prior to a Morris water maze retention test significantly impairs performance. Furthermore, these authors show that administration of metyrapone, which blocks corticosterone synthesis, blocks stress-induced memory impairments. In addition, in non-stressed rats administration of corticosterone prior to the test induces an impairment in memory retention. In contrast, Conboy and Sandi (2010) show that cold-temperature stress facilitates learning in a Morris water maze. These authors demonstrated that mice trained in colder water (22 °C) have increased post-training corticosterone levels and increased post-training learning relative to mice trained in warmer water (30 °C).

Related to these findings, the vSub has been shown to play a substantial role in stress integration. For instance, lesions of the vSub have been shown to have no effect on basal corticosterone level or the circadian pattern of corticosterone release (Herman et al., 1995). However, lesions of this structure increase CRF mRNA and CRF peptide expression in the paraventricular nucleus of the hypothalamus (PVN), increase restraint stress-induced CRF release, increase the depletion of PVN CRF following restraint or open field stress and increase ACTH response to a novel auditory stress while having no effect on HPA axis driven negative feedback (Herman et al., 1995; 1998; Nettles et al., 2000).

Collectively these studies suggest that the vSub acts to inhibit the actions of the HPA axis (Herman & Mueller, 2006). However, projections from the vSub have been

shown to innervate the BNST and amygdala, and it may be the case that the vSub influences stress responsivity through GABAergic projections from these structures to the PVN (Canteras & Swanson, 1992; Cullinan et al., 1993; Dworkin, Goeders, & Smith, 1985). Moreover, the vSub has been shown to interact with BNST in the formation of an aversive memory (Liu & Liang, 2009), suggesting a functional link between these structures which could potentially play a role in stress-induced reinstatement of drug seeking. As discussed in the general introduction, the effects of acute FD on the reinstatement of drug seeking are likely mediated by the actions of extra-hypothalamic CRF, particularly acting within the BNST (Dong et al., 2007; Donny, Bigelow, & Walsh, 2006; Erb & Stewart, 1999; Erb et al., 1998; Nettles et al., 2000).

Similarities Between Drugs and Stress with Regard to Synaptic Plasticity

As mentioned previously there is a limited amount of research focusing on synaptic plasticity within the vSub-NAc shell pathway in the context of drug-related effects. Dong et al. (2007) has demonstrated that acute withdrawal (12h) from 12 days of experimenter administered morphine impairs the induction of LTP within the vSub-NAc shell pathway; however, LTP induction is restored following 4 days of withdrawal. Moreover, stress-induced by 30 min on an elevated plus maze enabled low frequency stimulation (1 Hz), that was ineffective in non-stressed rats, to produce an LTD within the vSub-NAc shell pathway, following 12h but not 4 days of withdrawal.

Additionally, stress and drugs of abuse have been shown to produce similar changes in synaptic plasticity within the mesolimbic DA system. As mentioned above, a single exposure to a variety of abused drugs results in an increase in synaptic strength at VTA synapses, as indicated by an enhanced ratio of AMPA/NMDA receptor mediated

synaptic currents. Furthermore, there is an increase in the ratio of AMPA/NMDA receptor mediated synaptic currents, in the VTA, 24h after a single cold water forced swim stress suggesting that stress and drugs induce similar neuroplastic changes (Saal et al., 2003). Additionally, two days of cold water forced swim stress and novelty increase the ratio of AMPA/NMDA receptor mediated synaptic currents on excitatory glutamatergic synapses within the NAc. However, this effect is only observed on medium spiny neurons in the NAc shell and not in the NAc core (Campioni, Xu, & McGehee, 2009; Rothwell et al., 2011). Furthermore, repeated *in vivo* administration of cocaine has been shown to enhance NAc excitatory synaptic transmission following a protracted withdrawal period. However, if cocaine is administered during this withdrawal period, a reversal of potentiation and synaptic depression is observed (Kourrich et al., 2007). Similarly, a reinstatement of CPP following a priming injection of cocaine or a forced swim stress is associated with a reduction in synaptic strength within the NAc shell. Thus, triggers associated with the reinstatement of drug seeking, such as drug-priming and stress, appear to demonstrate a common form of synaptic plasticity in the NAc, particularly within the NAc shell (Rothwell et al., 2011).

However, it is worth noting that in the studies above, which discuss synaptic plasticity at excitatory synapses, within the NAc, with the exception of Dong et al. (2007), the stimulating electrode was targeted at fibers projecting from the PFC to the NAc, not from the vSub to the NAc.

The Current Study

Despite the demonstrated involvement of the NAc shell and the vSub in drug seeking behavior, there are relatively few studies investigating changes in synaptic

plasticity in the vSub-NAc shell pathway following opiate use. One notable exception is the study of Dong et al. (2007) in which rodents were administered morphine twice a day for a period of 12 days. In this study an impairment in the induction of LTP within the vSub-NAc shell pathway was observed following 12h, but not 4 days, of withdrawal. Here we expand upon the results of Dong et al. (2007) by investigating changes in the vSub-NAc shell pathway following heroin self-administration, extinction, and exposure to acute FD, a stressor known to reliably reinstate drug seeking (Shalev et al., 2000). Acute FD stress is functionally distinct from other forms of stress in that it represents a challenge to metabolic homeostasis. Thus, it is unclear what, if any, effect acute FD will have on synaptic plasticity in the vSub-NAc shell pathway. However, given that experimenter administered morphine and subsequent withdrawal impairs the induction of LTP in the vSub-NAc shell, an impairment in LTP within this pathway is expected in rats trained to self-administer heroin. Furthermore, acute stress and drugs of abuse have been shown to have similar effects on synaptic plasticity; thus an impairment in the induction of LTP following acute FD stress is expected. However, in rats with a history of both drug self-administration and acute FD-induced reinstatement it is expected that stress will reverse the effects of previous drug exposure which should allow for the induction of LTP in previously drug-trained rats.

METHODS

Subjects

Subjects consisted of 63 male Long Evans rats (Charles River, St Constant, QC, Canada) weighing 350-450 g at the start of experimentation. Prior to surgery rats were given 1 week to habituate to daily handling and facility light (normal 12:12 h light-dark

cycle, lights on at 8:00 a.m.; lights off at 8:00 p.m.) and temperature conditions (21°C). Following IV catheterization and electrode implantation surgery and 1-2 weeks recovery, rats were transferred to self-administration chambers or remained in the animal care facility. With the exception of tests for acute FD-induced reinstatement (see below), food and water were available *ad libitum*. All experimental procedures were approved by the Concordia University Animal Research Ethics Committee.

Surgery

Rats were implanted with bipolar electrodes under anesthesia (xylaxine+ketamine, 10+100 mg/kg, IP or inhaled Isoflurane 5%-1.5%). Bipolar electrodes were constructed from two segments of Teflon-coated stainless steel twisted-wire (125 um tips separated by 1.0 mm). Electrode leads were connected to gold-plated Amphenol pins and lowered through burr holes into the vSub (AP: -6.5 mm, ML: 5.0 mm, DV: 5.5-6.0 mm) and the NAc shell (AP: +1.6 mm, ML: +1.0, DV: 5.5-7.3 mm). The vertical position of both the stimulating electrode (vSub) and recording electrode (NAc shell) was adjusted to optimize the amplitude of field EPSPs (fEPSP) in the NAc shell. Once both electrodes were lowered to the target location an electrical stimulation, in most cases 1000 uA, was delivered to the vSub, and the NAc shell response was observed. Electrodes were adjusted to ensure the largest peak amplitude and a 15-25 ms response latency (see traces below). In addition to the electrodes, two stainless-steel screws were placed in the contralateral skull and served as reference electrode and as a ground. Finally, electrode leads, and wires from the reference and the ground screws were inserted into a 9-pin Macintyre connector and the connector was secured to the skull using jewelers' screws and dental cement.

Immediately prior to, or 1 week following electrode implantation, an IV silastic catheter (Dow Corning, Midland, MI) was inserted into the right jugular vein as described previously in the general methodology.

Apparatus

Following recovery from surgery, rats assigned to the heroin-training groups were moved from the animal care facility to operant self-administration chambers. These chambers are described in the general methodology; however, for this experiment the inactive lever was not retractable. Rats that remained in the animal care facility were singly housed in standard shoebox cages throughout experimentation.

For electrophysiological recordings rats were placed in a 30.0 X 60.0 X 30.0 cm Plexiglas chamber. Between testing sessions the floor, plastic grill flooring, and all walls were cleaned with 70% ethanol to minimize odor from the previous rat. fEPSPs were recorded from the NAc Shell using Datawave Software (DataWave Technologies, Loveland, CO, USA).

Drugs

As described in the general methodology, heroin (diacetylmorphine HCL) was dissolved in physiological saline. However, as a result of different infusion pumps being used in this experiment, the volume of heroin delivered per infusion was reduced by approximately half; thus, the drug syringe concentration was increased to account for this difference.

Procedure

The behavioral portion of this study consists of three phases: heroin self-administration training, extinction training, and reinstatement testing. These phases are

described in detail (see general methodology). Therefore, only procedural modifications and a brief procedural description are noted here. Additionally, prior to, and during each of these phases, electrophysiological recording were made at regular intervals. Control animals were treated in a similar manner with regard to daily maintenance and electrophysiological procedures.

Heroin Training, Extinction Training & Reinstatement

Following electrode implantation and IV catheterization surgery rats were assigned to one of four training and reinstatement conditions: heroin self-administration and acute FD (Her-FD), heroin self-administration and sated (Her-Sated), drug-naïve and acute FD (Naïve-FD) and drug-naive and sated (Naïve-Sated). All drug-naïve rats remained in the animal colony and were never exposed to heroin.

For animals in the heroin self-administration conditions, training was conducted over a period of 10-14 days. Throughout training, rats received three 3-h drug sessions beginning at 1:00 p.m. Following training the heroin syringes were removed and rats underwent a period of extinction training. Rats were given three-3h extinction sessions on the first day and one session thereafter. Once an extinction criterion of 15 or less active lever responses (per 3-h session) was reached, rats were tested for reinstatement of drug seeking, under extinction conditions. Rats were given one reinstatement test, which was preceded by a 48h 'off' period during which time no extinction sessions were conducted and rats were either food deprived or had unrestricted access to food.

Electrophysiology

For each rat, monopolar and bipolar electrophysiological responses were recorded. Thus, in cases where I was unable to obtain a bipolar recording, a monopolar

recording was used. However this recording was compared to the reference to reduce interference. Electrical stimuli were generated using a digital to analog channel (50 kHz) and a stimulus isolation unit (A-M Systems, Model 2200). NAc shell responses were amplified, digitized at 1024 Hz, and stored for analysis.

Prior to, and throughout all experimental phases, rats received input/output tests to monitor synaptic responses, fEPSP, in the NAc shell, evoked by stimulation of the vSub (see data analysis). Input/output tests consisted of removing the rat from the operant chamber, placing it in the electrophysiological chamber, and attaching the Macintyre plug to a lead. For the input/output test rats received 5 stimulus sweeps consisting of three pulse intensities (100, 500, and 1000 uA). Following the reinstatement tests, LTP was induced using high-frequency stimulation consisting of 10 trains, separated by an inter-train interval of 2 s, with 20 pulses at 200 Hz per train (Dong et al., 2007). In order to optimize the induction of LTP, response intensities were adjusted for each rat, such that 60% of the maximal response was evoked prior to the delivery of the train.

Data Analysis

To assess the effect of acute FD on the reinstatement of heroin seeking behavior, a series of ANOVAs were preformed using *deprivation state* (sated or FD) as a between subjects factor and *day* (baseline or test day) as a within subjects factor.

Electrophysiological responses were analyzed using DataWave Software (DataWave Technologies, Loveland, CO, USA). For the analysis of input/output tests and the induction of LTP, standardized rather than raw data was assessed. This was done for ease of comparison and is a standard practice. For the input/output test, raw fEPSP amplitude was standardized to the highest intensity response, which was observed at

1000 uA, on the second baseline recording day, conducted prior to the start of heroin training. Changes in the input/output test were assessed using separate repeated measures ANOVAs for the heroin-trained animals and for the drug-naïve rats. For these ANOVAs, *day* was used as a within subjects comparison and *deprivation state* (sated or FD) was used as a between subjects factor. Analyses were performed on input/output data from the following days: two days of baseline recordings (prior to drug self-administration; BL1 and BL2), early self-administration (SA days 1-2), mid-self-administration (SA days 2-4), late self-administration (SA days 8-12), the first extinction day (Ext 1), the last extinction day before the 48h "off" period (Ext-Pre), and immediately prior to the LTP test (Test). In cases where an animal had multiple input/output tests, during a given time point, the most internally consistent response was chosen. For drug-naïve rats, data from the two days of baseline recordings (BL1 and BL2), the day before the 48h "off" period (Pre-Test) and immediately prior to the induction LTP (Test) were analyzed.

Furthermore, since the most interesting day with regard to the input/output tests is immediately before the induction of LTP, an additional ANOVA was preformed on the input/output data at this time using *drug history* (heroin or drug-naive) and *deprivation state* (sated or FD) as between subjects factors.

For LTP, raw fEPSP amplitude measures were standardized to the average of the 10 min prior to the delivery of the stimulus train. % fEPSP amplitude was used for all statistical analyses. Additionally, LTP magnitude was assessed by comparing the average % fEPSP amplitude for the last 10 min of recording to the average % fEPSP for the 10 min baseline period using a repeated measures ANOVA. *Drug history* (heroin or drug-naive) and *deprivation state* (stated or FD) were considered between subjects factor and

time (baseline or final 10 min) was considered a within subjects factor. To assess the relationship between acute FD-induced reinstatement and the magnitude of LTP, a drug seeking score was computed using the following formula: Test day active lever responding – baseline active lever responding. Drug seeking score was correlated with average % fEPSP amplitude during the last 10 min using a Pearson's product moment correlation. For all comparisons significance was evaluated at $p < .05$ and all significant ANOVA results were followed-up by Sidak corrected t-tests.

RESULTS

Six rats did not survive the surgeries, 3 were removed due to the lose of a headcap, 1 rat was removed from the self-administration group due to a leaky catheter, 2 rats were removed due to a failure to train (defined as less than 15 active lever responses over 3h on the last day of training), 1 rat was removed because of a procedural error, and 22 rats were removed because of an overly variable or poor electrophysiological response to stimulation. Thus, the final number of rats in each of the experimental conditions was: HER-FD $n = 6$; HER-Sated $n = 5$; Naive-FD $n = 9$ and Naive-Sated $n = 8$.

Behavioral Performance

As demonstrated in *Figure 1*, rats showed a clear preference for the active, drug-paired lever over the inactive non-drug-paired lever. On the last training day the mean (+/- *SEM*) number of infusions, active lever responses and inactive lever responses were 48.81 (+/- 10.41), 109.91 (+/-18.93) and 28.91 (+/-12.88), respectively. Following training rats received a mean of 7.40 (*SEM* = 1.06) extinction days and a 48h "off" period. An effort was made to match heroin-trained rats to the sated and FD conditions based upon drug infusions ($M_{Sated} = 43.16$, $SEM = 7.41$; $M_{FD} = 53.07$, $SE = 18.57$),

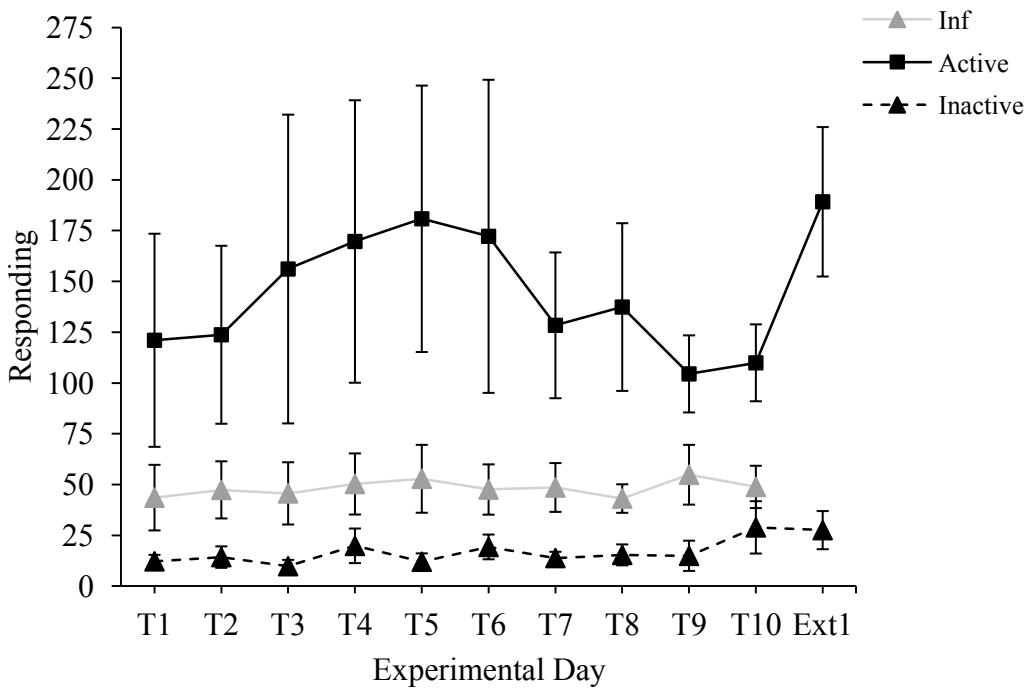

Figure 1.

Mean (+/- *SEM)*, number of active lever presses, inactive lever presses and infusions (0.1 mg/kg/infusion) for the 11 rats trained to self-administer heroin. Rats were trained to self-administer heroin over a minimum of 10 training days (T1-10; three-3h session per day) and the first extinction day (Ext 1). A significant difference existed between active and inactive lever responding on all training days and during the first day of extinction ($p <$ .05).

$F(1, 9) = 0.21$, $p = .66$, $\eta_p^2 = .06$, active lever responding (M_{Sated} =160.93, SEM = 7.70; M_{FD} =138.93, SE = 5.16), $F(1, 9) = 5.98$, $p = .04$, $\eta_p^2 = .40$, and inactive lever (M_{Sated} = 24.96, SEM = 0.77; M_{FD} =23.51, SE = 1.78, $F(1, 9) = 0.48$, $p = .51$, $\eta_p^2 = .05$ based on the mean of the last 5 days of training. However, as can be seen above, since some animals were removed at a later phase of the experiment, the groups are not well matched.

A mixed factorial ANOVA investigating active lever responding during the reinstatement phase revealed a significant effect of *day* (baseline or Test), $F(1, 9) = 11.77$, $p < .01$, $\eta_p^2 = .57$, a significant effect of *deprivation state* (sated or FD), $F(1, 9) = 10.75$, $p = .01$, $\eta_p^2 = .54$, and a significant *day X deprivation state* interaction, $F(1, 9) = 10.49$, $p = .01$, $\eta_p^2 = .54$ (see *Figure 2A*). Follow-up analyses, to investigate the interaction between *day* X *deprivation state* revealed a significant increase in responding on the active lever compared to the baseline extinction session in the FD rats, and a significantly higher rate of response in the FD rats compared to the sated ones on the test day, ps < .05. This is despite the higher response rate on the active lever for the sated group during self-administration training. For the responses on the inactive lever, there was no significant effect of *day*, $F(1, 9) = 0.01$, $p = .91$, $\eta_p^2 = .01$, *deprivation state,* $F(1, 9) = 0.20$, $p = .67$, $\eta_p^2 = .02$ or *day X deprivation state* interaction, $F(1, 9) = 1.00$, $p = .34$, $\eta_p^2 = .10$ (see *Figure 2B*).

Electrophysiology

The data from the input/output tests include the two days of baseline recordings, early self-administration, mid-self-administration, late self-administration, the first extinction day, the last extinction day before the 48h “off” period, and immediately prior

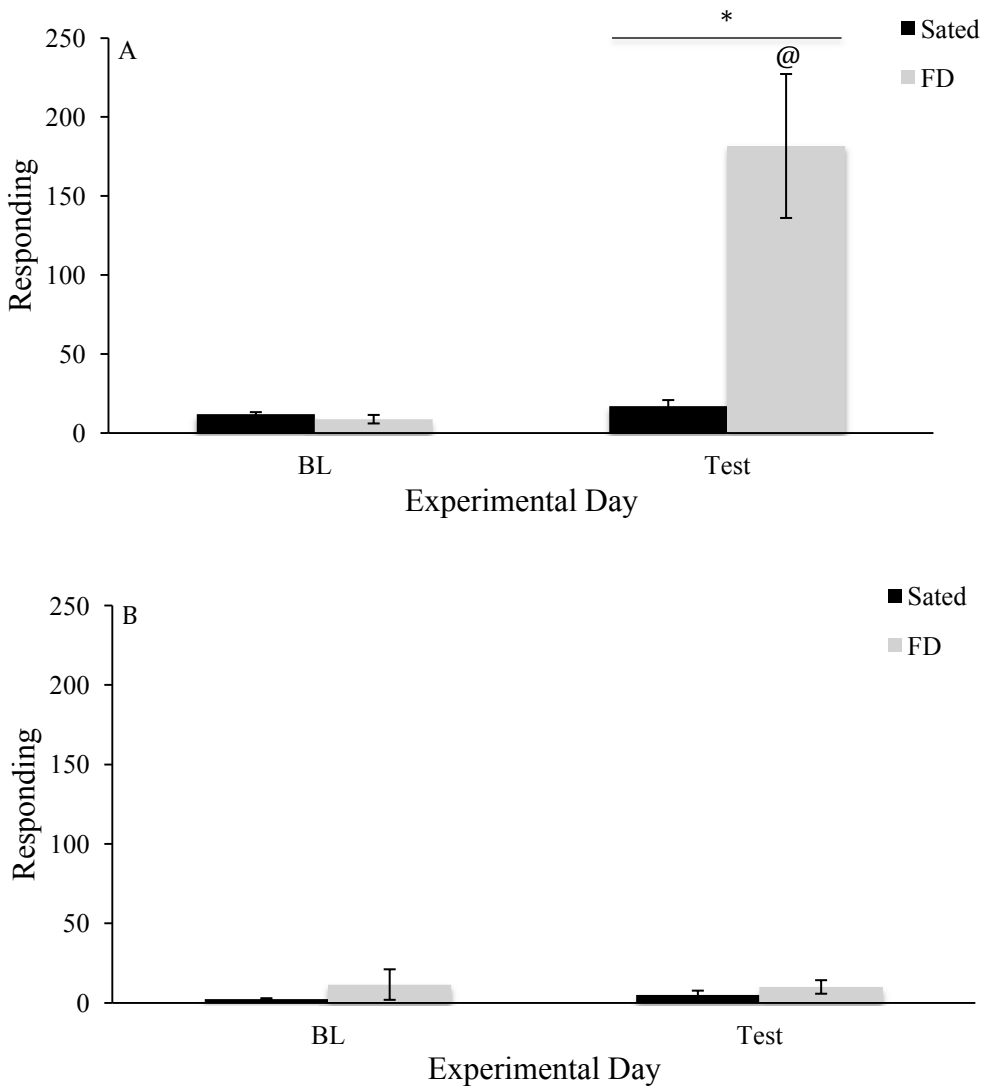

Figure 2.

The effect of an acute 48h FD on heroin seeking in abstinent animals. Data are mean (+/- *SEM*) number of active (A) and inactive lever (B) responses made during the baseline (BL) extinction session and following 48h unlimited access to food (Sated, $n = 5$) or food deprived (FD, $n = 6$). * $p < .05$ relative to the baseline condition. @ $p < .05$ compared to the BL and compared to sated rats on the test day.

to the LTP test are displayed in *Figure 3A*. For drug-naïve rats, baseline recordings, the day before the 48h sated or FD period and immediately prior to the LTP test are displayed in *Figure 3B*. As is apparent in *Figure 3*, there was no significant effect of *time*, $F(7,56) = 0.53$, $p = .81$, $\eta_p^2 = .06$, *deprivation state*, $F(7,56) = 1.75$, $p = .22$, $\eta_p^2 = .18$, or *time X deprivation state* interaction, $F(1,8) = 1\ .75$, $p = .22$, $\eta_p^2 = .18$ across input/output tests for rats trained to self-administer heroin. Similarly, for drug-naïve rats there was no significant effect of *time*, $F(3, 45) = 1.24$, $p = .30$, $\eta_p^2 = .08$, *deprivation state*, $F(1,15) = 1.43$, $p = .25$, $\eta_p^2 = .09$, or *time X deprivation state* interaction, $F(3,45) = 1.45$, $p = .25$, $\eta_p^2 = .09$. An additional ANOVA preformed on the test day also revealed no significant effect of *drug history*, $F(1, 24) = 0.81$, $p = .38$, $\eta_p^2 = .03$, *deprivation state*, $F(1, 24) = .55$, $p = .47$, $\eta_p^2 = .02$, or *drug history* X *deprivation state* interaction, $F(1, 24) = 2.82$ $p = .13$, $\eta_p^2 = .10$.

In order to assess any differences in LTP across conditions, a repeated measures ANOVA was preformed as described above. This ANOVA revealed a significant effect of *time*, $F(1, 24) = 13.93$, $p < .001$, $\eta_p^2 = .37$, a significant *time X deprivation state* interaction, $F(1,24) = 4.78$, $p = .04$, $\eta_p^2 = .17$, and a tendency toward a significant effect of *deprivation state*, $F(1,24) = 4.05$, $p = .06$, $\eta_p^2 = .15$ (see *Figure 4*). Further analyses revealed no significant difference between food deprived and sated animals during the baseline period ($M_{FD} = 100.67$, $SEM = 0.30$; $M_{Sated} = 100.10$, $SEM = 0.26$), $p > .05$. However, only animals in the sated condition had a significant increase in % fEPSP amplitude during the last 10 min of recording ($M = 126.86$, $SEM = 6.54$), compared to the baseline average, $p < .05$. Thus, animals exposed to acute FD showed no significant

change in % fEPSP amplitude, while sated animals showed an approximately 26% facilitation (see *Figure 5*).

Despite the apparent difference between groups in terms of the immediate response to the stimulus train, there was no significant difference between groups in terms of length of time to return to baseline, as measured by the first time point at, or exceeding 100% fEPSP amplitude following the stimulus pulse ($M_{\text{Naive-Sated}}$ = 4.31 min, *SEM* = 1.36; $M_{\text{Naive-FD}}$ = 4.61 min, *SEM* = 1.98; $M_{\text{Heroin-Sated}}$ = 10.20, *SEM* = 3.36; $M_{\text{Heroin-FD}}$ = 4.83 min, *SEM* = 2.76), F(3,24) = 1.25, p = .21, η_p^2= .14. Finally, as shown in *Figure 6*, the magnitude of LTP decreased, within heroin-trained rats, as drug seeking behavior increased, r (9) = -.72, p = .01.

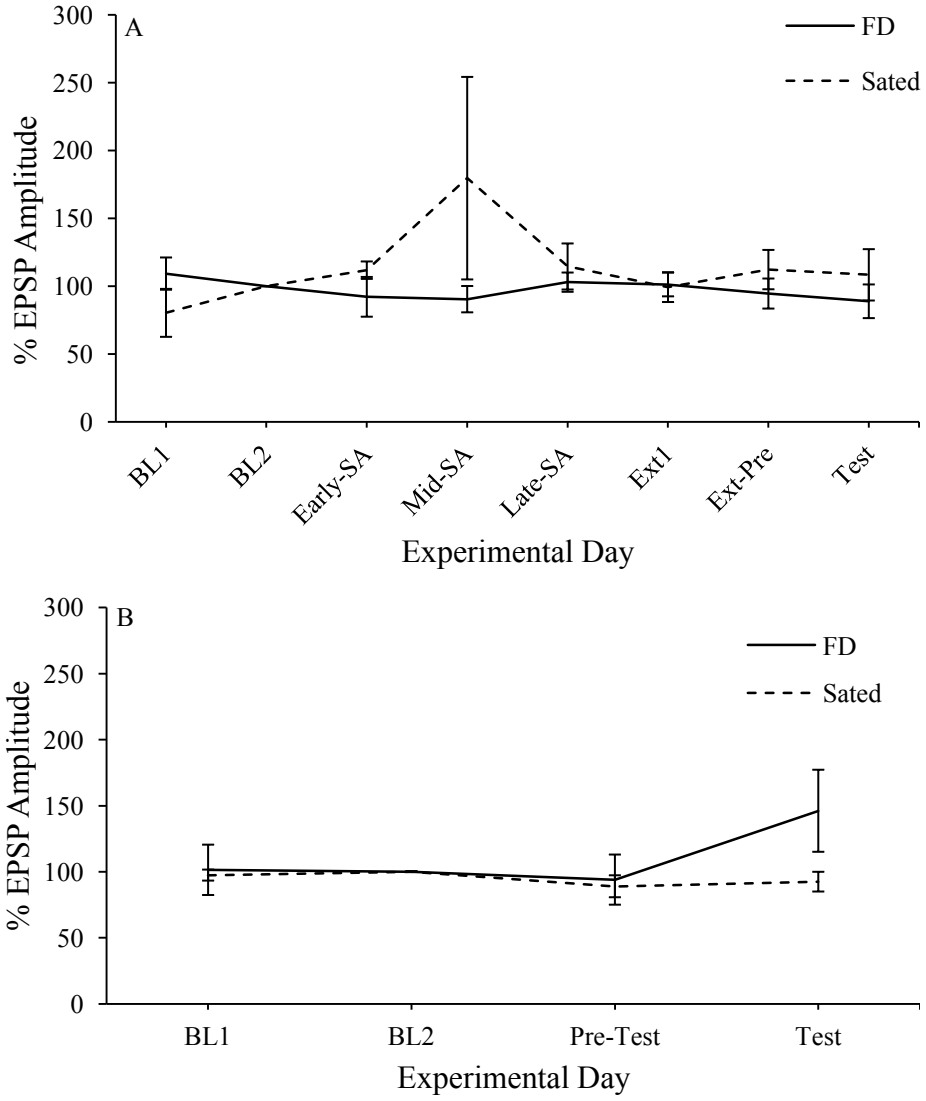

Figure 3.

Input/Output tests recording across experimental days for heroin-trained (A) and drug-naïve rats (B) animals, that were tested under sated or food deprived (FD) conditions. All data points are standardized to the 1000 uA response on the second day of baseline recording.

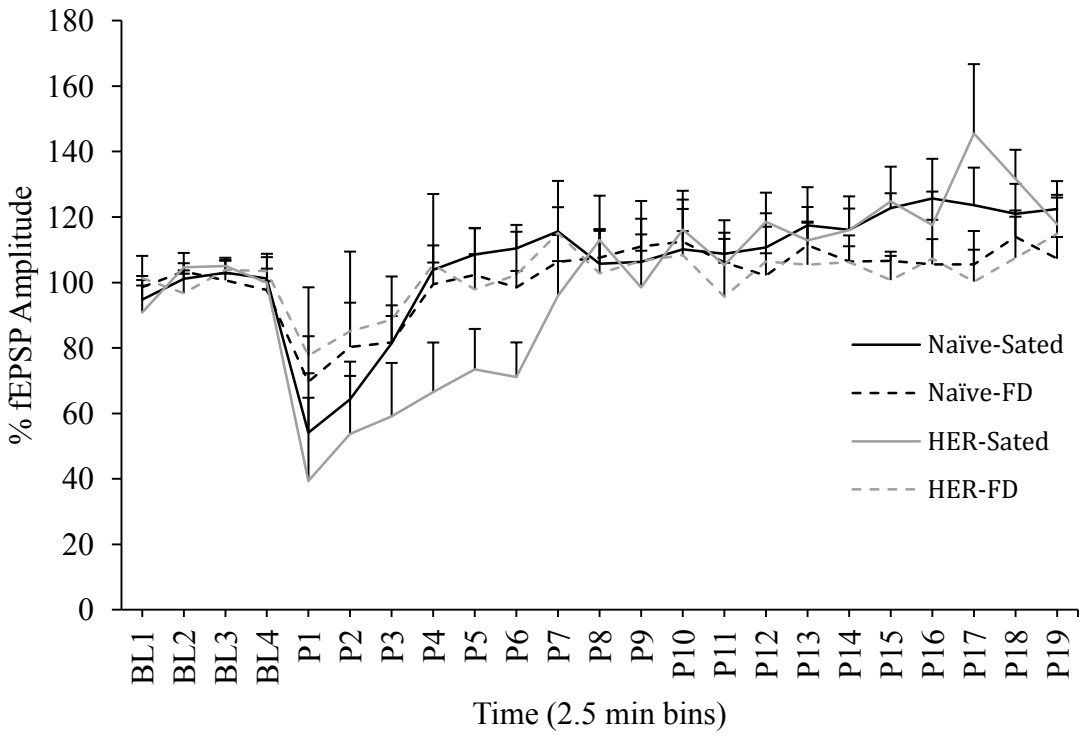

Figure 4.

% fEPSP amplitude relative to baseline. Data are mean (+*SEM*) % fEPSP amplitude standardized to the mean of the baseline period. Data are shown in 2.5 min segments consisting of the average of 5 recording points over the course of a 10 min baseline and 48 min of recording after the stimulus train delivery. BL1-4 correspond to the 4 baseline records and P1-19 correspond to the 19 recording periods following delivery of the tetanic train.

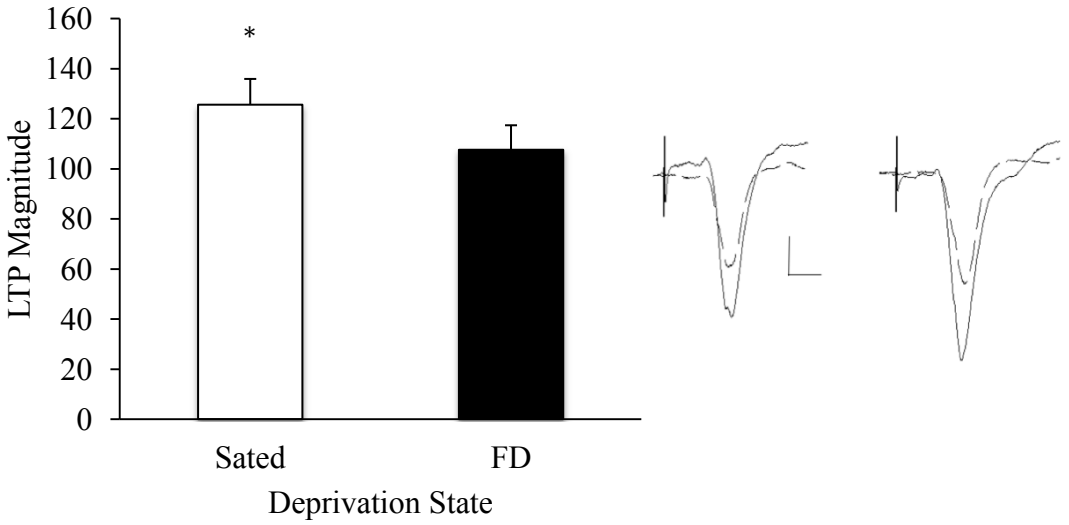

Figure 5.

Mean (+*SEM*) % fEPSP amplitude expressed as the percent change from baseline for the average of the last 10 min of recording. Bars correspond to the mean (+*SEM*) for sated rats (heroin-trained and naïve sated; white) and food deprived (heroin-trained and naïve food deprived, FD; black) rats. * significantly greater than baseline, $p < .05$. Additionally, a sample traces from the baseline (left trace) and final 10 min of recording (right trace) are shown for a sample FD rat (dashed line) and a sample sated (solid line) rat. Traces were created using a mean of 5 traces from each time period. Calibration: Vertical scale bar = 0.2 mV, horizontal scale bar = 10 ms.

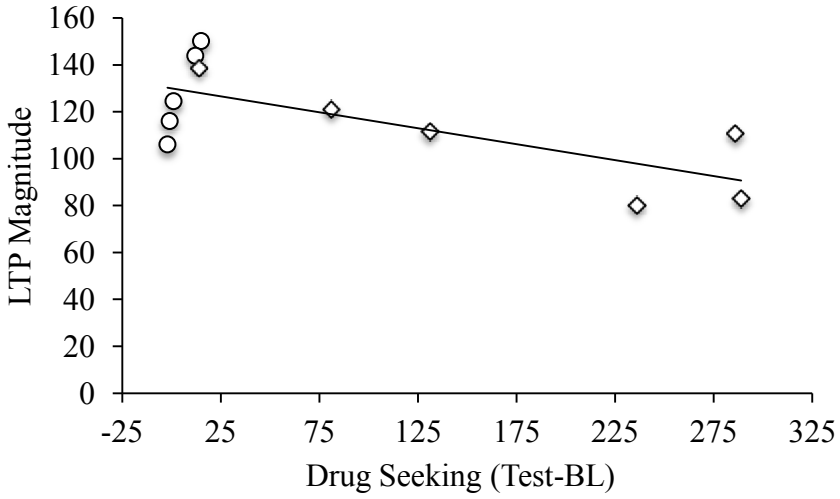

Figure 6.

Scatterplot and trend line demonstrating the relationship between LTP magnitude (the average of the last 10 min of recording during the LTP test) and drug seeking behavior (Active lever responding on the test day – Active lever responding on the baseline day prior to the food deprivation or sated test). Rats that were food deprived on the test day are indicated by diamonds, and sated rats are indicated by circles. A significant correlation is present, r (9) = -.72, p = .01.

DISCUSSION

The main finding of the current study is that acute FD impairs the ability of a high-frequency stimulation to induce LTP in the vSub-NAc shell pathway. This impairment in LTP induction was seen in both heroin-trained and drug-naïve food deprived rats. Furthermore, there was no impairment in the induction of LTP in heroin-trained sated rats, indicating that a period of acute FD, and not drug history, is associated with a reduction in the induction of LTP. In addition, drug exposure also appears to have no effect on synaptic plasticity in the vSub-NAc shell pathway during heroin self-administration training and extinction, as evidenced by the lack of change in the input/output tests. Finally, amongst heroin-trained rats, a significant inverse correlation was found between drug seeking on the reinstatement test day and LTP magnitude. Thus, rats that showed higher levels of reinstatement of heroin seeking also showed impaired LTP.

The Lack of Effect for Drug Self-Administration Training on Synaptic Plasticity

The finding that previous drug history was not associated with changes in synaptic plasticity, as measured by input/output tests and the induction of LTP, is somewhat surprising. Previously, it has been shown that drug administration and subsequent withdrawal can impair the induction of LTP in the vSub-NAc pathway in animals that have been chronically treated with morphine. This impairment was evident 24h after the last morphine infusion. However, 4 days after the last infusion of morphine LTP was induced to a similar degree in morphine-withdrawn and saline-treated rats (Dong et al., 2007). In contrast, in adolescent rats administered the cannabinoid CB1/CB2-receptor agonist WIN55,212-2 for a period of 2 weeks, an impairment in the

induction of LTP in the vSub-NAc pathway was evident 24h and 10 days, but not 30 days, after the last drug infusion (Abush & Akirav, 2012). Thus, it may be the case that drug training and the subsequent withdrawal-induced impairment in LTP induction is sensitive to the type of drug administered, the age of the animal, and the length of the withdrawal period. In the current study, I attempted to induce LTP in adult rats which were without drug for nearly 10 days prior to the reinstatement test. Given the design of the current experimental procedure, an earlier LTP test would not have been possible.

Alternatively, it may be the case that procedural differences account for the fact that an impairment in the induction of LTP was not observed in heroin-trained sated rats. In the current study animals were trained to self-administer heroin and then exposed to a period of behavioral extinction prior to a reinstatement test. In contrast, the vast majority of the relevant electrophysiological studies involve animals that have received experimenter-administered, non-response-contingent drug delivery. Numerous studies have shown that these two procedures are not equivalent even when time of drug delivery and drug dose are held constant. For instance, among human cocaine users the cardiovascular effects of cocaine are more pronounced following yoked as compared to self-administered drug (Donny et al., 2006). Similarly, increased mortality is observed in yoked rodents given cocaine at the same time and dose as their self-administering counterparts (Dworkin et al., 1985). In addition to general physiological differences resulting from yoked and self-administered drug, there are also substantial differences in how these two routes of administration affect the brain. NAc DA release, which is strongly associated with abuse potential and the reinforcing value of abused drugs, is higher following response contingent cocaine delivery than non-response contingent

cocaine delivery (Hemby, Co, Koves, Smith, & Dworkin, 1997; Lecca et al., 2007a). Moreover, changes in dendritic spine density following morphine exposure differ as a function of brain region and mode of drug administration. Thus, following 22 days of self-administered or experimenter administered morphine, and a one-month withdrawal period, a decrease in spine density was observed in numerous brain regions, including the NAc shell. However, this decrease was significantly greater in animals having previously self-administered morphine. Furthermore, a decrease in spine density in the CA1 and dentate gyrus of the hippocampus was only observed in animals that self-administered morphine (Robinson, Gorny, Savage, & Kolb, 2002). These differences in spine density may be interpreted as drug-induced changes in synaptic reorganization, which may underlie some of the long-term effects associated with opiate drug use.

Exposure to drug abstinence versus extinction of drug seeking may also have differing effects on synaptic plasticity. To the best of my knowledge there have been no studies investigating these differences within the vSub-NAc shell pathway. However, previous studies have demonstrated differential changes in the NAc following extinction or abstinence. For instance, inactivation of the dmPFC, which projections to the NAc core, blocks the reinstatement of cocaine seeking, in rodents which have undergone extinction training, but has no effect on cocaine seeking in rodents which have been exposed to 2-3 weeks of abstinence (Fuchs, Branham, & See, 2006; McLaughlin & See, 2003). Further evidence for extinction training-induced plasticity was recently presented by Knackstedt and colleagues (2010) who report a blunting of LTD elicited in the NAc core by PFC stimulation in extinguished, but not abstinent, cocaine-trained rats; however, in contrast they show a reduction in LTP in both extinguished and abstinent rats.

The Relationship between FD and Synaptic Plasticity

Interestingly, acute FD, but not previous drug history, was shown to prevent the induction of LTP within the vSub-NAc pathway. The impairment in the induction of LTP following stress is not entirely unexpected, although the effect of stress on synaptic plasticity is complicated. Stressors such as restraint, restraint + tail shock, and inescapable footshock have been shown to impair the induction of LTP in the hippocampal CA1 region (Joëls, Krugers, & Karst, 2007; Joëls & Krugers, 2007), which projects to the vSub. However, Diamond et al. (1992) have shown an inverse relationship between hippocampal synaptic plasticity, as measured by hippocampal primed burst potentiation, and corticosterone. Thus, it may be the case that mild stressors potentiate the induction of LTP while more severe stressors impair the induction of LTP (Joëls et al., 2007). Additionally, stress exposure and drug exposure have been shown to have similar effects on synaptic plasticity in the NAc, particularly the NAc shell. For example, stress exposure, in cocaine-administered mice, resulted in reduced excitatory synaptic strength in the NAc, and the effect was mirrored by re-exposure to cocaine which also induced reinstatement of CPP (Rothwell et al., 2011).

To the best of my knowledge the only study addressing the role of stress in modulating synaptic plasticity within the vSub-NAc pathway is that of Dong et al. (2007). In rats repeatedly treated with morphine or saline and abstinent for 12h, stress (exposure to an elevated platform) facilitated the induction of LTD in the NAc following low frequency (1 Hz) stimulation of the vSub. However, prolonged abstinence (4 days) impaired LTD in the stressed rats. Similarly, in the current study, the effects of FD on

LTP, in heroin-trained rats, was found following a prolonged extinction period (an average of 7.40 days), plus a 48h "off" period.

Importantly, Dong et al. (2007) did not investigate the effects of stress on LTP in the vSub-NAc pathway following prolonged withdrawal, making the generalization of their findings to the current study difficult. In addition, acute FD differs from other forms of stress in two main ways. First, "acute" FD is a relatively long lasting and perhaps a more severe form of stressor. Thus, it may be the case that the neuronal response to a brief stressor, such as 30 min exposure to elevated platform, is different than that produced by 48h without food. Second, acute FD differs from other forms of stress in that its effects are complex and include additional brain systems to the "classic" stress-responsive systems. For example, it has been shown that acute FD-induced reinstatement, but not footshock stress-induced reinstatement, of heroin seeking can be attenuated by administration of the anorexigenic hormone leptin (Shalev et al., 2001). I am unaware of any reports on the effects of leptin in the vSub-NAc shell pathway, and studies on the involvement of leptin in synaptic plasticity present conflicting data. For instance, leptin administration, in vitro, has been shown to result in either a facilitation or reversal of the expression of LTP in CA1 pyramidal neurons, when applied at a similar dose range (Moult, Milojkovic, & Harvey, 2009; Oomura et al., 2006).

The Significance of an Impairment in the Induction of LTP Following Acute FD

Prior to assessing the significance of the impairment in LTP observed following acute FD-induced reinstatement it is important to mention that a potentiation of % fEPSP amplitude was observed in non-FD rats and that the same stimulation protocol has been shown to induce a similar magnitude of LTP in drug naïve rats, rats exposed to a single

morphine injection, and rats repeatedly exposed to morphine but given 5 days of abstinence (Dong et al., 2007). Thus, the currently observed impairment in the induction of LTP is not due a non-effective procedure.

Numerous researchers have demonstrated an impairment in the induction of LTP, mainly within hippocampal synapses, following a variety of stressors (i.e., restraint, restraint + footshock, sleep deprivation) (Campbell, Guinan, & Horowitz, 2002; Foy et al., 1987; Kim et al., 1996). However, there are very few studies investigating synaptic plasticity following stress in regions outside of the hippocampus (see examples above). Moreover, few if any, of the reports on plasticity in the NAc and the vSub-NAc shell pathway were performed in awake, behaving animals. Thus, the results described above were obtained *in vitro* (e.g., Rothwell et al. 2011), or in anesthetized animals (e.g., Dong et al. 2007). Consequently, the current finding of a reduction of LTP in the vSub-NAc pathway are cautiously interpreted based on the results of studies on the effect of stress on synaptic plasticity in the hippocampus.

Within the hippocampal literature, stress-induced impairments in LTP have been associated with two time dependent processes: a rapid non-genomic process and a slower genomic process (see Joëls et al., 2007). Within minutes of stress-exposure, rapid non-genomic processes leading to an impairment in the induction of LTP occur within the hippocampus. This process is thought to rely on the actions of glucocorticoids at mineralocorticoid receptors, which enhances the probability of glutamate release facilitating glutamate transmission and producing an endogenous form of LTP, which prevents further induction of LTP by tetanic stimulation. Furthermore, stress responsive genes in the hippocampus, and perhaps elsewhere, are transcriptionally regulated by

glucocorticoids acting at both mineralocorticoid and glucocorticoid receptors leading to a prolonged change in the properties of neurons (de Kloet, Karst, & Joëls, 2008; Joëls et al., 2007; Karst et al., 2005; Venero & Borrell, 1999).

Here, we demonstrate an impairment in the induction of LTP which is highly correlated with acute FD-induced reinstatement, suggesting that acute FD induces an 'LTP-like' effect which simultaneously impairs the induction of LTP by high-frequency stimulation ("saturation effect"), and facilitates drug seeking. This interpretation is supported by the findings of Campioni et al (2009), which demonstrated a glucocorticoid receptor-dependent stress-induced increase in synaptic strength in the NAc, in mice.

However, as mentioned previously in this thesis, acute FD-induced reinstatement is not dependent upon a stress-induced increase in adrenal corticosterone release (Shalev, et al., 2006). On the other hand, it is important to note that Shors et al. (1990) showed that adrenalectomy did not hinder the ability of stress to impair the induction of LTP in the hippocampus. Furthermore, in rats exposed to controllable and uncontrollable footshock, corticosterone is increased to a similar extent; yet, stress-induced impairment in the induction of LTP was only observed in rats unable to control the shock (Shors et al., 1989). Conversely, intra-hippocampal CRF infusion has been shown to produce a long-lasting enhancement of synaptic efficacy in the dentate gyrus, as indicated by an increase in population spikes and an increased fEPSP amplitude and slope. Moreover, intra-hippocampal infusion of the CRF antagonist, α-helical CRF, caused a dose dependent reduction in LTP-induced by tetanic stimulation (Wang, Wayner, Chai & Lee, 1998). Therefore, it can be suggested that CRF-induced synaptic plasticity and the induction of LTP via a tetanic stimulus-train involve similar mechanisms. Thus, stress-

induced CRF release, rather than corticosterone, appears to results in the induction of 'LTP-like' process within the dentate gyrus, and possibly the vSub synapses on the NAc shell.

The Significance of an Impairment in the Induction of LTP Following Acute FD: Caveat

While it is likely that acute 48h FD induces an 'LTP-like' effect which blocks the induction of LTP by tetanic stimulation (see above), there is one rather important caveat to consider. Throughout experimentation, input/output tests were performed, whereby the vSub was stimulated and responding in the NAc shell was recorded. Had acute FD induced an 'LTP-like' state, then an increase in the % fEPSP amplitude following acute FD would be expected due to an increase in synaptic strength within this pathway. Interestingly, I did not observe an increase in % fEPSP amplitude in drug-naïve FD rats prior to the induction of LTP; however, responding in this group is not statistically significantly different from any of the other conditions. In addition, no effect across the input/output measuring times was observed, which is unusual. As mentioned previously, synaptic plasticity is altered following drug self-administration, withdrawal, and extinction; yet, at no point were changes in the response of the NAc shell to vSub stimulation observed. The flattened nature of the input/output tests may indicate a lack of sensitivity in this measure, possibly due to low subject numbers and the high variability in the data set that is typical to recordings made in non-anesthetized rats, making the findings of the input/output data less informative.

Given previous findings, I suggest that acute FD stress, like other forms of stress, induces an 'LTP-like' effect which leads to a "saturation" of the LTP response which inhibits the induction of LTP. An important concern is how to interpret a potential 'LTP-

like' effect in food deprived rats. Shors and Matzel (1997) have suggested a role for LTP in attention to stimuli. Thus, the presence of an 'LTP-like' state in rats that are acutely food deprived can increase the salience of environmental cues. In previously heroin-trained rats, this increase in salience may trigger a return to drug seeking when drug-associated cues are presented under acute FD conditions. Alternatively, it may be the case that the endogenous LTP generated by acute FD induces amnesia for the previously learned extinction contingency (Diamond, Park, & Woodson, 2004).

Concluding Remarks

In the current experiment the role of the vSub-NAc pathway in acute FD-induced reinstatement was investigated by electrically stimulating the vSub and recording the response in the NAc shell. This pathway was chosen because of the modulatory influence exerted by the vSub over the mesolimbic DA system. Furthermore, an impairment in the induction of LTP within this pathway has been observed in rats experiencing the stress of early withdrawal from repeated morphine administration (Dong et al., 2007).

Given the above findings, it was expected that previous heroin training would impair the induction of LTP. Instead, previous drug history was shown to have no effect on LTP, while acute FD was shown to impair the induction of LTP regardless of drug history. I cautiously conclude that the impairment in the induction of LTP observed in the current experiment might be due an acute FD-induced 'LTP-like' state which results in an impairment in subsequent LTP-induction. The mechanism through which such LTP triggers the reinstatement of heroin seeking is unclear; however, it is possible that acute FD interferes with extinction learning thereby triggering a return to the previously well learned behaviors, in this case drug seeking.

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CHAPTER 3

GLUTAMATERGIC RECEPTOR EXPRESSION FOLLOWING HEROIN SELF-ADMINISTRATION AND ACUTE FOOD DEPRIVATION-INDUCED REINSTATEMENT OF HEROIN SEEKING

ABSTRACT

The study of addiction has tended to focus on the neurotransmitter DA. However, drugs of abuse also modulate the release of glutamate, particularly within brain regions associated with drug reinforcement, such as the mesocorticolimbic DA pathway. Glutamate transmission has been implicated in most, if not all, forms of synaptic plasticity and is likely to underlie the persistent vulnerability to relapse observed in drug addicts. Similarly, in rodents, glutamate agonists and antagonists have been shown to trigger or block, respectively, the reinstatement of extinguished drug seeking, a model of human drug relapse. Thus, in the current study I investigated changes in the AMPA GluA1 and GluA2, and the NMDA NR1 receptor-subunits, in rats trained to self-administer heroin and drug naïve rats. Following a minimum of 10 days of heroin self-administration (0.1 mg/kg/infusion), drug seeking was extinguished by removing the drug. Under extinction conditions rats were tested for acute 21h FD-induced reinstatement or remained sated. Immediately following the end of a 2h reinstatement test, brains were removed and Western Immunobloting was preformed on tissue from the dmPFC, NAc, VTA and BLA. Drug naïve rats were treated in a similar manner. The results of this study revealed a tendency toward a reduction in GluA2 and NR1 expression in the PFC and NAc, respectively, of heroin-trained food deprived rats, compared to heroin-trained sated rats, as well as a significant increase in GluA2 expression in the VTA of heroin-trained sated rats compared to drug-naïve sated rats. Finally, FD increased GluA2 expression, in the BLA, in drug-naïve but not heroin-trained rats. Thus, heroin self-administration and acute FD may act jointly or in isolation to modulate iGluR subunit expression, a critical component of synaptic plasticity. It is

therefore concluded that adaptation in glutamate synapses might be involved in acute FD-induced reinstatement of extinguished heroin seeking.

INTRODUCTION

Traditionally, the study of addiction has tended to focus on the role of DA. A reason for this is that most, if not all, drugs of abuse facilitate the release of DA in the NAc, an effect which is directly linked to the rewarding value of these substances (Di Chiara & Imperato, 1988; Di Chiara et al., 2004; Herman et al., 1995). However, repeated drug use leads to an enhanced motivation to administer the drug and a decreased ability to adapt behavior in response to non-drug related environments and stimuli (Kalivas, LaLumiere, Knackstedt, & Shen, 2009). This persistence in drug seeking and the vulnerability to relapse long after the acute effects of drug taking have waned, suggest that drug use results in long-term changes in the neuronal circuitry mediating drug-seeking (Kalivas, 2004; Wolf, 2002).

Glutamate has been shown to play a central role in plasticity within the brain, including drug-induced neuroplasticity (Kalivas, 2004; 2009; Wolf, 2002). In fact, addiction has been characterized as a form of pathological synaptic plasticity resulting, at least in part, from drug-induced changes in glutamate synapses (Gass & Olive, 2008; Kalivas & Volkow, 2005; Kalivas:2009; McEntee & Crook, 1993). Such changes result in the abnormal strengthening of neuronal pathways that are involved in mediating the effects of abused drugs. Thus, in the current chapter, changes in the expression of GluRs subunits, within regions of the mesocorticolimbic DA pathway, are investigated following heroin self-administration, extinction, and acute FD-induced reinstatement, a rodent model of stress-induced relapse. Here, it is expected that heroin self-administration, and subsequent drug removal, will lead to enduring changes in GluR subunit expression that contribute to the propensity to reinstate drug seeking under

abstinent conditions. Furthermore, as demonstrated in chapter 2, acute FD can induce changes in synaptic plasticity; thus, it is expected that acute FD will also modulate GluR subunit expression. The direction of these changes (ie., upregulation or downregulation) and whether or not there is an interaction between a history of heroin self-administration and acute FD stress may differ depending on the region of interest and the role of this region in drug seeking.

The Nature of Glutamate Receptors

Glutamate is one of the most abundant excitatory neurotransmitters, in the mammalian brain, mediating as much as 70% of synaptic transmission in the central nervous system (Fonnum, 1984; Gass & Olive, 2008; Headley & Grillner, 1990). Once released into the synaptic cleft glutamate can exert its effect by binding to one of two classes of receptors: iGluRs and mGluRs. These receptors differ in terms of both their location and function. The iGluRs are ligand gated ion channels that mediate fast excitatory neurotransmission and are located primarily on the head of the postsynaptic spine (Gass & Olive, 2008; Guo et al., 2009). In contrast, mGluRs, which are located both pre- and postsynaptically (Gass & Olive, 2008) are directly coupled to second messengers via excitatory G-proteins and thus modulate less rapid synaptic processes (Conn & Pinn, 1997; Gass & Olive, 2008; Guo et al., 2009).

Each class of GluR encompasses numerous receptor subtypes. For instance, there are three groups of mGluRs (Group 1-3), which include the $mGluR_1$-$mGluR_8$ receptors (Gass & Olive, 2008). Furthermore, there are three iGluR receptor families: AMPA, NMDA and kainite, each of which is composed of a number of subunits (Dingledine, et al., 1999; Gass & Olive, 2008).

AMPA receptors are protein complexes that form ligand-gated ion channels composed of a variety of four non-covalently bound subunits termed GluA1-4 (Dingledine et al., 1999; Gass & Olive, 2008; Guo et al., 2009). NMDA receptors, like AMPA receptors, are heterotetrameric protein complexes that form ligand-gated ion channels and are composed of three different receptor subtypes: NR1, NR2 and NR3. There are a variety of different forms of each of these subunits. For instance, there are eight different NR1 subunits generated by alternative splicing from a single gene, four different NR2 subunits (A, B, C and D) and two NR3 subunits (A and B) (Dingledine et al., 1999). However, unlike, the AMPA receptor, the NMDA receptor requires co-activation by both glutamate, which binds the NR2 subunit and glycine, which binds to the NR1 and NR3 receptor subunits, to function. The exact composition of the NMDA receptor is somewhat debated. However, NR1 is an obligatory receptor subunit with most researchers assuming the classical NMDA receptor is composed of two NR1 and two NR2 subunits of the same or different subtypes (Dingledine et al., 1999). Similar to NMDA and AMPA receptors, kainite receptor are tetrameric protein complexes that form ligand-gated ion channels composed of various subunits termed GluA5-7, KA1, and KA2 (Dingledine et al., 1999; Pinheiro & Mulle, 2006). For the purpose of the current experiment only AMPA and NMDA receptor subunits will be considered. This is because AMPA and NMDA receptors have been extensively investigated for their role in synaptic plasticity. For a review on the function of kainite receptors see Pinheiro and Mulle (2006).

As previously mentioned, AMPA receptors are composed of subunits which are assembled together to produce an ion channel (Dingledine et al., 1999; Gass & Olive, 2008; Guo et al., 2009). Each AMPA receptor subunit contains a glutamate binding site,

and once activated, these sites allow the ion channel to become permeable to a variety of cations including calcium, sodium, and potassium. Activation of AMPA receptors contributes to the depolarization of the postsynaptic membrane and the activation of NMDA receptors by the subsequent removal of the ion-channel blockade by magnesium. Removal of this blockade allows an influx of ions, particularly calcium into the cell (Gass & Olive, 2008). This rise in calcium has been linked to both LTP and LTD. LTD occurs when magnesium ions are only partially displaced and less calcium is allowed to enter the cell (Derkach, Oh, Guire, & Soderling, 2007).

Glutamate Transmission and Psychoactive Drugs

The study of addiction has revealed many parallels between addiction and learning and memory. As such, numerous theories postulate that addiction is a disorder of 'overlearning' whereby, with repeated drug use, drug seeking becomes automatic and contexts and cues associated with drug taking become more salient (Gass & Olive, 2008). Thus, as in learning and memory, drugs of abuse induce forms of synaptic plasticity. Consequently, there has been a great deal of research investigating changes in AMPA and NMDA receptors following drug exposure.

Drug-Induced Sensitization

The first experiments to demonstrating a role for glutamate in drug addiction focused on the role of glutamate in the induction of behavioral sensitization, an animal model of the intensification of craving observed in human drug addicts (Wolf, 1998). Behavioral sensitization, which is typically studied with regard to psychostimulant drugs such as cocaine or amphetamine, refers to the augmentation of a motor-stimulant response following repeated drug exposure (Steketee & Kalivas, 2011).

Studies of this nature have showed that daily pretreatment with the NMDA receptor antagonist MK-801 blocks cocaine- and amphetamine-induced behavioral sensitization (Karler, Calder, Chaudhry, & Turkanis, 1989), an effect which seems to be mediated largely by the actions of glutamate in the VTA. For instance, repeated intra-VTA infusion of the NMDA receptor antagonist 3-(*R*-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid has been shown to block the induction of behavioral sensitization following repeated intra-VTA infusions of amphetamine (Cador, Bjijou, Cailhol, & Stinus, 1999). Moreover, two days of intra-VTA infusion of L-trans-pyrollidine-2,4-dicarboxylic acid, a glutamate reuptake inhibitor, leads to the development of a sensitized locomotor response to a single injection of amphetamine, an effect which can be blocked by administering an NMDA receptor antagonist (Aked, Coizet, Clark, & Overton, 2005). Additionally, lesions of the mPFC, but not amygdala, have been shown to block both behavioral sensitization and the facilitation of VTA glutamate release following repeated amphetamine administration (Cador et al., 1999), suggesting an important role for the glutamatergic projection from the mPFC to the VTA in behavioral sensitization.

Drug-Reinforced Behaviors

Drugs of abuse function as 'instrumental reinforcers' in that they increase the frequency of behaviors associated with their acquisition. As such, drugs facilitate self-administration and foster a preference for places associated with their administration (Everitt & Robbins, 2005). The literature regarding the role of glutamate in drug-self administration and CPP is somewhat mixed in nature. For instance, reducing glutamate transmission via intra-VTA administration of the NMDA antagonist LY235959 (Kenny,

Chartoff, Roberto, Carlezon, & Markou, 2009) or the mGluR agonist LY379268 has been shown to decrease nicotine and cocaine self-administration (Baptista, Martin-Fardon, & Weiss, 2004; Liechti, Lhuillier, Kaupmann, & Markou, 2007). However, intra-VTA administration the non-competitive NMDA receptor antagonist dizocilpine increases the rate of heroin self-administration; whereas, the competitive NMDA receptor antagonist AP-5 or the non-competitive NMDA receptor antagonist, ketamine, dose dependently blocks heroin self-administration. Similarly, intra-VTA infusion of the AMPA/kainite receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) significantly reduces heroin self-administration (Xi & Stein, 2002). The interpretation of these findings is somewhat difficult, since it is not clear if the changes in drug intake are compensatory to the changes in the rewarding value of the drug. Thus, an increase in drug-intake could be explained by either an increase in its rewarding value, or by a compensatory response to reduced value.

With regard to CPP, both the NMDA antagonist AP-5 and the AMPA antagonist CNQX, have been shown to induce a mild CPP. In contrast, AP-5 and CNQX block the development and expression of morphine CPP as well as the development of cocaine CPP when injected into VTA (Harris & Aston-Jones, 2003; Harris, Wimmer, Byrne, & Aston-Jones, 2004). Similarly, systemic injection of the non-competitive NMDA receptor antagonist MK-801 has been shown to block the induction of CPP when given prior to training injections of cocaine but have no effect when given acutely before the CPP test. In contrast icv infusion of the AMPA/kainite receptor antagonist DNQX, blocks the expression, but not acquisition, of cocaine CPP. Thus, at least for cocaine, it appears that specific GluRs, most likely in the VTA, mediate different aspects of CPP. More

specifically, NMDA receptors mediate the primary rewarding properties of cocaine (as assessed by the acquisition of CPP); whereas, non-NMDA receptors are important for the expression of behavior elicited by stimuli previously associated with drug reinforcement (as assessed by the expression of CPP) (Cervo & Samanin, 1995). Here, it is also important to remember that the actions of glutamate in the VTA may be region specific. For instance, CNQX, administration into the anterior VTA has been shown to block the rewarding effects of morphine in a CPP paradigm and heroin in a self-administration paradigm; whereas, CNQX administration into the posterior VTA blocks the locomotor effect of opiates but has no effect on the rewarding properties of either drug (Shabat-Simon, Levy, Amir, Rehavi, & Zangen, 2008).

Synaptic Plasticity in Glutamate Synapses

The activation of AMPA and NMDA receptors as well as the trafficking of these receptors is highly related to their subunit composition (Collingridge, Isaac, & Wang, 2004; Derkach et al., 2007). For instance, the presence or absence of the GluA2 receptor subunit is critical in determining the calcium permeability of AMPA receptors. AMPA receptors containing the GluA2 receptor, the majority of AMPA receptors in the brain, are highly impermeable to calcium due to the presence of arginine in the ion pore channel (Derkach et al., 2007; Gass & Olive, 2008; Hollmann, Hartley, & Heinemann, 1991; Vandenberghe, Robberecht, & Brorson, 2000).

Furthermore, phosphorylation of AMPA receptor subunits and trafficking of AMPA receptor subunits to and from the membrane plays an important role in the regulation of the AMPA receptor functioning. For instance, the GluA1 receptor subunit can be phosphorylated at three serine residues: serine (Ser) 831, Ser 845 and Ser 818

(Santos, Carvalho, Caldeira, & Duarte, 2009). In mice lacking these phosphorylation sites there are deficits in the induction of both LTP and LTD (Lee et al., 2003). Moreover, the induction of LTP increases phosphorylation of GluA1 at Ser831 (Mammen, Kameyama, Roche, & Huganir, 1997).

In the absence of stimuli capable of inducing synaptic plasticity, AMPA receptors undergo continual cycling, a process of regulating the density of these receptors at the plasma membrane. However, following the activation of NMDA receptors the cycling of AMPA receptors is altered in a manner which is dependent upon subunit composition. For instance, the GluA1 receptor subunit is critically involved in the insertion of AMPA receptors into the plasma membrane during LTP (Derkach et al., 2007; Hayashi et al., 2000).

Drug-induced Adaptations in Glutamatergic Synapses

Research investigating changes in the functioning of the glutamatergic system, following drug use, has revealed substantial neuroadaptations in the mesocorticolimbic pathway. For instance, a reduction in basal extracellular glutamate is observed in the NAc core of rats trained to self-administering cocaine, cocaine-yoked rats, and rats treated with cocaine to induce behavioral sensitization, relative to drug naïve rats (McFarland, Lapish, & Kalivas, 2003; Pierce et al., 1996). In contrast, a reduction in basal NAc core glutamate is not observed in rats trained to self-administer heroin or in heroin yoked rats (LaLumiere & Kalivas, 2008). The reason for the dissociation between heroin and cocaine in terms of NAc glutamate is unclear. However, systemic administration of N-acetylcysteine, a prodrug for the amino acid cysteine, which is involved in the regulation of extracellular glutamate, has been shown to restore the cocaine-induced reduction in

NAc glutamate and prevent both cocaine and heroin priming-induced reinstatement (Zhou et al., 2008).

Numerous changes in iGluR subunit expression have also been noted following the discontinuation of drug use. For example, Ghasemzadeh et al. (1999) used in situ hybridization and real time-PCR to investigate changes in iGluR subunit expression following a week of consecutive cocaine injections, 3 weeks of abstinence, and a single priming injection of cocaine. In cocaine-naïve rats, a single cocaine injection produced a reduction in GluA3, GluA4, and NR1 mRNA in the NAc, and a reduction in NR1 mRNA in the dorsal striatum and VTA. However, in the previously cocaine exposed rats, cocaine injection increased GluA3 and GluR2 mRNA in in the NAc and PFC, respectively (also see (Ghasemzadeh et al., 1999) for findings with regard to mGluRs). In contrast, Fitzgerald and colleagues (1996), used immunoblotting to show an increase in the expression the GluA1 and NR1 subunits in the VTA, but no changes were found in the levels of GluA1, GluA2/3 or NR1 protein in the NAc, 1 day after the termination of chronic cocaine administration.

Changes in iGluR expression have also been observed following opiate drug exposure. For instance, 6 days of chronic morphine, via a morphine pellet, has been shown to increase the expression of NR1 and NR2 NMDA receptor subunits in NAc (Bajo, Crawford, Roberto, Madamba, & Siggins, 2006). Similarly, 48h with a morphine pellet increases NR1 and NR2 receptor subunit expression in the NAc, and decreases NR1 expression in the mPFC and hippocampus (Murray, Harrison, Grimwood, Bristow, & Hutson, 2007). In contrast, 10 days of intermittent morphine injection, but not 5 days of continuous exposure to morphine, results in an elevation in GluA1 receptor subunit

expression in the VTA (Fitzgerald et al., 1996). Furthermore, morphine sensitization followed by a 7-day abstinence period has been shown to decrease GluA2 and increase in NR1 and NR2 receptor subunit mRNA in the amygdala (Sepehrizadeh, Bahrololoumi Shapourabadi, Ahmadi, Hashemi Bozchlou, Zarrindast, & Sahebgharani, 2008a; Sepehrizadeh, Sahebgharani, Ahmadi, Shapourabadi, Bozchlou, & Zarrindast, 2008b).

Importantly, most of the studies described above investigated drug-exposure and abstinence-induced changes in the expression of GluRs subunits in animals that have experienced passive administration, rather than self-administration, of the drugs. However, as detailed below, similar changes in the expression of GluRs subunits have been observed in abstinent animals following self-administration training.

The Role of Glutamate in the Reinstatement of Extinguished Drug Seeking

Studies investigating the reinstatement of drug seeking behavior consistently demonstrate a role for NAc glutamate transmission. For example, in rats trained to self-administer cocaine, a decrease in basal extracellular NAc glutamate is observed during drug abstinence. However, when exposed to cocaine, during abstinence, there is an augmentation of NAc glutamate release, relative to drug naïve animals, and a reinstatement of drug seeking (McFarland et al., 2003). Thus, drug-induced changes in glutamatergic transmission may, at least in part, mediate the propensity to relapse to drug seeking. As further evidence for the role of glutamate transmission in reinstatement, Cornish and Kalivas (2000) have demonstrated the reinstatement of cocaine seeking-behavior following systemic injection of cocaine or intra-NAc infusion of DA or AMPA. The more interesting aspect of this study is that intra-NAc infusion of the mixed DA receptor antagonist fluphenazine blocked DA-induced reinstatement; whereas, the AMPA

receptor antagonist CNQX, but not the NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid, blocked cocaine- and intra-NAc DA or AMPA-induced reinstatement.

With regard to the role of specific GluR subunits in the reinstatement of drug seeking, the GluA1 subunit has received a considerable amount of attention. Infusion of antisense oligonucleotides, directed against mRNA for the GluA1 subunit, into the NAc shell or core, causes an approximately 36% reduction in the expression of the GluA1 subunit and a pronounced reduction in cocaine priming- and AMPA-induced reinstatement (this effect is more pronounced in the NAc shell) (Ping, Xi, Prasad, Wang, & Kruzich, 2008). Similarly, Anderson et al. (2008) has demonstrated that the reinstatement of cocaine-seeking behavior is associated with an increase in GluA1 phosphorylation on Ser831 and enhanced cell-surface expression of GluA1-containing AMPA receptors in the NAc shell, but not NAc core. Furthermore, these authors found that cocaine priming-induced reinstatement could be attenuated by intra-NAc shell administration of the viral vector AAV10-GluR1- C99, which impairs the transport of GluA1-containing AMPA receptors to the cell surface.

Glutamate neurotransmission is also involved in the reinstatement of heroin seeking behavior. Microdialysis studies have shown an increase in extracellular NAc core glutamate release in rodents trained to self-administer heroin and subsequently tested for cue- and priming-induced reinstatement (LaLumiere & Kalivas, 2008). Furthermore, heroin priming and cue-induced reinstatement of heroin seeking is blocked by intra-NAc core infusion of the AMPA/kinate receptor antagonist CNQX; whereas, contextual cue-induced reinstatement of heroin seeking is attenuated by inhibition of glutamate

transmission in the NAc shell or VTA by infusion of the mGluR receptor agonist LY379268 (Bossert et al., 2006a; Bossert, Liu, Lu, & Shaham, 2004; LaLumiere & Kalivas, 2008). Furthermore, re-exposure to cues previously associated with heroin self-administration has been shown to produce a downregulation of the GluA2 receptor subunit in the mPFC. This downregulation is associated with a nearly 25% reduction in AMPA/NMDA current ratio, a measure of synaptic depression. Furthermore, blockade of clathrin-dependent GluA2 endocytosis, in the mPFC, prevents mPFC synaptic depression and reduces heroin priming and the heroin cue-induced reinstatement (Van den Oever et al., 2008).

Rationale for the Current Studies

To the best of my knowledge there are currently no studies investigating changes in iGluRs following heroin self-administration and subsequent acute FD-induced reinstatement. However, there are several reasons to expect that the expression of the AMPA receptor subunits GluA1 and GluA2, as well as the NMDA receptor subunit NR1, will be modulated following acute FD-induced reinstatement of heroin seeking. As discussed above, drug self-administration and subsequent reinstatement tests modulate glutamatergic neurotransmission and the expression of iGluRs. Furthermore, in drug naïve rats, 10 days of repeated restraint stress or 10 days of unpredictable stress increase VTA GluA1 and VTA GluA1 and NR1 receptor subunit expression, respectively (Fitzgerald et al., 1996). Therefore it was hypothesized that if acute FD-induced reinstatement of heroin seeking is mediated, at least in part, by adaptations in glutamatergic synapses, specific changes in iGluRs subunits in the heron-trained, food deprived rats compared to heroin-trained sated and drug-naïve rats should be expected.

METHODS

Subjects

Subjects consisted of 42 male Long Evans rats (Charles River, St Constant, QC, Canada) run in two experimental replications and weighing 300-421 (M = 358.59, SEM = 3.90) g at the start of experimentation. Prior to and post-surgery animals were housed under constant temperature (21°C) and on a reverse 12h/12h light/dark cycle (lights off at 9:30 am). Following IV catheterization rats were randomly assigned to one of two heroin self-administration groups or to one of two drug-naïve groups (see below).

Surgery

All rats were implanted with an IV silastic catheter (Dow Corning, Midland, MI) into the right jugular vein, as previously described in the general methodology.

Apparatus

Following recovery from surgery, rats assigned to the heroin-training groups were moved from the animal care facility to the operant chambers, described in detail in the general methodology, for the duration of experimentation. Drug-naïve rats remained in the animal care facility and were singly housed in standard shoebox cages throughout experimentation.

Drug

As described in previously, heroin (diacetylmorphine HCL) was dissolved in physiological saline and delivered at a concentration of 0.1 mg/kg per infusion.

Procedure

Heroin Self-administration Training, Extinction Training & Reinstatement

The behavioral portion of this study consists of three phases: heroin self-

administration training, extinction training, and reinstatement testing. These phases are described in detail in the general methodology; however, there are some procedural modifications. Following IV catheterization surgery rats were assigned to one of four training and reinstatement conditions: heroin self-administration and acute FD (Her-FD), heroin self-administration and sated (Her-Sated), drug-naïve and acute FD (Naïve-FD) and drug-naive and sated (Naïve-Sated). Drug-naïve animals were handled daily in a similar manner to those in the operant chambers except they were never exposed to heroin or the drug-training environment.

For animals in the heroin self-administration groups, training was conducted over a period of 10 days. Throughout the training phase, rats received three 3-h drug (days 1-5, FR-1; days 6-10, FR-3) sessions beginning shortly after at the onset of the dark phase (approximately 10 am). Following training the heroin syringes were removed and rats underwent a period of extinction training. Beginning on the second extinction day, session length was reduced to one-3 h session/day. Once an extinction criterion of 15 or less active lever responses (per 3-h session) was reached, rats were tested for reinstatement of drug seeking, under extinction conditions. Each rat was given one 2h reinstatement test, which was preceded by 21h of FD or 21h stated. Immediately following the end of the 2h reinstatement test, rats were removed from the operant chamber and decapitated. Brains were removed and flash frozen in isopentane for 5s before being transferred to a -80°C freezer for storage. Rats in the colony were food deprived or remained sated and were killed at the same time as rats which had been trained to self-administer heroin.

Western Immunoblotting

Brains were removed from a -80°C freezer and placed in a cryostat set to -13°C. After approximately 1h in the cryostat, brains were mounted, and two 500 µm slices, corresponding to each of the regions of interest were taken. Over dry ice, a blunted 18G needle was used to take bilateral tissue punches 0.83 mm in diameter from the BLA, NAc and VTA and two 0.83 mm bilateral tissue punches were taken from the dmPFC. All areas were identified using the atlas of Watson and Paxinos (see *Figure 1*). Tissue punches were prepared for analysis by adding 75 ul of lysis buffer (10 mM Tris-HCl, pH 7.5, 2% SDS, Complete Mini Protease Cocktail Inhibitor (Roche Diagnostics, Laval, QC, Canada) to each set of punches and sonicating each of the samples. Following centrifuging (10, 000 RPM) the supernatant was collected and protein quantification was preformed using the BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Based upon this quantification each supernatant was aliquoted into vials with 15 µg of protein and frozen at – 80°C. Prior to immunoblotting aliquots were removed from the freezer, placed on ice and prepared for immunoblotting by adding 6 ul of sample treatment buffer (Invitrogen Life Technologies, Burlington, ON, Canada), 2.4 ul of reducing agent (Invitrogen Life Technologies, Burlington, ON, Canada) and distilled water to ensure a consistent volume across aliquots. All samples were heated to 70°C for 10 min and loaded into individual lanes of 4-12% Bis Tris gels (Invitrogen Life Technologies, Burlington, ON, Canada). In addition to loading the samples, each gel also had 3-5 ul of

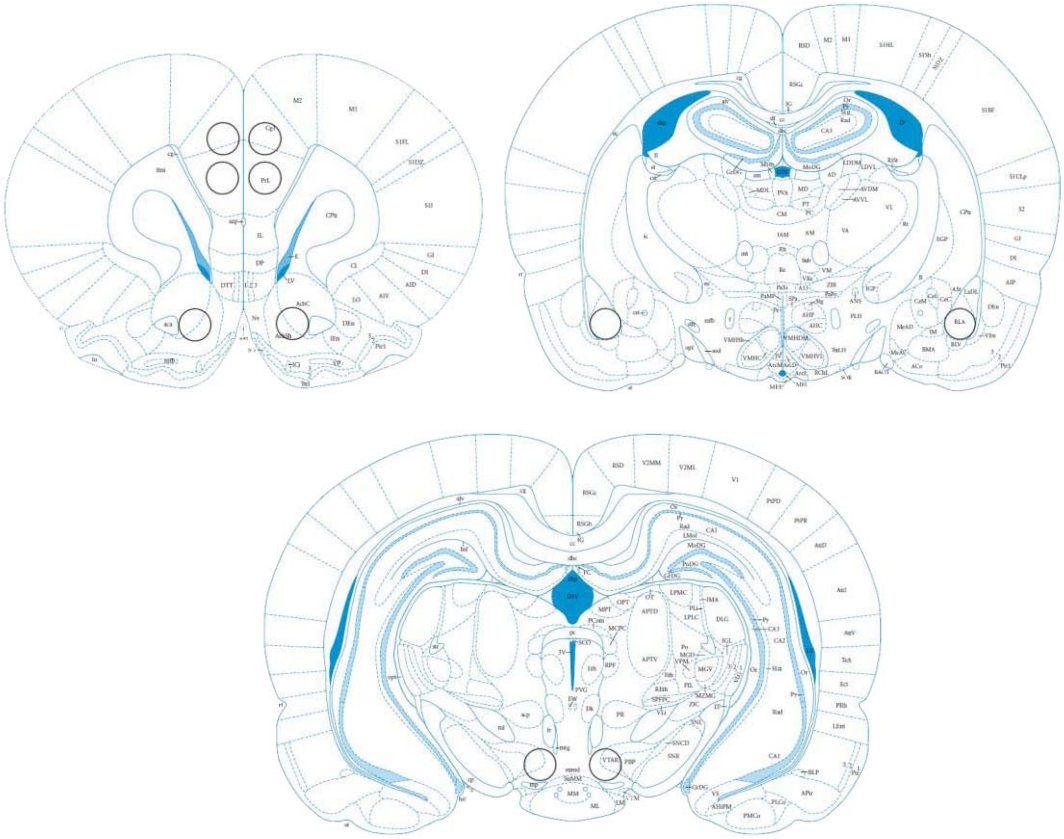

Figure 1.

Western Immunoblot Punches. Displayed above are the starting point and location of the dmPFC (top left), NAc (top left), BLA (top right) and VTA (bottom center) punches. For each region, with the exception of the dmPFC, two 500 µm thick, 0.83 mm in circumference bilateral punches were taken. For the dmPFC an additional set of punches were taken. Circles correspond to the appropriate region and size of the punch.

Precision Plus Protein Western C Standard (BioRad, Mississauga, ON, Canada) loaded. Electrophoresis was run using the electrophoresis insert of the XCell SureLock Mini-Cell System (Invitrogen Life Technologies, Burlington, ON, Canada) set to run at 200 V for 50 min with a running buffer consisting of 5% NuPage MOPS SDS (20X) in distilled water (Invitrogen Life Technologies, Burlington, ON, Canada). Additionally, 500 ul of NuPage antioxidant (Invitrogen Life Technologies, Burlington, ON, Canada) was added to the inner chamber of the electrophoresis cell prior to the start of running. Following gel electrophoresis, protein was transferred to a nitrocellulose membrane (0.45 um pore size; Invitrogen Life Technologies, Burlington, ON, Canada) using the Xcell II Blot Module component of the SureLock Mini-Cell System and a transfer buffer consisting of distilled water, 10% methanol, 5% NuPage transfer buffer (20X) and 0.1% antioxidant.

Membranes were blocked for 1h in milk (5% milk in tris-buffered saline + tween (.5%), TBS-T) and incubated overnight at 4°C in 5% milk TBS-T and the primary antibodies for GluA1 (1:1000), GluA2 (1:1000) or NR-1 (1:1000) and the loading control tubulin (1:1000 or 1:4000). All primary antibodies were purchased from Millipore, (Temecula, CA, USA). Following incubation in the primary antibody, membranes were washes several times in TBS-T and incubated with a secondary antibody to the primary antibodies and loading control (anti-beta actin; 1:5000, Abcam, Cambridge, MA, USA) and a secondary antibody to the standard (Strep Tactin-HRP conjugate; 1:5000, BioRad Mississauga, ON, Canada).

Following secondary antibody incubation protein was visualized by exposing Kodak Biomax or HyBlot CL film (Sigma Aldrich, Oakville, ON, Canada or Harvard Apparatus, Saint-Laurent, QC, Canada, respectively), to membranes treated with Western

Lightning Reagents (PerkinElmer Life Sciences, Inc., Boston, MA, USA).

Data Analysis

Behavioral Data

The effect of test condition (21h FD or sated) on heroin seeking behavior was assessed using SPSS Software v. 19.0.0 for Mac (IBM Inc., Armonk, New York, USA). A repeated measures ANOVA was performed using *Day* (Baseline (day prior to reinstatement test) or Test Day) as a within subjects factor and *Deprivation State* (FD or Sated) as a between subjects factor. Significant effects were followed up with Sidak corrected t-tests comparisons. All analyses were evaluated for significance at $\alpha = .05$.

Western Immunoblotting

Optical density was measured for the band migrating at the approximate molecular weight for each GluR subunit (GluA1: 106 kDa; GluA2: 108 kDa; NR1: 120 kDa) antibody and the tubulin (50 kDa) antibody using ImageJ software (National Institute of Health, Bethesda, Maryland, USA). In brief, a rectangular selection approximately double the height of the protein band in the first lane was drawn around each of the glutamate or tubulin bands within each gel. Protein expression was then analyzed, within each rectangle by looking at peak band intensity, determined as the area under the curve, relative to the film background. The measure resulting from this analysis was divided by the total protein expression across the bands of interest for each gel to give a measure of % GluR subunit expression or % tubulin expression per gel. Finally, % GluR subunit expression was divided by the % tubulin expression to correct for differences in protein loading across the samples. This final result was divided by the mean GluR subunit expression in the control (Naive-Sated) group (Miller, 2010). This

final step was preformed to allow for ease of visual comparison in figures. This method of quantification was chosen to account for differences in the running of gels, primary incubation time, and exposure time.

As mentioned previously, a great deal of research has shown a change in the expression of GluR subunit expression following chronic drug use. In order to test the hypothesis that heroin self-administration, in the current experiment, produced similar changes, a planned comparison assessing GluR subunit expression in the Her-Sated and Naive-Sated rats, was preformed for each of the regions investigated. Furthermore, stress has also been shown to alter the expression of GluR subunits. Consequently, a major focus in this study was to assess the effect of acute-FD stress on GluR subunit expression in rats trained to self-administer heroin as compared to drug-naïve rats. Thus, two planned comparisons were conducted to compare the effect of FD in heroin-trained rats (Her-FD compared to Her-Sated) and in drug naïve (Naïve-FD compared to Naïve-Sated). Additionally, since glutamate was expressed as a function of tubulin, to control for differences in loading, the above planned comparisons were repeated for % control tubulin expression. All comparisons were evaluated at $\alpha = .05$.

RESULTS

Of the 42 rats that received intravenous catheterization surgery, 20 rats were assigned to self-administer heroin and 22 rats were assigned to remain in the colony and remain heroin naive. Furthermore, 10 rats in the heroin group were assigned to the food deprived condition (Her-FD) and 10 rats were assigned to the sated condition (Her-Sated). Of the rats assigned to the Her-Sated condition one rat was removed due to a failure to train, defined as an average of less than 20 responses on the ‘active’ drug-paired

lever, throughout training. Thus, the total number of rats included in the Her-Sated group is 9. For the drug-naïve rats, 10 rats were assigned to the Naïve-Sated group and 12 rats were assigned to the Naïve-FD group.

Behavioral Data

As demonstrated in *Figure 2*, rats showed a clear preference for the 'active' drug-paired lever over the 'inactive' non-drug paired lever throughout training. On the last training day the mean (+/- *SEM*) number of infusions, active lever responses, and inactive lever responses were 38.32 (7.45), 294.37 (126.70) and 19.21(6.20), respectively. Following training, rats experienced a mean of 11.79 (*SEM* = 1.92) extinction days prior to the reinstatement test.

For the purposes of reinstatement testing, rats that were assigned to either the HER-FD group or the HER-Stated group were matched in terms of infusions (M_{FD} = 37.18, *SEM* = 8.54; M_{Sated} = 34.71, *SEM* = 4.77), $F(1,17) = 0.06$, $p = .81$, $\eta_p^2 = .01$, active lever responding (M_{FD} = 247.08, *SEM* = 94.44; M_{Sated} = 210.22, *SEM* = 40.31), $F(1,17) = 0.12$, $p = .73$, $\eta_p^2 = .01$, and inactive lever responding (M_{FD} = 21.56, *SEM* = 6.96; M_{Sated} = 17.87 *SEM* = 5.61), $F(1,17) = 0.17$, $p = .69$, $\eta_p^2 = .01$, during the last 5 days of training.

Repeated measures ANOVA performed on the number of active lever responses made during the baseline and test days revealed a significant effect of *day* with rats responding more on the reinstatement test day (*M* = 41.90; *SEM* = 10.73) than on the baseline day (*M* = 11.37; *SEM* = 0.82), $F(1,17) = 8.24$, $p = .01$, $\eta_p^2 = .33$. A significant

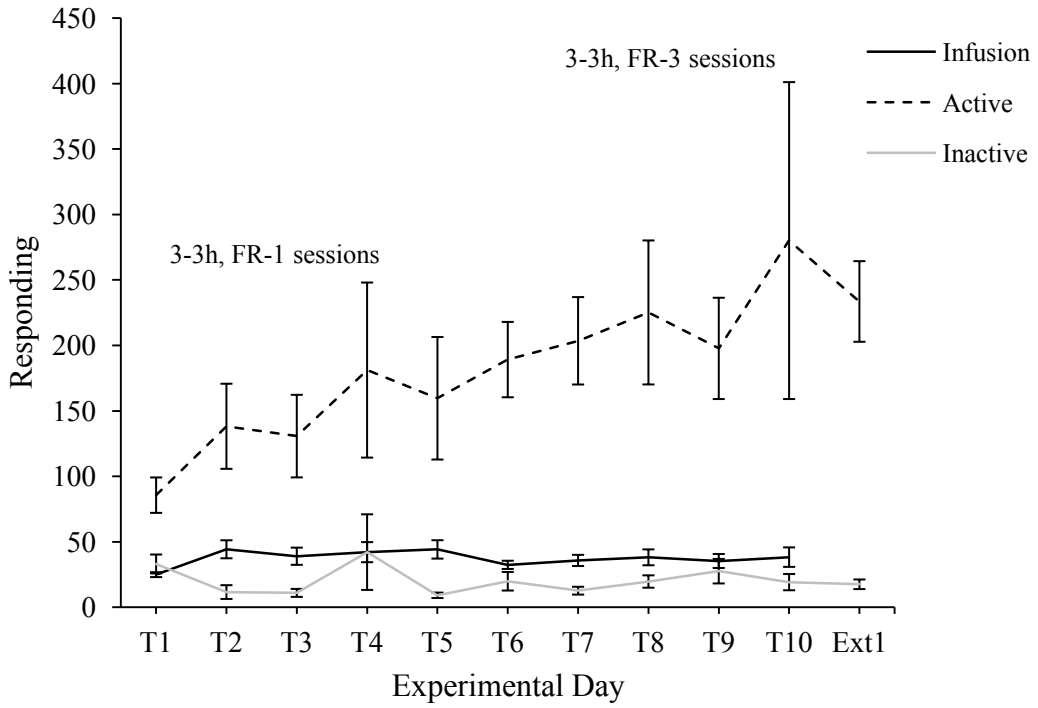

Figure 2.

Mean (+/- *SEM*), number of active lever presses, inactive lever presses, and heroin infusions. Rats were trained to self-administer heroin (0.1 mg/kg/infusion) over a 10-day training period (days 1-5: three 3h sessions, FR-1; days 6-10: three 3h sessions, FR-3). Also shown is the number of active and inactive lever responses for the first day of extinction. A significant difference existed between active and inactive lever responding on all training days and during extinction ($p < .05$).

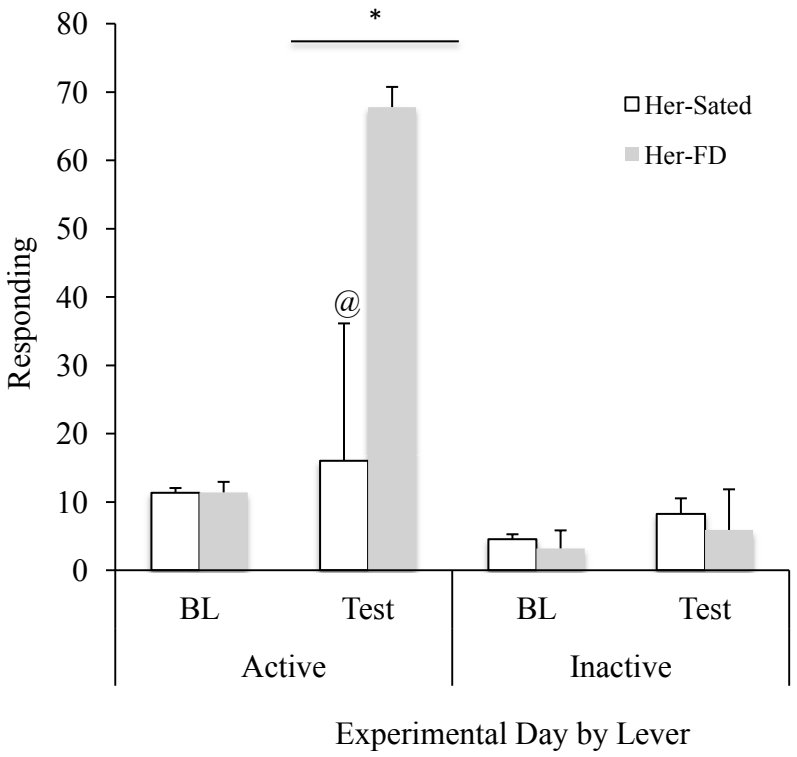

Figure 3.

The effect of acute food deprivation (FD) on the reinstatement of heroin seeking behavior. Data are mean (+*SEM*) number of active (left) and inactive (right) lever responses made during baseline (BL) and following 21h FD or unlimited access to food (Sated) for rats trained to self-administer heroin for 10 days and subsequently given extinction training. * $p < .05$ when compared to baseline; @ $p < .05$ when compared to FD condition.

effect of *deprivation state* was also observed, with food deprived rats (M = 39.60; SEM = 7.49) responding more than sated rats (M = 13.67; SEM = 7.90), $F(1,17) = 5.67$, $p = .03$, $\eta_p^2 = .25$. Additionally, a significant *day* X *deprivation state* interaction was observed, $F(1,17) = 5.92$, $p = .03$, $\eta_p^2 = .26$. Analysis of this interaction revealed that the FD rats had a significantly higher number of responses on the active lever during the test day compared to their responding on the baseline day, and compared to the sated rats responding on the test day, ps < .05 (see *Figure 3*). For the responses on the inactive lever, there was no significant effect of *day*, $F(1, 17) = 0.87$, $p = .36$, $\eta_p^2 = .05$, *deprivation state,* $F(1, 17) = 0.32$, $p = .58$, $\eta_p^2 = .001$ or *day X deprivation state* interaction, $F(1, 17) = 0.02$, $p = .89$, $\eta_p^2 = .001$ (see *Figure 2B*).

Western Immunoblotting

In the results reported below there are minor differences in subject numbers across regions and subunits of interest. These differences are due to a limited number of sample aliquots, finding a low level of protein during the BCA protein assay quantification, or problems during gel loading.

Dorsal Medial Prefrontal Cortex (dmPFC)

Thirty-nine rats were included in the analysis of GluA1 receptor-subunit expression (Her-Sated: n = 8; Her-FD: n = 10; Naïve-Sated: n = 9; FD: n = 12). One rat was removed from the analysis because the standard ladder was over-exposed and interfered with the GluA1 band. The second rat's data was not analyzed because of a limited number of protein aliquots. Using planned comparisons, no significant effect of drug history was revealed when comparing the Her-Sated group to the Naïve-Sated group, t (15) = -0.07, p = .95. Moreover, no significant differences were observed when

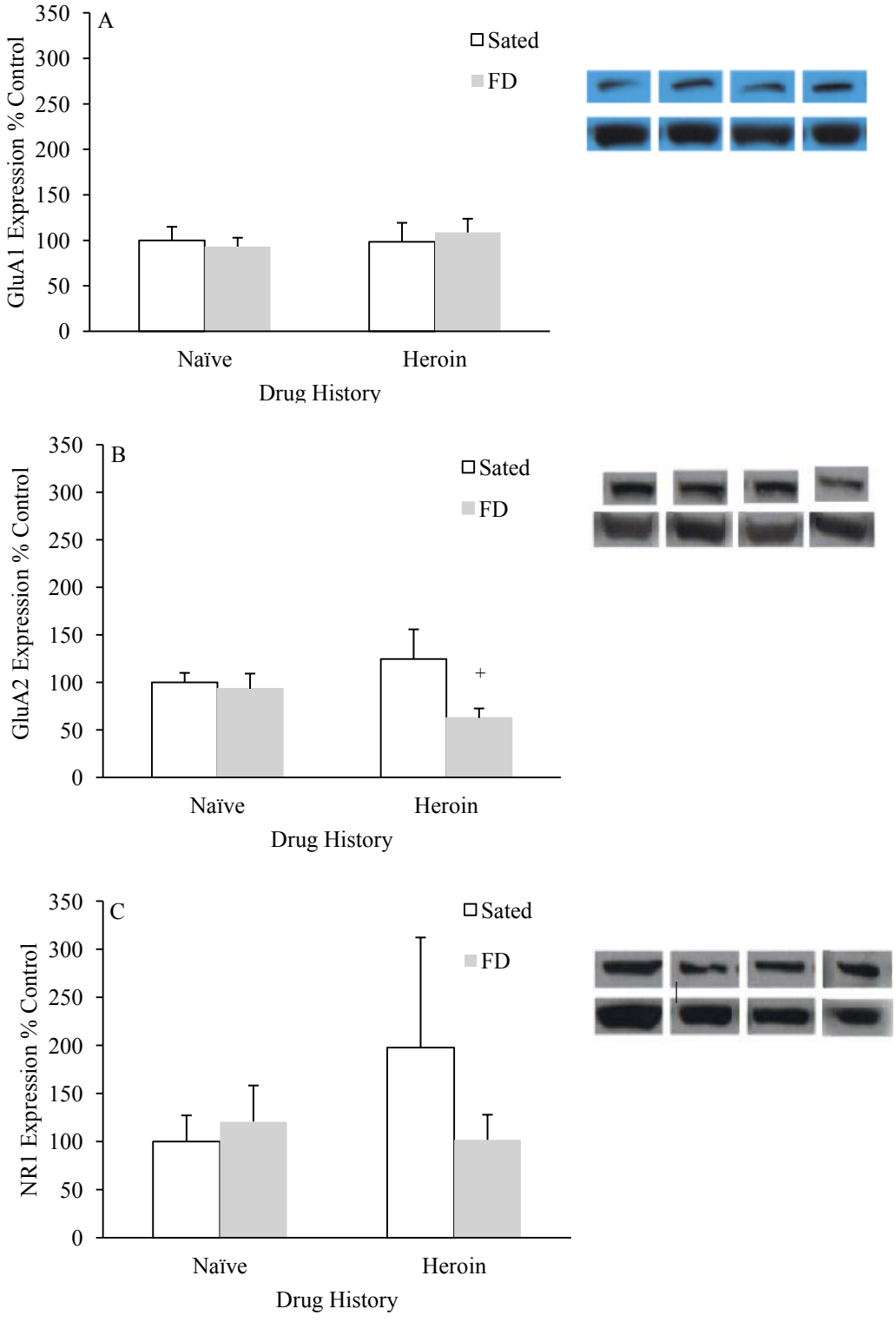

Figure 4. (caption on next page)

Figure 4.

Mean (+*SEM*) GluA1 (A), GluA2 (B), and NR1 (C) GluR subunit expression in the dmPFC for rats trained to self-administer heroin (0.1 mg/kg for a minimum of 10 days; 3-3h sessions per day) or drug naïve rats. Heroin-trained rats received extinction training and were tested for a reinstatement of drug seeking (2h test) under sated or 21h food deprivation (FD) conditions. Drug-naïve rats were sated or food deprived for 23h before their brains were collected. GluR subunit expression is presented as percent change from the drug-naïve-sated group. + $p = .08$ relative to heroin-trained sated rats. Blots to the right show a sample band for Naïve-Sated, Naïve-FD, Heroin-Sated and Heroin-FD, in that order.

assessing the effect of FD in the heroin-trained rats, t (16) = 0.41, p = .69, and in drug naïve rats, t (19) = 0.40, p = .69 (see *Figure 4A*).

Thirty-nine rats (Her-Sated: n = 9; Her-FD: n = 9; Naïve-Sated: n = 9; Naïve-FD: n = 12) were included in the analysis of GluA2 receptor subunit expression. Two rats were removed from the analysis due to an absence or great reduction in the tubulin band for these animals, indicating a problem in the loading of the lanes corresponding to these rats. Planned comparisons of GluA2 receptor-subunit expression revealed no significant effect of drug history for sated rats, t(16) = 0.75, p = .46. With regard to the effect of FD, planned comparisons of GluA2 receptor-subunit expression revealed a tendency toward a significant reduction in subunit expression in the Her-FD rats, as compared to the Her-Sated rats, t (16) = -1.89, p = .08. However, no such tendency was observed when comparing the FD and Naive-Sated rats, t (19) = -0.32, p = .75 (see *Figure 4B*).

NR1 receptor subunit expression was analyzed in 40 rats (Her-Sated: n = 9; Her-FD: n = 10; Naive-Sated: n = 9; FD: n = 12). One rat was eliminated from the analysis due to an insufficient number of protein aliquots. No significant differences in the expression of the NR1 subunit were observed when comparing the Her-Sated and Naive-Sated groups, t (16) = 0.83, p = .42. Also, planned comparison investigating the effect of FD in rats trained to self-administer heroin, t (17) = -0.86, p = .40, and naïve rats, t (19) = 0.42, p = .68, showed no significant difference in NR1 receptor-subunit expression (see *Figure 4C*).

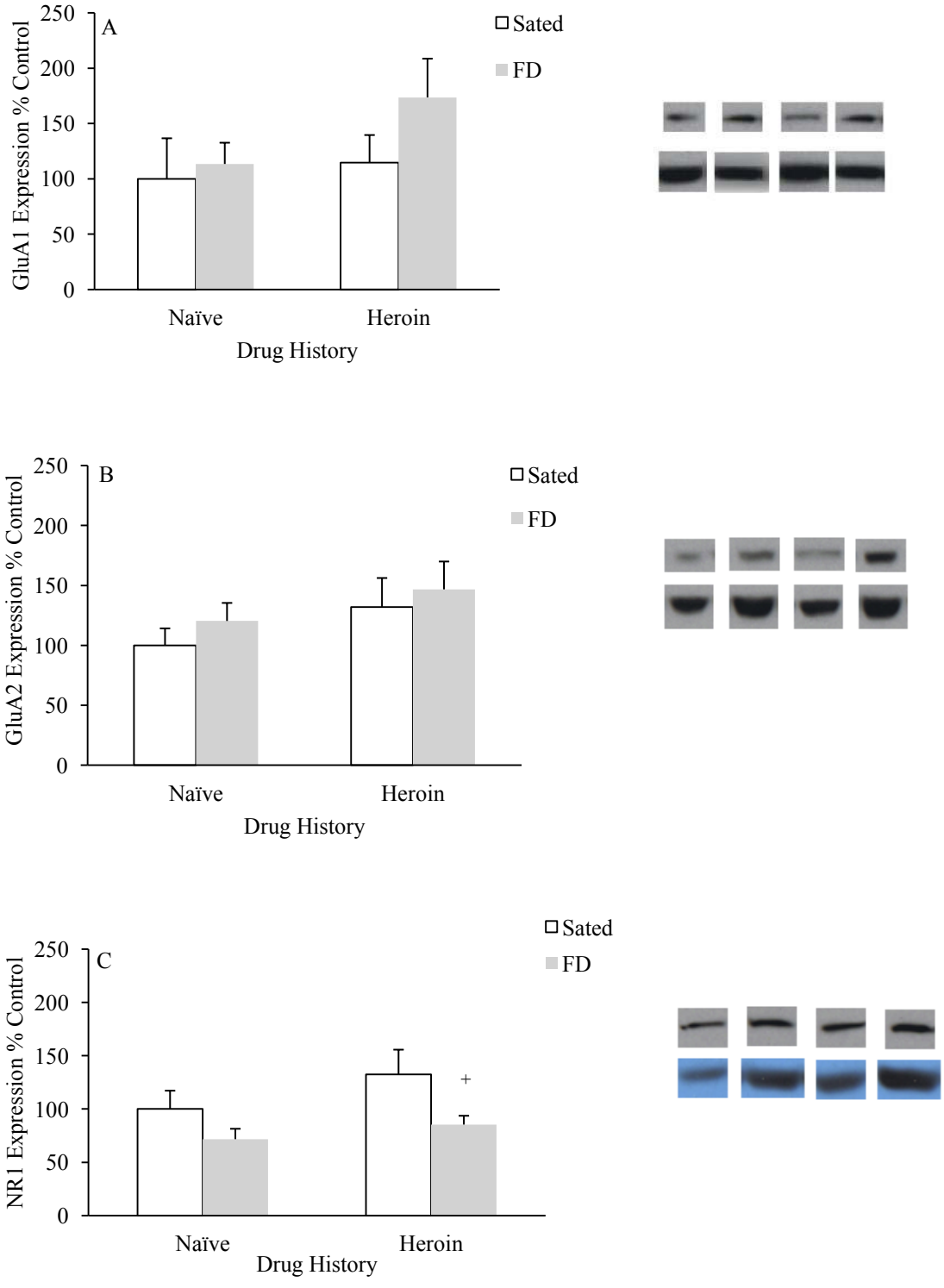

Figure 5. (Caption on the next page)

Figure 5.

Mean (+*SEM*) GluA1 (A), GluA2 (B), and NR1 (C) GluR subunit expression in the NAc for rats trained to self-administer heroin (0.1 mg/kg for 10 days) or drug naïve colony rats. Heroin-trained rats received extinction training and were tested for a reinstatement of drug seeking under sated or 21h food deprivation (FD) conditions. Drug-naïve rats were sated or food deprived for 23h before their brains were collected. GluR subunit expression is presented as percent change from the drug-naïve-sated group. + $p = .08$ relative to heroin-trained sated rats. Blots to the right show a sample band for Naïve-Sated, Naïve-FD, Heroin-Sated and Heroin-FD, in that order.

Nucleus Accumbens (NAc)

GluA1 receptor subunit expression was assessed in 34 rats (Her-Sated: $n = 8$; Her-FD: $n = 9$; Naive-Sated: $n = 8$; FD: $n = 9$). Rats were removed from the analyses due to an insufficient number of protein aliquots ($n = 4$), over-exposure of the standard in the adjacent lane making analysis impossible ($n = 2$), or identification as a statistical outlier ($n = 1$; z-score = 4.05). Planned comparisons assessing GluA1 receptor subunit expression in the NAc revealed no significant effect of heroin exposure in sated rats, t (14) = 0.36, $p = .73$. Furthermore, there was no significant effect of FD observed in heroin-trained, t (17) = -0.86, $p = .40$, or drug-naïve rats, t (19) = 0.42, $p = .68$ (see *Figure 5A*).

For the GluA2 receptor-subunit, 39 rats (Her-Sated: $n = 9$; Her-FD: $n = 9$; Naive-Sated: $n = 10$; FD: $n = 11$) were included in the following analyses. Two rats were not run due to an insufficient number of protein aliquots. Planned comparisons assessing GluA2 receptor-subunit expression in the NAc revealed no significant effect of heroin exposure, in sated rats, t (17) = 1.16, $p = .26$. Additionally, FD was shown to have no effect on GluA2 receptor-subunit expression in heroin-trained, t (16) = 0.44, $p = .67$, or drug-naïve rats, t (19) = 0.99, $p = .34$ (see *Figure 5B*).

NR1 receptor-subunit expression was analyzed for 37 rats (Her-Sated: $n = 9$; Her-FD: $n = 9$; Naive-Sated: $n = 8$; FD: $n = 11$). The samples from 4 rats were not run due to an insufficient number of protein aliquots. Planned comparisons showed no significant effect of drug history in sated rats, t (16) = 0.83, $p = .42$, and no significant difference between heroin-trained FD and heroin-trained sated rats, t (17) = -0.86, $p = .40$. However, a tendency toward a significant reduction in NR1 receptor-subunit expression was

observed in the Her-FD group relative to the Her-AL group, t (16) = -1.91, p = .08 (see *Figure 5C*). In contrast to all the other analyses described in this chapter, a significant difference in tubulin expression was observed when comparing the FD (M = 181.06, SEM = 16.57) and Naive-Sated (M = 100.00, SEM = 15.27) rats, t (17) = 3.46, p < .01, as well as when comparing Her-FD (M =188.44, SEM = 26.43) and Her-Sated (M = 97.26, SEM = 14.00) rats, t (16) = 3.05, p < .01. Given these differences in tubulin expression the above planned comparisons were repeated using the non-tubulin corrected glutamate measure. The results of these comparisons were exactly the same as those described above.

Ventral Tegmental Area (VTA)

Thirty-seven rats were included in the analysis of GluA1 receptor-subunit expression (Her-Sated: n = 7; Her-FD: n = 10; Naive-Sated: n = 9; FD: n = 11). Two rats were removed from the analysis because of a limited number of protein aliquots and two rats were not included due to a low BCA quantification. Planned comparisons assessing differences in GluA1 receptor-subunit expression revealed no significant effect of drug history, t (14) = -0.03, p = .98. Moreover, acute FD had no effect on GluA1 receptor-subunit expression in drug-naive, t (18) = -1.29, p = .21, or heroin-trained rats, t (15) = 0.52, p = .52 (see *Figure 6A*).

For the GluA2 receptor-subunit, the data from 35 rats were analyzed (Her-Sated: n = 6; Her-FD: n = 11; Naive-Sated: n = 8; FD: n = 10). Two rats were not considered in the analysis because of low a low BCA protein quantification, one rat was removed due to a loading error, one rat was removed due to the presence of an air bubble and two rats were not analyzed due to a limited number of samples. Planned comparisons of GluA2

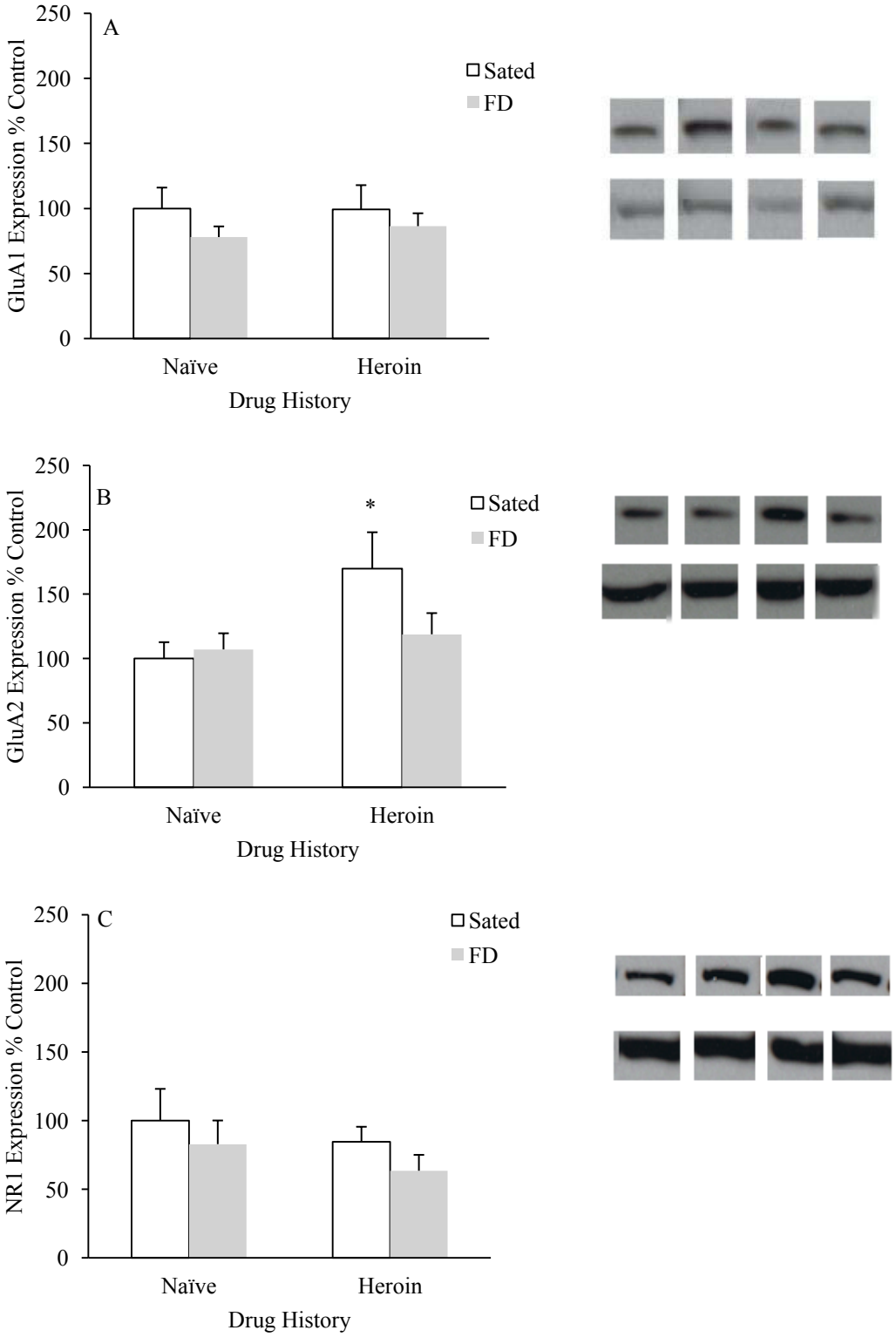

Figure 6. (Caption on next page)

Figure 6.

Mean (+*SEM*) GluA1 (A), GluA2 (B), and NR1 (C) GluR subunit expression in the VTA for rats trained to self-administer heroin (0.1 mg/kg for 10 days) or drug naïve colony rats. Heroin-trained rats received extinction training and were tested for a reinstatement of drug seeking under sated or 21h food deprivation (FD) conditions. Drug-naïve rats were sated or food deprived for 23h before their brains were collected. GluR subunit expression is presented as percent change from the drug-naïve-sated group. * $p < .05$ relative to drug-naïve sated rats. Blots to the right show a sample band for Naïve-Sated, Naïve-FD, Heroin-Sated and Heroin-FD, in that order.

receptor subunit expression revealed a significant increase in GluA2 receptor-subunit expression in the Her-Sated group relative to Naive-Sated group, suggesting an effect for drug exposure, $t(12) = 2.47$, $p = .03$. Acute FD was shown to have no effect on GluA2 receptor-subunit expression in heroin trained, $t(15) = -1.68$, $p = .11$, and heroin-naïve rats ($M_{Sated} = 100.00$, $SEM = 12.65$; $M_{FD} = 107.03$, $SEM = 12.55$), $t(16) = 0.39$, $p = .70$ (see *Figure 6B*). NR1 receptor-subunit expression was analyzed in 34 rats (Her-Sated: $n = 6$; Her-FD: $n = 10$; Naive-Sated: $n = 8$; FD: $n = 10$). One rat was eliminated due to an insufficient number of protein aliquots. Two rats were not considered in the analysis because of a low BCA protein quantification, one rat was identified as an outlier and removed from the data set (z-score = 5.32), and three rats were not run due to an insufficient number of samples. Planned comparisons of NR1 receptor subunit expression revealed no statistically significant effect of previous drug history, $t(12) = -0.54$, $p = .60$. Additionally, acute FD had no effect on NR1 receptor-subunit expression in heroin-trained rats, $t(14) = 0.31$, $p = .76$, or in drug-naïve rats, $t(16) = -0.61$, $p = .55$ (see *Figure 6C*).

Basolateral Amygdala (BLA)

GluA1 receptor subunit expression in the BLA was not analyzed due to technical difficulties resulting in unintelligible data (multiple bands at very similar molecular weight positions).

GluA2 receptor subunit expression was assessed in 39 rats (Her-Sated: $n = 9$; Her-FD: $n = 10$; Naive-Sated: $n = 9$; FD: $n = 11$). One rat was eliminated from this analysis due to a lack of protein following BCA quantification and one rat was eliminated due to a loading error. Planned comparisons revealed that acute FD significantly increased GluA2

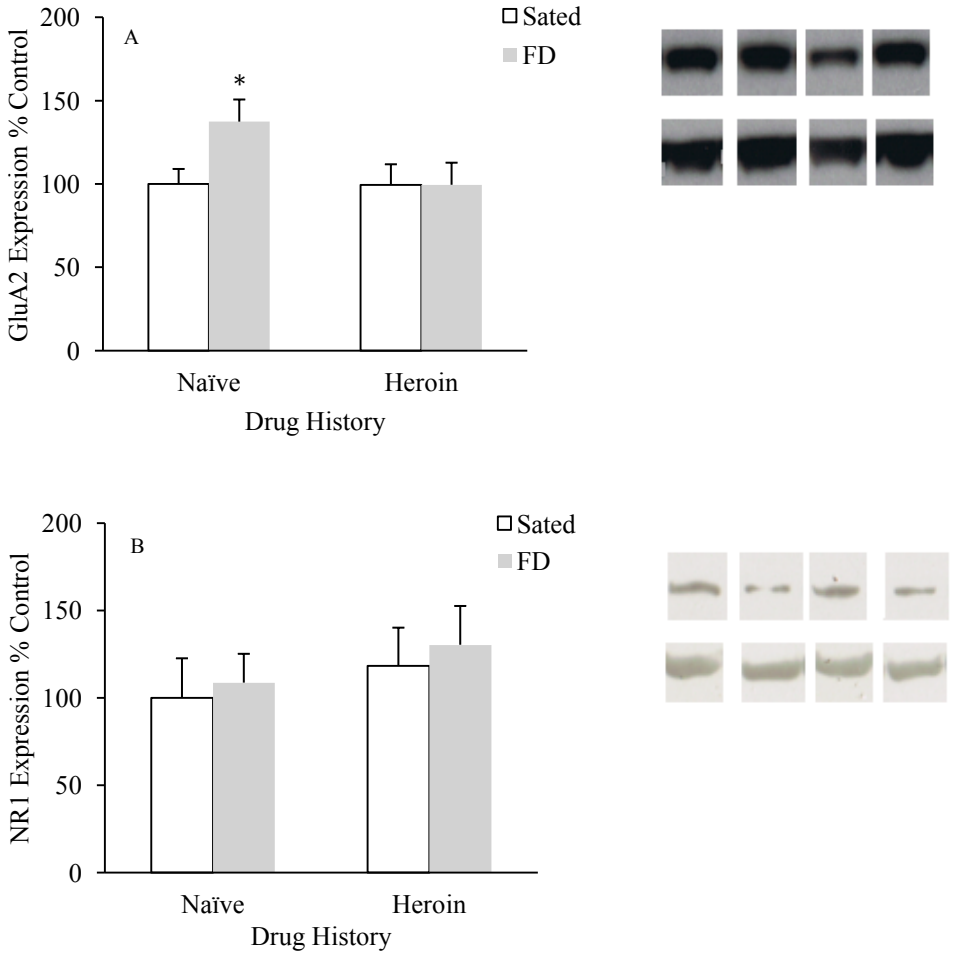

Figure 7.

Mean (+*SEM*) GluA2 (A) and NR1 (B) GluR subunit expression in the VTA for rats trained to self-administer heroin (0.1 mg/kg for 10 days) or drug naïve colony rats. Heroin-trained rats received extinction training and were tested for a reinstatement of drug seeking under sated or 21h food deprivation (FD) conditions. Drug-naïve rats were sated or food deprived for 23h before their brains were collected. GluR subunit expression is presented as percent change from the drug-naïve-sated group. * $p < .05$ relative to drug-naïve sated rats. Blots to the right show a sample band for Naïve-Sated, Naïve-FD, Heroin-Sated and Heroin-FD, in that order.

receptor subunit expression in drug naïve, t (18) = 2.23, p = .04, but not in heroin-trained rats, t (17) = 0.06, p = .95. In addition, GluA2 receptor subunit expression did not differ between rats in the Her-Sated and Naive-Sated groups, t(16) = -0.03, p = .97 (see *Figure 7A*).

The NR1 receptor subunit expression was analyzed in 33 rats (Her-Sated: n = 8; Her-FD: n = 7; Naïve -Sated: n = 10; Naïve-FD: n = 8). One rat was removed from the analysis due to an air bubble and seven rats were not run due to an insufficient number of protein aliquots. Planned comparisons revealed no significant differences for FD-induced changes in NR1 receptor-subunit expression in drug naïve, t (16) = 0.30, p = .77, and heroin-trained rats ($M_{\text{Her-Sated}}$ = 118.24, SEM = 21.79; $M_{\text{Her-FD}}$ = 130.32, SEM = 22.19), t (13) = 0.34, p = .71. Additionally, there was no significant difference in NR1 receptor subunit expression between the Her-Sated and Naive-Sated rats, $p > .05$ (see *Figure 7B*).

DISCUSSION

As previously discussed, drug addiction can be conceptualized as a form of aberrant learning resulting from maladaptive synaptic plasticity. As such, it was expected that 10 days of heroin self-administration, followed by extinction of drug seeking, and a subsequent test of acute 21h FD-induced reinstatement would alter iGluR subunit expression, an indicator of changes in the strength of glutamate synapses. Here I report a tendency toward a reduction AMPA GluA2 receptor-subunit expression in the dmPFC in heroin-trained FD rats relative to heroin-trained sated rats, and a tendency toward a reduction in the NMDA NR1 receptor-subunit expression in the NAc in heroin-trained FD rats, relative to heroin-trained sated rats. Furthermore, a significant increase in GluA2 receptor expression was observed in the VTA of heroin-trained sated rats as compared to heroin naïve sated rats. Finally, heroin-naïve FD rats were found to have an increased GluA2 receptor-subunit expression in the BLA, as compared to drug naïve sated rats.

In interpreting the significance of these findings there is obvious need for caution with regard to the discussion of statistical tendencies. However, with this in mind, the observed tendencies will be interpreted within context of the prevailing literature addressing iGluR subunit expression and drug exposure.

Acute FD-Induced Reinstatement and Expression of the NMDA NR1subunit in the NAc

A tendency toward a reduction in the NMDA NR1 receptor subunit was only observed in the NAc of heroin-trained food deprived rats, as compared to heroin-trained sated rats. This result is in contrast with previous findings demonstrating that 18 months of cocaine self-administration in rhesus monkeys and cocaine overdose in human cocaine addicts is associated with an increase in NAc NR1 receptor expression (Hemby et al.,

2005). Furthermore, NAc NR1 expression is also upregulated in rats made dependent on morphine by chronic morphine exposure (Bajo et al., 2006).

One major difference exists between the current study and the studies cited above. This experiment utilized the reinstatement procedure, in which rats were trained to self-administer heroin for a period of 10 day followed by a prolonged abstinence and extinction training period. In contrast, in Bajo et al.'s study (2006) rats were killed immediately after morphine treatment. In Hemby et al.'s report (2005) it is stated that monkeys were killed after drug self-administration studies ended, but it is not clear what this timeframe means. However, since this experiment was intended to compare iGluR subunit-expression in human overdose victims and primates it can be assumed that heroin was onboard at the time of death.

Similar to the current study, a reduction in NR1 subunit mRNA is observed in the NAc of rats given experimenter administered injections of cocaine for 14 days and exposed to 24h of withdrawal (Yamaguchi et al., 2002). However, here reduction in the expression of NR1 receptor subunit is only observed in heroin-trained food deprived rats. Thus it may be the case that extinction training reversed the effects of heroin self-administration on the NR1 receptor subunit in the NAc (see Self, 2004 for a discussion of extinction-induced reversal of drug-induced neuroplasticity). However, following acute-FD and subsequent reinstatement of heroin seeking, these changes were once again apparent. In support of this conclusion NAc NR1 receptor subunit expression is reduced in the NAc shell following 1 week of forced abstinence from cocaine self-administration an effect which is associated with a withdrawal induced LTD in the NAc shell. However, extinction training has been shown to normalize both NR1 receptor subunit expression

and reverse LTD in the NAc shell (no effect of extinction on NR1 regulation was observed in the core), a process which is involved in restoring inhibitory control over behavior (Self, 2004).

Acute FD-Induced Reinstatement and Expression of the AMPA GluA2 Receptor Subunit

A tendency toward an increase in GluA2 receptor subunit expression was observed in the dmPFC of heroin-trained FD rats relative to heroin-trained sated rats. Furthermore, a statistically significant increase in the GluA2 receptor subunit was observed in the VTA of heroin-trained stated rats, relative to heroin naïve sated rats, and in the BLA of drug naïve FD rats relative to drug naïve sated rats.

The Dorsal Medial Prefrontal Cortex (dmPFC)

While caution must also be exercised in interpreting the tendency toward a reduction in GluA2 receptor expression in the dmPFC, the current findings are in agreement with those of Van den Oever et al. (2008) which demonstrate a downregulation of the GluA2 receptor subunit in mPFC synaptic membranes following cue-induced reinstatement of heroin seeking. Also, these authors assessed synaptic plasticity within the mPFC, 30 min after exposure to drug cues, and found a significantly lower AMPA/NMDA current ratio suggesting a decrease in the strength of glutamate synapses onto neurons in the vmPFC.

In contrast, in the current study tissue punches were taken from the dmPFC, corresponding to the infralimbic and cingulate cortex. Thus, the current experimental design does not allow any conclusions regarding the contribution of glutamatergic transmission in the vmPFC to acute FD-induced reinstatement of heroin seeking. However, using the same rational as Van den Oever et al. (2008), my findings suggest

that a reduction in GluA2 receptor-subunit expression in the dmPFC, leading to inhibited synaptic transmission in the dmPFC, underlies acute FD-induced reinstatement of extinguished heroin seeking.

However, this interpretation does contradict with the suggested role of the dmPFC, as described by Kalivas and colleagues (Peters et al., 2008; 2009), whereby activation of this region drives, and inhibition attenuates, the reinstatement of drug seeking. Interestingly, as mentioned above, the over-expression of GluA2-lacking AMPA receptors (as would be expected by the reduced expression of GluA2, but not GluA1, subunits) could be interpreted as an increase in the excitability of the relevant synapses (Conrad et al. 2008; Liu et al. 2007). Consequently, the current findings suggest that the exposure to FD in heroin-trained rats resulted in an excitation of the dmPFC-NAc pathway, resulting in the reinstatement of drug seeking (Peters et al., 2008; 2009),

The Ventral Tegmental Area (VTA)

Within the VTA, a significant increase in GluA2 receptor-subunit expression was observed in in heroin-trained sated rats as compared to heroin naïve sated rats suggesting a drug-induced change in synaptic plasticity. Similarly, in rats trained to self-administer cocaine and exposed to forced abstinence, an increase in GluA2 receptor-subunit was observed in the VTA following 24h of withdrawal. However, GluA2 receptor-subunit expression was not elevated after 30 days of withdrawal, suggesting a transient elevation of VTA GluA2 receptor subunits in cocaine-trained rats. In the current study, rats were killed after approximately 12 days of extinction, thus it appears that the increase in GluA2 receptor subunit, in the VTA of heroin-trained rats, is relatively long-lasting. However, in contrast to the current findings, 10 days of intermittent morphine

injections resulted in an elevation of GluA1, but not GluA2, in the VTA. As discussed above, these discrepant results may be the result of differences in self- versus experimenter-administered drug. Alternatively, it may be the case that the increase in GluA2 receptor expression observed in the current study is the results of learning that drug predictive cues no longer signal drug availability. Thus, increased GluA2 receptor expression may signal that a long-term change in synaptic plasticity has already occurred at glutamate synapses in the VTA. For instance, following the induction of LTP, a transient increase in synaptic GluA2-lacking AMPA receptor subunits is observed. However, these subunits are replaced by GluA2 receptor subunits following a short period of time (Plant et al., 2006). Thus, increased GluA2 subunits may indicate a strengthening of synapses in the VTA. However, the nature of this potential synaptic change is unclear (i.e., drug- or extinction-induced). The data also suggest that the observed change in the expression of GluA2 subunit in the VTA is not related to FD-induced reinstatement, since the levels of GluA2 were not changed in FD rats.

The Basolateral Amygdala (BLA)

Within the BLA, acute FD, was shown to increase GluA2 receptor subunit expression in the food deprived drug-naïve rats relative to sated drug-naïve animals. This finding is somewhat unexpected. Given that morphine-induced behavioral sensitization is associated with a reduction in BLA GluA2 expression (Sepehrizadeh et al., 2008), a drug-dependent change in GluA2 receptor-subunit expression was expected. However, in rats trained to self-administer cocaine and subsequently exposed to withdrawal, an increase in GluA1, but not GluA2, expression in the BLA was observed (Lu, Dempsey, Shaham, & Hope, 2005). Thus, neuroadaptions in glutamate synapses

may differ in animals trained to self-administer drug and animals passively exposed to drug.

Despite the extensive literature regarding the involvement of the BLA in stress responsivity, there appear to be no studies addressing the effect of acute stress on GluA2 receptor subunit expression in the BLA. However, in knockout mice with a targeted deletion of the gene encoding the GluA2 receptor, an impairment in the performance of a conditional emotional response and a reduced anxiety in an elevated plus maze is observed (Mead et al., 2006). Thus, while GluA2 receptors are typically associated with an inhibition of synaptic plasticity, these receptors appear to be necessary for learning. In particular, as discussed above, learning depends upon the maintenance of LTP which relies upon GluA2-dependent trafficking of AMPA receptors to the synapse (Yao et al., 2008). Moreover, corticosterone, which is released as a result of the HPA axis response to stress, has been shown to increase the trafficking of GluA2 receptors to the synapse in the hippocampus, a process which may also occur in the BLA (Groc, Choquet, & Chaouloff, 2008).

Methodological Considerations

Finally, some methodological considerations should be presented. First, beta-tubulin was used to control for differences in the loading of samples, as is the standard practice (see Edwards, Graham, Whisler, & Self, 2009; Fourgeaud, 2004; Levine et al., 2005). However, beta-tubulin is a scaffolding protein involved in the development of new synapses. Recently, it has been shown that tubulin is downregulated following cocaine self-administration and extinction (Reissner et al., 2011). In contrast, binge cocaine administration and chronic morphine exposure have been shown to have no effect on

tubulin expression in the rat (Beitner-Johnson, Guitart, & Nestler, 1992; Perrine, Miller, & Unterwald, 2008), and long-term heroin use has been shown to have no effect on tubulin in humans (Sastre, Ventayol, & García-Sevilla, 1996 but see Reynolds, Mahajan, Sykes, & Nair, 2006). Thus, it seems unlikely that differences in the expression of tubulin are masking differences in GluR subunit expression in the current study. However, it must be noted that a difference in tubulin express was observed in the Western immunoblots assessing the NR1 receptor-subunit in the NAc. This finding was surprising since it was not replicated in blots assessing the expression of GluA1 and GluA2 subunits in the same samples. I therefore believe that the observed difference in tubulin in these particular analyses were not due to a real effect of heroin exposure, but rather a result of a coincidental sample-loading-related variance.

Second, the current analysis did not distinguish between the GluR subunit protein expressed on the cellular membrane and intracellular GluR expression. The actions of glutamate are determined by GluRs expressed at the synapse; thus, it is surface rather than intracellular iGluR subunits, which regulate the actions of glutamate and it is the trafficking of these receptors into and out of the synapse that is critical for synaptic plasticity (Boudreau & Wolf, 2005). Over the past few years, several methods have been developed that allow dissociation between membrane bound and cytoplasmic receptor pools. Future follow-up studies in our laboratory will utilize the BS^3 protein cross-linking assay (Boudreau & Wolf, 2005) to address this issue, as well as assess AMPA receptor phosphorylation-state, as an indication of receptor functionality.

Conclusion

The findings of the current studies suggest a role for adaptations in glutamate

synapses in drug addiction. Here, I observe changes in the AMPA and NMDA receptors subunits, iGluRs which have been extensively investigated for their role in synaptic plasticity. While a significant increase in the expression of the GluA2 subunit in the BLA was observed in FD rats, my most interesting results relate to changes in GluA2 and NR1 receptor expression in dmPFC, VTA, and NAc. These changes are particularly interesting within the context of long-term changes in synaptic plasticity, such as LTP, that may underlie the long-lasting risk for relapse in drug addicts.

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GENERAL DISCUSSION

The current series of experiments expand upon previous work demonstrating a reinstatement of heroin and cocaine seeking following acute 21-48h FD (Shalev et al., 2000; 2001; Shalev, Marinelli, Baumann, Piazza, & Shaham, 2003; Tobin et al., 2009). The precise nature of this form of reinstatement has not been fully elucidated. However, acute FD-induced reinstatement has been conceptualized as a type of stress-induced reinstatement. Yet, acute FD differs substantially from footshock-induced reinstatement, a more common model of stress-induced relapse. In particular, acute FD-, but not footshock-induced reinstatement, is attenuated by systemic administration of the DA D1 receptor antagonist SCH 23390 (Tobin et al., 2009), and central administration of the anorexigenic hormone, leptin (Shalev et al., 2001). Thus, in the experiments described in chapter 1, I administered SCH 23390 locally to elucidate the neuronal circuitry mediating the effect of DA, acting on the D1 receptor, in acute FD-induced reinstatement. The results of these experiments show that acute FD-induced reinstatement relies upon the activation of DA D1 receptors in the NAc shell, dmPFC, and BLA.

Moreover, as previously discussed, drug addiction can be conceptualized as a perversion of normal learning resulting from maladaptive neuroplasticity (Kalivas, 2004). Consequently, recent research has highlighted a role for glutamate as well as an interaction between the DAeric and glutamatergic systems in the reinstatement of drug seeking behavior. Thus, in chapters 2 and 3 I investigated the role of adaptations in glutamatergic pathways and synapses in acute FD-induced reinstatement. These studies employed two distinct approaches. In chapter 2, synaptic plasticity was investigated in the glutamatergic projection from the vSub of the hippocampus to the NAc shell. This

pathway provides a direct link between the neuronal circuitry involved in memory and learning, and the neuronal circuitry involved in drug reward (Dong et al., 2007; Sesack & Grace, 2010; Setlow, 1997). In this chapter, an impairment in the induction of LTP, a cellular correlate of learning and memory (Bliss & Collingridge, 1993), within the vSub-NAc shell pathway, was observed in rats exposed to 48h of FD regardless of previous drug history. However, amongst drug-trained rats, a significant inverse correlation was observed between drug seeking on the reinstatement test and the magnitude of LTP. Thus, acute FD-induced reinstatement of heroin seeking may depend upon changes in synaptic plasticity within the vSub-NAc shell pathway. As a complement to these findings, in chapter 3 changes in the expression of iGluR subunits, known to be associated with synaptic plasticity, were investigated (Malinow & Malenka, 2002). Here I showed a significant effect of drug history in sated, but not in FD rats, with heroin-trained sated rats demonstrating greater GluA2 receptor subunit expression in the VTA compared to drug-naive sated rats. Additionally, acute FD, in the absence of drug self-administration, increased BLA GluA2 receptor subunit expression. Furthermore, amongst heroin-trained food deprived rats a tendency toward a reduction in the expression of GluA2 and NR1 receptor subunits was observed in the dmPFC and the NAc, respectively.

Collectively these studies suggest that an understanding of the role played by the DAergic and glutamatergic systems, as well as an understanding of the interactions between these systems is necessary to fully elucidate the neuronal mechanisms through which acute FD induces a reinstatement of heroin seeking. In particular, glutamate and

DA have numerous regulatory interactions and DA, predominantly acting at the D1 receptor, has been implicated in the regulation of synaptic plasticity.

Acute Food Deprivation-Induced Reinstatement is a Unique Form of Stress-Induced Reinstatement

Understanding the neuronal mechanisms mediating stress-induced relapse is of the utmost importance as stress is a frequently cited reason for relapse amongst human drug abusers (see Bradley, Phillips, Green, & Gossop, 1989; Litman et al., 1977; McKay et al., 1996; Wallace, 1989). In the rodent literature there are two main models of stress-induced relapse: footshock-induced reinstatement (Shaham & Stewart, 1995) and acute FD-induced reinstatement (Shalev et al., 2000; Tobin et al., 2009). A third reinstatement trigger, yohimbine, a norepinephrine α_2 receptor antagonist, has also been conceptualized as a model of stress-induced relapse. Yohimbine has been shown to reinstate cocaine (Kupferschmidt, Tribe, & Erb, 2009; Lee, Tiefenbacher, Platt, & Spealman, 2004), methamphetamine (Shepard et al., 2004), and alcohol (Dzung Lê, Funk, Harding, Juzytsch, & Fletcher, 2009) seeking in rodents and induce drug craving in opiate dependent individuals (Stine et al., 2002). However, yohimbine has been shown to produce anxiety and panic attacks in human drug abusers (McDougle et al., 1994; Stine et al., 2002). Thus, yohimbine-induced reinstatement may differ in a physiological and qualitative sense from 'true' models of stress-induced relapse.

Furthermore, as alluded to previously, acute FD-induced reinstatement, as a model of stress-induced relapse, differs from other forms of reinstatement. Firstly, acute FD-induced reinstatement involves a single 21-48h deprivation. This timeframe contrasts drastically with footshock stress, which typically involves a 10-15 min intermittent stress

exposure (for examples see Buffalari & See, 2009; McFarland et al., 2004; Shaham & Stewart, 1995). Secondly, footshock stress occurs within the previously drug-paired context but terminates prior to the start of the reinstatement test. In contrast, rats in the current study remained food deprived throughout the reinstatement test. Finally, acute FD, unlike other forms of stress-induced reinstatement, represents a homeostatic metabolic challenge. As such, a period of acute 48h FD has been shown to dramatically reduce body weight and alter the release of neuropeptides involved in the regulation of feeding. For instance, rats euthanized after 48h of FD have lower levels of circulating leptin, glucose and insulin. Furthermore, acute 48h FD causes a significant increase in arcuate nucleus neuropeptide Y and agouti-related peptide mRNA, two peptides which stimulate appetite, and a decrease in Pro-opiomelanocortin mRNA, a peptide which inhibits appetite (Bi, Robinson, & Moran, 2003).

Also relevant to the current studies is the finding that plasma leptin level and cerebral spinal fluid DA concentration are both reduced, in a significantly correlated manner, in human participants asked to fast for 12-20h, suggesting that acute FD may cause a reduction in the synthesis of DA (Hagan et al., 1999). These findings might indicate that an acute FD-induced increase in DA transmission is not involved in acute FD-induce reinstatement. However, DA transmission is dependent upon other factors in addition to DA synthesis. For example, acute 24-36h FD has been shown to decrease DA transporter (DAT) mRNA in the VTA/SN of rats. However, no change was found in the concentration of DAT protein (B_{max}) in the major target areas of the mid-brain DA neurons, the NAc and dorsal striatum (Patterson et al., 1998). Surprisingly, DAT activity was reduced in the dorsal striatum of FD rats, suggesting that the effect of FD on the

function of DAT is mediated by posttranslational mechanisms, possibly through changes in the phosphorylation state of the DAT protein (Patterson et al., 1998). Reduced DAT activity in DA terminal areas could result in an overall increase in DA function due to an increase in the length of time DA remains in the extracellular space. It is intriguing, however, that no change in DAT activity was found in the NAc of the FD rats (Patterson et al., 1998). This lack of effect might be explained by limitations in the method used, which did not allow for a differentiation between the shell and core compartments of the NAc, areas known to be differentially innervated by mid-brain DA neurons (Ikemoto, 2007).

While it is unclear to what degree acute FD and chronic food restriction are similar, it is worth noting that chronic food restriction augments morphine- and cocaine-induced DA release in the NAc (Piazza & Le Moal, 1998; 1996), increases amphetamine-induced NAc c-fos expression (Carr & Kutchukhidze, 2000), and increases self-administration of electrical BSR (Fulton et al., 2000). Furthermore, central administration of leptin causes a reversal of chronic food restriction-induced sensitization of lateral hypothalamic BSR, possibly by interaction with DA neurons (Hommel et al., 2006). Similarly, leptin has been shown to block acute FD-induced reinstatement while having no effect on footshock- or heroin priming-induced reinstatement of heroin seeking (Shalev et al., 2001).

The Relationship Between the DA D1 Receptor and Feeding

Given that the current studies employed acute FD and the above findings, demonstrating that manipulations of the DAergic system can modulate the effects of acute FD stress, it is interesting to consider the possibility that SCH 23390 attenuated

drug seeking by acting directly on the neuronal circuitry mediating feeding. For example, systemic administration of SCH 23390 has been shown to produce a dose-dependent reduction in feeding (Terry & Katz, 1992). Furthermore, treatment with SCH 23390 has been shown to in inhibit Tetrhydrocannabinol-induced feeding and decrease FR responding for food, at a drug dose that did not attenuate feeding when given alone (Verty, McGregor, & Mallet, 2004). Similarly, DA depletion via icv 6-OHDA infusion, produces a marked decrease in 24h food consumption (Breese, Smith, Cooper, & Grant, 1973) and the DA D1 receptor agonist, SKF 38393, has been shown to increase highly palatable chocolate food consumption, in chronically food-restricted rats (Cooper, Al-Naser & Clifton, 2006b). Collectively, these findings suggest that activation of the DA D1 receptor facilitates feeding while blocking the D1 receptor inhibits feeding. Given that FD is thought to increase the immediate valence of food, and presumably drug cues due to a shared neuronal substrate (Kenny, 2011), my findings seem to support this conclusion.

However, the role of DA in feeding is not simple and presents somewhat of a paradox. For instance, systemic administration of the DA D1 receptor agonists SKF 82958, SKF 77434 or SKF 38393 suppressed feeding in food restricted rats, in a manner which was related to their efficacy and selectivity to the DA D1 receptor. Thus, SKF 82958 was shown to have the lowest effective dose 50 (ED_{50}) for inducing anorexia and the greatest efficacy. Furthermore, SKF 82958-induced anorexia, but not anorexia induced by other DA D1 receptor agonists, was surmounted by the DA D1 receptor antagonist SCH 23390 (Terry & Katz, 1992). Thus, SKF 77434- and SKF 38393-induced anorexia is not mediated by the DA D1 receptor exclusively (for a discussion of the DA

D2 and 5-HT receptors see Terry & Katz, 1992; Zarrindast, Owji, & Hosseini-Nia, 1991). Similarly, the DA D1 receptor agonist, A-77636, administered just prior to the start of a nightly feeding period produces a reduction in meal size and duration of meal consumption (Cooper et al., 2006b). The apparent dissociation between the role of DA observed in the above studies may relate to procedural differences, such as DA agonist or antagonist dose, and metabolic state (i.e., food restricted or sated).

Furthermore, the effect of systemically administered DA D1 receptor antagonists, on feeding behavior, may differ from the effect of these antagonists on feeding when administered locally, as is the case in chapter 1. The vast majority of research investigating DAergic modulation of feeding has focused on the role of DA in the NAc. For instance, microdialysis studies have shown an increase in extracellular NAc DA prior to lever pressing for food reinforcement (Kiyatkin & Gratton, 1994) and during food delivery (McCullough & Salamone, 1992). Moreover, when focusing on the differential role of NAc subregions, glutamate receptor antagonists or GABA agonists have been shown to produce an intense hyperphagia following infusion into the NAc shell, but not NAc core (Maldonado-Irizarry, Swanson, & Kelley, 1995; Stratford & Kelly, 1997). Similarly, first exposure to a highly palatable food elevates extracellular DA release to a much greater extent in the NAc shell than in the NAc core (Bassareo & Di Chiara, 1999). In contrast, conditioned stimuli associated with palatable food increase DA in the NAc core, but not shell (Bassareo & Di Chiara, 1999). Furthermore, antagonism of DA D1 or D2 receptors in the NAc shell or NAc core, with SCH 23390 or raclopride, respectively, has been shown to have no effect on total food intake or latency to eat in rats exposed to chronic food restriction. However, raclopride infusion into the NAc core and raclopride

or SCH 23390 infusion into the NAc shell results in a reduction in the number of feeding bouts with a significant increase in feeding bout duration observed following raclopride infusion in the NAc core and SCH 23390 infusion in the NAc shell (Baldo, Sadeghian, Basso, & Kelley, 2002). It is worth noting that in Baldo et al.'s (2002) study, SCH 23390 was infused bilaterally at a dose of 1.0 or 2.0 ug/side; whereas, in the experiments described in chapter 1 SCH 23390 was infused at a dose of 0.3 or 0.6 ug/side. It is therefore unclear how SCH 23390, at the current dose range, may have affected hunger during the FD test.

To the best of my knowledge, there are no reported findings on the effect of SCH 23390 infusion into the dmPFC or BLA, on feeding. However, both the dmPFC and the BLA have been implicated, to some degree, in feeding. For instance, in a modified reinstatement model, SCH 23390 infused into the dmPFC blocked yohimbine- and pellet priming-induced reinstatement of food seeking (Nair et al., 2011). Furthermore, the BLA is implicated in neuropeptide regulation of feeding behavior and may act as a satiety center (Vigh, Lenard, & Fekete, 1999). Thus, intra-BLA bombesin infusion, a homologue of gastrin-releasing peptide, a proposed satiety signal released peripherally during feeding, reduces food consumption in rats that have been food deprived for 24h (Vigh et al., 1999).

Acute FD-Induced Reinstatement: Potential Neuronal Circuitry

As demonstrated in chapter 1, DA transmission within the NAc shell, dmPFC and BLA, is critical for the induction of acute FD-induced reinstatement. Thus, my results are in agreement with those of Park et al. (2002) who demonstrate the importance of DA transmission in the mPFC in the reinstatement of cocaine seeking. In particular, these

authors show that priming-induced reinstatement of cocaine seeking is blocked by intra-mPFC administration of the mixed DA receptor antagonist, flupenthixol. Furthermore, this study also demonstrates that intra-mPFC or NAc injection of the DA agonist, cocaine, reinstates drug seeking, supporting the critical role of DA transmission in these regions. Moreover, intra-mPFC cocaine-induced reinstatement was blocked by infusion of the AMPA receptor antagonist CNQX into the NAc demonstrating the importance of a glutamatergic projection from the mPFC to the NAc in the reinstatement of drug seeking.

Kalivas and his colleagues (Peters et al., 2008) have proposed a model of drug seeking which involves two parallel circuits: a 'Go circuit' involved in driving drug seeking, and a 'Stop circuit' involved in inhibiting drug seeking (Peters et al., 2008, 2009). The 'Go circuit' consists of the prelimbic cortex (located in the dmPFC) and the, NAc core; whereas, the 'Stop circuit' consists of the infralimbic cortex (located in the vmPFC) and NAc shell. In line with this proposed circuitry, TTX infusions into the prelimbic cortex has been shown to block both footshock and priming-induced reinstatement of cocaine seeking (Capriles et al., 2003). Furthermore, in the current thesis and in Capriles et al.'s study (2003), infusion of SCH 23390 into the dmPFC, but not vmPFC, was shown to block acute FD- and footshock-induced reinstatement, respectively. Additionally, a direct interaction between the BLA and the NAc core, has been demonstrated by Di Ciano and Everitt (2004). In an elegant neuropharamacological disconnection study, these authors showed that unilateral infusion of α-flupenthixol, a non-selective DA receptor antagonist, into the BLA, and contralateral infusion of LY293558, an AMPA receptor antagonist, into the NAc core produced a reduction in cocaine seeking under a second order schedule of reinforcement. Thus, this finding in

conjunction with the proposed model of Kalivas and his colleagues (Peters et al., 2008, 2009) highlight the importance of a limbic-cortical-ventral striatal circuit in drug seeking.

The notion of two parallel and functionally distinct circuits in drug seeking and the inhibition of drug seeking is intuitively appealing and relatively well supported by studies of reinstatement in cocaine-trained rats (see examples cited throughout this thesis). Furthermore, a limbic-cortical-ventral striatal drug seeking circuit supports the current findings with regard to the importance of the dmPFC and BLA, in acute FD-induced reinstatement. Moreover, a role for DA in the NAc shell, rather than NAc core, in acute FD-induced reinstatement of heroin seeking was shown. Similarly, McFarland et al. (2004) have demonstrated that it is glutamate transmission in the NAc core, and not DA, that is critically involved in footshock-induced reinstatement of cocaine seeking. Finally, these authors also report that transient inactivation of the NAc shell attenuates footshock-induced reinstatement, suggesting a critical role for the NAc shell in stress-induced reinstatement of drug seeking. The current results, therefore, are in good agreement with the model suggested by Kalivas and colleagues.

The Gating of Glutamatergic Inputs to the NAc

As discussed in chapter 2, the NAc receives glutamatergic input from the mPFC, BLA and vSub (French & Totterdell, 2002; French et al., 2003). As a result of this converging information, the NAc must integrate limbic input from the BLA and vSub and cortical input from the mPFC (Goto & Grace, 2008). The balance between the relative importance of these inputs into the NAc has been shown to depend on the two activity states of VTA DA neurons: phasic bursting and slow tonic firing (Grace, 1991), and the subsequent pattern of NAc DA release. For instance, phasic DA release facilitates the

vSub input to the NAc, by activating DA D1 receptors. In contrast, an increase or decrease in tonic DA release attenuates or facilitates, respectively, mPFC input to the NAc (Goto & Grace, 2005a). Thus, DA transmission in the NAc serves to regulate the influence of input from the vSub and mPFC regions involved in context-dependent processing and executive functions such as action monitoring, respectively (Goto & Grace, 2008). It is not yet clear how tonic and phasic DA release in the NAc modulate sensory and emotional input from the BLA (Davis, 1992; Ledoux, 1993) to the NAc. However, this input has been shown to be sensitive to DA D1 receptor activation and antagonism (for further details see Charara & Grace, 2003).

The regulation of glutamatergic input from the mPFC and vSub to the NAc, by DA receptor subtypes, suggests a DA dependent balance between mPFC control of behavioral flexibility and vSub control of context related behavior (Belujon & Grace, 2008; Grace, Floresco, Goto, & Lodge, 2007). Related to these findings, cocaine sensitization has been shown to block vSub/CA1 stimulation-induced LTP at vSub/CA1-NAc shell synapses and prevent vSub/CA1 stimulation-induced LTD in mPFC-NAc synapses. Thus, repeated cocaine exposure may disrupt cellular mechanisms involved in the induction of LTP. Alternatively, repeated drug use may induce an 'LTP-like' state in the vSub-NAc pathway which would prevent the induction of LTP due to the mechanisms of LTP already being engaged (Goto & Grace, 2005b see chapter 3 for a discussion of the importance of this dissociation). Similarly, an impairment in the induction of LTP, at vSub-NAc shell synapses, has been observed in chronically morphine-treated rats 12h after withdrawal. However, LTP was restored and LTD was impaired in this pathway following a 4-day withdrawal period (Dong et al., 2007). In

chapter 2, I expand upon these findings by demonstrating an impairment in the induction of LTP, in the vSub-NAc shell pathway, following acute FD. However, this effect was observed in both heroin-trained and heroin-naïve rats, suggesting that the observed modulation in synaptic plasticity occurred as a result of FD and was not related to previous drug history.

Nevertheless, I also found an inverse correlation between LTP in the vSub-NAc shell pathway and active lever pressing on the reinstatement test, suggesting that acute FD-induced plasticity in this pathway may play an important role in the reinstatement of heroin seeking. Using the current experimental procedure it is not possible to say with certainty that the impairment in LTP-induction, observed in food deprived rats, was due to the previous induction of an 'LTP-like' state. However, it is not unreasonable to speculate that this indeed was the case. For instance, if acute FD does produce an 'LTP-like' effect in the vSub-NAc shell pathway, it can be expected that this pathway will be strengthened, and as a result of this strengthening cortical regulation would be dampened. Related to this hypothesis, tetanic stimulation of the vSub induces an LTP of vSub-evoked EPSPs in the NAc shell and core, as well as a persistent decrease in mPFC evoked NAc EPSPs (Goto & Grace, 2005a). Furthermore, inactivation of the mPFC, with TTX, blocks the ability of stimulation to the fimbria, which carries glutamatergic fibers from the vSub to the NAc, to increase spike firing in individual NAc neurons (localized mainly within the shell). However, following the induction of LTP in the vSub-NAc pathway, mPFC TTX infusion was shown to have no effect on fimbria-induced NAc spike firing (Belujon & Grace, 2008). Thus, it appears that mPFC initially plays a permissive role in allowing the vSub to stimulate the NAc; however, following repeated

activation of this pathway the mPFC no longer exerts control over the functioning of the vSub-NAc pathway allowing for a reduction in cortical control of contextually driven behavior (Belujon & Grace, 2008). Related to this finding, the transition from voluntary drug use, motivated by the acutely rewarding effects of the drug, to more compulsive and habitual drug use, as is the case in drug addiction, involves a shift away from cortical control of behavior (Everitt & Robbins, 2005).

The Involvement of DA in the Trafficking of iGluR Subunits

The interaction between DA and glutamate systems is likely mediated, at least in part, by the downstream effects of DA D1 receptor activation. For example, within the NAc, stimulation of DA D1 receptors activates cAMP, a second messenger involved in intracellular signal transduction, and PKA, a family of enzymes activated by cAMP and critical to protein phosphorylation (Anderson et al., 2008). Activation or inactivation of NAc PKA has been shown to increase and decrease, respectively, responding for cocaine on a progressive ratio schedule of reinforcement (Lynch & Taylor, 2005). Moreover, inactivation of NAc PKA has been shown to reduce cocaine self-administration, shift the dose response curve for cocaine to the left, and reinstate drug seeking in abstinent rats (Stewart & Vezina, 1988). The intracellular effects of NAc PKA are numerous; however, a particularly interesting target of PKA is voltage-gated L-type calcium channels. Calcium influx through these channels activates the CaMKII, which play a central role in transporting AMPA glutamate receptors, particularly the GluA1 receptor subunit, to the surface of the cellular membrane (Anderson et al., 2008; Boehm & Malinow, 2005). The relevance of these effects to drug seeking has been demonstrated by Anderson et al. (2008) who have reported that the reinstatement of cocaine seeking is associated with a

DA D1 receptor dependent increase in NAc shell CaMKII and an increase in membrane surface GluA1 receptor expression. Furthermore, these authors have shown that impairing the transport of the GluA1 receptor subunit causes an attenuation of cocaine seeking.

Acute FD-Induced Reinstatement and iGluR trafficking

Interestingly, no change in GluA1 receptor subunit expression in the NAc, or in any of the other regions investigated was observed in the current manipulation. This lack of effect may be the result of a pharmacological difference between repeated cocaine and heroin self-administration. An indication that this may be the case comes from studies addressing drug-induced changes in dendritic spine morphology, a reflection of synaptic strength (Alvarez & Sabatini, 2007; Holtmaat & Svoboda, 2009). For instance, stimulant drugs increase dendritic spine density in the NAc and mPFC; whereas, the opiate drug morphine decreases spine density in these regions (Robinson & Kolb, 2004). Furthermore, as discussed in chapter 3, I assessed total iGluR subunit expression, rather than membrane surface and intracellular iGluR subunit expression. Thus, a change in the configuration of membrane-embedded AMPA receptors might not be reflected in the amount of receptor subunit expressed in the whole cell. However, a near significant reduction in NR1 receptor subunit expression in the NAc of heroin-trained food deprived rats relative to heroin-trained sated rats was observed. As the NR1 subunit is an obligatory subunit of the NMDA receptor (Gass & Olive, 2008) this indicates a probable reduction in NMDA receptors within the NAc of heroin-trained rats exposed to acute FD. Interestingly, NAc infusion of the NMDA receptor antagonist, AP-5, dose dependently increases cocaine self-administration, suggesting a reduction in the rewarding value of

cocaine, but has no effect on ongoing heroin self-administration (Pulvirenti, Maldonado-Lopez, & Koob, 1992). Moreover, AP-5 causes a dose dependent potentiation of restraint stress-induced DA release in the NAc (Doherty & Gratton, 1997). In contrast to my findings, NAc NR1 receptor subunit expression is not altered following priming-induced reinstatement of an amphetamine induced CPP; however, GluA1 receptor subunit expression is decreased (Cruz, Marin, & Planeta, 2008). Thus, it may be the case the NR1 receptor subunit is selectively involved in stress-induced reinstatement. It is tempting to suggest the existence of a relationship between the reduction in NAc NR1 receptors, observed following acute FD-induced reinstatement, and activation of DA D1 receptors. However, existing evidence, although supporting NMDA-DA D1 receptor interactions, suggests a synergistic, rather than opposing effect. Thus, it has been demonstrated that in the striatum, DA D1 receptors are co-localized with NMDA receptors (Fiorentini, Gardoni, Spano, Di Luca & Missale, 2003).

However, in neostriatal slice preparations, DA and DA D1 receptor agonists have been shown to potentiate responses made by activation of NMDA receptors, an effect which is markedly decreased in mutant mice lacking the DA D1 receptor (Levine et al., 1996). Furthermore, activation of neostriatal NMDA receptors has been shown to recruit functional DA D1, but not DA D2, receptors to the plasma membrane (Scott et al., 2002). It is possible, though, that opiate drugs, which do not act directly on DA neurons, have a different impact on NMDA receptors.

In addition to a potential change in the expression of the NR1 receptor subunit I also found a significant increase in GluA2 receptor subunit expression in the BLA of drug-naïve food deprived rats, relative to drug-naïve sated rats, and a significant increase

in GluA2 receptor subunit expression in the VTA of heroin-trained sated rats relative to drug naïve sated rats. Finally, a tendency toward a reduction in GluA2 receptor subunit expression was observed in the dmPFC of heroin-trained food deprived rats relative to heroin-trained sated rats. Changes in the GluA2 AMPA receptor subunit are of particular interest because as mentioned in the introduction, this subunit regulates AMPA receptor calcium permeability (Derkach et al. 2007; Gass & Olive, 2008; Hollmann et al., 1991, Vandenberghe, et al., 2000). Although there are a few exceptions (see Dickinson et al., 2009; Fitzjohn et al., 2001), it is widely accepted that synaptic plasticity, both LTP and LTD, rely upon calcium-dependent mechanisms (Kerrigan, Daniel, Regan, & Cho, 2012; Malenka, 1994). Thus, the presence or absence of the AMPA GluA2 receptor subunit prevents or allows for calcium influx through the AMPA receptor (Mead & Stephens, 2003). AMPA receptor mediated calcium influx can influence synaptic plastic by regulating AMPA receptor trafficking, thereby producing long-term changes in synaptic strength, and/or can induce a change in the subunits of the AMPA receptor, thereby modifying the electrophysiological properties of the AMPA receptor (Liu & Zukin, 2007).

During the induction of LTP, GluA2-lacking receptors are transiently incorporated into the synapse; however, following the induction of LTP there is an increase in GluA2-containing AMPA receptors (Plant et al., 2006). Thus, an increase in the expression of the GluA2 receptor subunit, within a particular region, may indicate a strengthening of synapses within this area. In the current study, the increased expression of the GluA2 subunit, within the BLA, was observed only in the food-deprived drug-naive, but not the heroin-trained rats, suggesting that drug self-administration might

interfere with this FD-induced adaptation.

In contrast, the finding of an increase in GluA2 receptor subunit expression in the VTA of heroin-trained sated rats relative to drug-naive sated rats suggests that FD stress reverses the heroin-induced increased expression of GluA2 subunits in this area, or blocks the changes in GluA2 subunit expression that occur following exposure to the drug self-administration environment. The former idea is in agreement with the reported increase in VTA GluA2 receptor subunit expression in cocaine-trained rats, that is found following 30 days of forced abstinence (Lu, Grimm, Shaham, & Hope, 2003).

Finally, within the dmPFC, a tendency toward a reduction in GluA2 receptor subunit expression was observed in heroin-trained food deprived rats compared to heroin-trained sated rats. Similarly, cue-induced reinstatement of heroin seeking is associated with a reduction in GluR2 receptor subunit expression and a decrease in synaptic strength in mPFC pyramidal neurons, which was indicated by a reduction in AMPA/NMDA current ratio (Van den Oever et al., 2008). Unfortunately, these authors do not distinguish between dmPFC and vmPFC with regard to the GluA2 receptor subunit. However, they do show that blocking GluA2 receptor endocytosis in the ventral, but not dorsal, mPFC attenuates cue-induced reinstatement. Thus, these authors suggest a critical role for endocytosis of the GluA2 subunit in the vmPFC, i.e., increased proportion of GluA2-lacking AMPA receptors, in cue-induced reinstatement. The current findings suggest an important role for dmPFC GluA2-lacking AMPA receptors in acute FD-induced reinstatement of heroin seeking; however, I cannot comment on the involvement of the vmPFC (see above for the importance of this distinction).

Conclusion

The results of the current thesis do no fully elucidate the neuronal circuitry involved in acute FD-induced reinstatement of heroin seeking; however, it does highlight several key components of this circuitry. In particular, the current study highlights the importance of DA acting at the D1 receptor in the NAc shell, dmPFC, and BLA, but not the NAc core or vmPFC. As discussed previously, acute FD has been shown to have a direct effect on the neuronal circuitry mediating reward. Thus, acute FD may enhance the effect of DA in the NAc in response to drug associated cues. Furthermore, the actions of DA, at the D1 receptor, in acute FD-induced reinstatement are likely mediated by an interaction between DA and glutamate. As evidence of the importance of glutamatergic inputs to the NAc, a change in synaptic plasticity in the gltuamatergic projection from the vSub to the NAc shell following acute FD was observed. Furthermore, it appears that acute FD and heroin self-administration serve to modify the expression of iGluR subunits in the BLA, VTA and dmPFC. In particular, acute FD is associated with changes, or the blocking of changes, in the AMPA GluA2 receptor subunit, the subunit that regulates the calcium permeability of AMPA receptors. Thus, acute FD stress may lead to changes in iGluR subunit expression, which contributes to the facilitation and inhibition of synaptic plasticity.

In order to further elucidate the interaction between DA and glutamate in acute FD-induced reinstatement of heroin seeking, future studies should investigate synaptic plasticity and iGluR subunit expression, in rats treated with the DA D1 receptor antagonist SCH 23390, prior to being tested for acute FD-induced reinstatement. Studies of this nature could demonstrate a functional link between DA acting at the D1 receptor

and drug- or FD-induced changes in glutamate synapses. Furthermore, to ensure that the methodology employed in the current studies did not obscure changes in iGluR subunit membrane expression or phosphorylation, future studies should include a measure of iGluR subunit expression in the surface and intracellular pools, as well as iGluR phosphorylation.

REFERENCES

Abraham, W. C. (2008). Metaplasticity: tuning synapses and networks for plasticity. *Nature Reviews Neuroscience*, *9*(5), 387–387. doi:10.1038/nrn2356

Abush H, Akirav I (2012). Short- and Long-Term Cognitive Effects of Chronic Cannabinoids Administration in Late-Adolescence Rats. PLoS ONE 7(2): e31731.doi:10.1371/journal.pone.0031731

Adinoff, B., Risher-Flowers, D., de Jong, J., Ravitz, B., Bone, G. H., Nutt, D. J., Roehrich, L., et al. (1991). Disturbances of hypothalamic-pituitary-adrenal axis functioning during ethanol withdrawal in six men. *The American Journal of Psychiatry*, *148*(8), 1023–1025.

Aguilar, M. A., Rodríguez-Arias, M., & Miñarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Research Reviews*, *59*(2), 253–277. doi:10.1016/j.brainresrev.2008.08.002

Aked, J., Coizet, V., Clark, D., & Overton, P. G. (2005). Local injection of a glutamate uptake inhibitor into the ventral tegmental area produces sensitization to the behavioural effects of d-amphetamine. *Neuroscience*, *134*(2), 361–367. doi:10.1016/j.neuroscience.2005.04.044

Alderson, H. L., Robbins, T. W., & Everitt, B. J. (2000). The effects of excitotoxic lesions of the basolateral amygdala on the acquisition of heroin-seeking behaviour in rats. *Psychopharmacology, 153*(1), 111–119. doi:10.1007/s002130000527

Alvarez, V. A., & Sabatini, B. L. (2007). Anatomical and Physiological Plasticity of Dendritic Spines. *Annual Review of Neuroscience*, *30*(1), 79–97. doi:10.1146/annurev.neuro.30.051606.094222

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.

Anderson, S. M., Famous, K. R., Sadri-Vakili, G., Kumaresan, V., Schmidt, H. D., Bass, C. E., Terwilliger, E. F., et al. (2008). CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nature Neuroscience*, *11*(3), 344–353. doi:10.1038/nn2054

Anderson, S. M., Bari, A. A., & Pierce, R. C. (2003). Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. *Psychopharmacology*, *168*(1-2), 132–138. doi:10.1007/s00213-002-1298-5

Anderson, S. M., Schmidt, H. D., & Pierce, R. C. (2006). Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. *Neuropsychopharmacology*, *31*(7), 1452–1461. doi:10.1038/sj.npp.1300922

Bachtell, R. K., Whisler, K., Karanian, D., & Self, D. W. (2005). Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology, 183*(1), 41–53. doi:10.1007/s00213-005-0133-1

Baudry, M., & Lynch, G. (2001). Remembrance of arguments past: how well is the glutamate receptor hypothesis of LTP holding up after 20 years? *Neurobiology of* Learning and Memory, 76, 284–297. doi:10.1006/nlme.2001.4023

Bajo, M., Crawford, E. F., Roberto, M., Madamba, S. G., & Siggins, G. R. (2006). Chronic morphine treatment alters expression of N-methyl-D-aspartate receptor

subunits in the extended amygdala. *Journal of Neuroscience Research*, *83*(4), 532–537. doi:10.1002/jnr.20756

Baldo, B. A., Sadeghian, K., Basso, A. M., & Kelley, A. E. (2002). Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behavioural Brain Research*, *137*(1-2), 165–177.

Baptista, M. A. S., Martin-Fardon, R. & Weiss, F. (2004). Preferential Effects of the Metabotropic Glutamate 2/3 Receptor Agonist LY379268 on Conditioned Reinstatement versus Primary Reinforcement: Comparison between Cocaine and a Potent Conventional Reinforcer. *The Journal of Neuroscience*, *24*(20), 4723–4727. doi:10.1523/JNEUROSCI.0176-04.2004

Bassareo, V., & Di Chiara, G. (1999). Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neuroscience*, *89*(3), 637-641.

Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Current opinion in neurobiology*, *4*(3), 389–399. doi:10.1016/0959-4388(94)90101-5

Beitner-Johnson, D., Guitart, X., & Nestler, E. J. (1992). Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. *The Journal of Neuroscience*, *12*(6), 2165–2176.

Bellone, C., & Lüscher, C. (2005). mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. *European Journal of Neuroscience*, *21*(5), 1280–1288. doi:10.1111/j.1460-

9568.2005.03979.x

Belujon, P., & Grace, A. A. (2008). Critical Role of the Prefrontal Cortex in the Regulation of Hippocampus-Accumbens Information Flow. *The Journal of Neuroscience*, *28*(39), 9797–9805. doi:10.1523/JNEUROSCI.2200-08.2008

Berglind, W. J., Case, J. M. , Parker, M. P., Fuchs, R. A., & See, R. E. (2006). Dopamine D1 or D2 receptor antagonism within the basolateral amygla differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience, 137*, 699-706.

Berridge, K. C. (1996). Food reward: brain substrates of wanting and liking. *Neuroscience and Biobehavioral Reviews*, *20*(1), 1–25.

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*, *191*(3), 391–431. doi:10.1007/s00213-006-0578-x

Bi, S., Robinson, B. M., & Moran, T. H. (2003). Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *American journal of physiology Regulatory, integrative and comparative physiology*, *285*(5), R1030–R1036.

Blaha, C. D., Yang, C. R., Floresco, S. B., Barr, A. M., & Phillips, A. G. (1997). Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. *The European Journal of Neuroscience*, *9*(5), 902–911.

Black, Y. D., Green-Jordan, K., Eichenbaum, H. B., & Kantak, K. M. (2004). Hippocampal memory system function and the regulation of cocaine self-administration behavior in rats. *Behavioural Brain Research*, *151*(1-2), 225–238.

doi:10.1016/j.bbr.2003.08.020

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31–39. doi:10.1038/361031a0

Boehm, J., & Malinow, R. (2005). AMPA receptor phosphorylation during synaptic plasticity. *Biochemical Society Transactions*, *33*(Pt 6), 1354-1356.

Bossert, J. M., Ghitza, U. E., Lu, L., Epstein, D. H., & Shaham, Y. (2005). Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications. *European Journal of Pharmacology*, *526*, 36–50. doi:10.1016/j.ejphar.2005.09.030

Bossert, J. M., Gray, S. M., Lu, L., & Shaham, Y. (2006a). Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. *Neuropsychopharmacology*, *31*(10), 2197–2209. doi:10.1038/sj.npp.1300977

Bossert, J. M., Liu, S. Y., Lu, L., & Shaham, Y. (2004). A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. *The Journal of Neuroscience*, *24*(47), 10726–10730. doi:10.1523/JNEUROSCI.3207-04.2004

Bossert, J. M., Poles, G. C., Sheffler-Collins, S. I., & Ghitza, U. E. (2006b). The mGluR2/3 agonist LY379268 attenuates context- and discrete cue-induced reinstatement of sucrose seeking but not sucrose self-administration in rats. *Behavioural Brain Research*, *173*(1), 148–152. doi:10.1016/j.bbr.2006.06.008

Bossert, J. M., Poles, G. C., Wihbey, K. A., Koya, E., & Shaham, Y. (2007). Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. *The Journal of Neuroscience*, *27*(46), 12655–12663. doi:10.1523/JNEUROSCI.3926-

07.2007

Bossert, J. M., Stern, A. L., Theberge, F. R. M., Cifani, C., Koya, E., Hope, B. T., & Shaham, Y. (2011). Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. *Nature Neuroscience*, *14*(4), 420–422. doi:10.1038/nn.2758

Bossert, J. M., Stern, A. L., Theberge, F. R. M., Marchant, N. J., Wang, H.-L., Morales, M., & Shaham, Y. (2012). Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. *The Journal of Neuroscience*, *32*(14), 4982–4991. doi:10.1523/JNEUROSCI.0005-12.2012

Boudreau, A. C. & Wolf, M. E. (2005). Behavioral Sensitization to Cocaine Is Associated with Increased AMPA Receptor Surface Expression in the Nucleus Accumbens. *The Journal of Neuroscience*, *25*(40), 9144–9151. doi:10.1523/JNEUROSCI.2252-05.2005

Bourne, J. A. (2001). SCH 23390: The First Selective Dopamine D1-Like Receptor Antagonist. *CNS Drug Reviews*, *7*(4), 399–414.

Bradley, B. P., Phillips, G., Green, L., & Gossop, M. (1989). Circumstances surrounding the initial lapse to opiate use following detoxification. *The British Journal of Psychiatry*, *154*(3), 354–359. doi:10.1192/bjp.154.3.354

Breese, G. R., Smith, R. D., Cooper, B. R., & Grant, L. D. (1973). Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. *Pharmacology, Biochemistry, and Behavior*, *1*(3), 319–328.

Brewer, D. D., Catalano, R. F., Haggerty, K., Gainey, R. R., & Fleming, C. B. (1998). A

meta-analysis of predictors of continued drug use during and after treatment for opiate addiction. *Addiction*, *93*(1), 73–92.

Buczek, Y., Lê, A. D., Wang, A., Stewart, J., & Shaham, Y. (1999). Stress reinstates nicotine seeking but not sucrose solution seeking in rats. *Psychopharmacology*, *144*(2), 183–188.

Buffalari, D. M., & See, R. E. (2009). Footshock stress potentiates cue-induced cocaine-seeking in an animal model of relapse. *Physiology & Behavior*, *98*(5), 614–617. doi:10.1016/j.physbeh.2009.09.013

Cador, M., Bjijou, Y., Cailhol, S., & Stinus, L. (1999). D-amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. *Neuroscience*, *94*(3), 705–721.

Caine, S. B., Heinrichs, S. C., Coffin, V. L., & Koob, G. F. (1995). Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Research*, *692*(1-2), 47–56.

Caine, S. B., Negus, S. S., Mello, N. K., Patel, S., Bristow, L., Kulagowski, J., Vallone, D., et al. (2002). Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *The Journal of Neuroscience*, *22*(7), 2977–2988. doi:20026264

Caine, S. B., Thomsen, M., Gabriel, K. I., Berkowitz, J. S., Gold, L. H., Koob, G. F., Tonegawa, S., et al. (2007). Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *Journal of Neuroscience, 27*(48), 13140–13150. doi:10.1523/JNEUROSCI.2284-07.2007

Carroll, M. E. (1985). The role of food deprivation in the maintenance and reinstatement

of cocaine-seeking behavior in rats. *Drug and Alcohol Dependence*, *16*(2), 95–109.

Campbell, I. G., Guinan, M. J., & Horowitz, J. M. (2002). Sleep deprivation impairs long-term potentiation in rat hippocampal slices. *Journal of Neurophysiology*, *88*(2), 1073–1076. doi:10.1152/jn.00873.2001

Campioni, M. R., Xu, M., & McGehee, D. S. (2009). Stress-Induced Changes in Nucleus Accumbens Glutamate Synaptic Plasticity. *Journal of Neurophysiology*, *101*(6), 3192–3198. doi:10.1152/jn.91111.2008

Canteras, N. S., & Swanson, L. W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *The Journal of Comparative Neurology*, *324*(2), 180–194. doi:10.1002/cne.903240204

Capriles, N., Rodaros, D., Sorge, R. E., & Stewart, J. (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, *168*(1-2), 66–74. doi:10.1007/s00213-002-1283-z

Carr, K. D., & Kutchukhidze, N. (2000). Effect of chronic food restriction on Fos-like immunoreactivity (FLI) induced in rat brain regions by intraventricular MK-801. *Brain Research*, *873*(2), 283–286.

Carr, K. D., & Sesack, S. R. (2000). Projections from the Rat Prefrontal Cortex to the Ventral Tegmental Area: Target Specificity in the Synaptic Associations with Messoaccumbens and Mesocortical Neurons. *The Journal of Neuroscience, 20* (10), 3864-3873.

Cervo, L., Carnovali, F., Stark, J. A., & Mennini, T. (2003). Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine

receptors. *Neuropsychopharmacology*, *28*(6), 1150–1159. doi:10.1038/sj.npp.1300169

Cervo, L., & Samanin, R. (1995). Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Research*, *673*(2), 242–250.

Charara, A., & Grace, A. A. (2003). Dopamine Receptor Subtypes Selectively Modulate Excitatory Afferents from the Hippocampus and Amygdala to Rat Nucleus Accumbens Neurons. *Neuropsychopharmacology*, *28*(8), 1412–1421. doi:10.1038/sj.npp.1300220

Childress, A. R., Ehrman, R., McLellan, T., MacRae, J., Natale, M., & O'Brien, C. P. (1994). Can induced moods trigger drug-related responses in opiate abuse patients? *Journal of Substance Abuse Treatment*, *11*(1), 17–23. doi:10.1016/0740-5472(94)90060-4

Ciccocioppo, R., Sanna, P. P., & Weiss, F. (2001). Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D_1 antagonists. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(4), 1976–1981. doi:10.1073/pnas.98.4.1976

Collingridge, G. L., Isaac, J. T. R., & Wang, Y. T. (2004). Receptor trafficking and synaptic plasticity. *Nature Reviews Neuroscience*, *5*(12), 952–962. doi:10.1038/nrn1556

Conboy, L., & Sandi, C. (2010). Stress at learning facilitates memory formation by regulating AMPA receptor trafficking through a glucocorticoid action.

Neuropsychopharmacology, *35*(3), 674–685. doi:10.1038/npp.2009.172

Conn, P. J., & Pinn, J.-P. (1997). Pharmacology and functions of metabotopic glutamate receptors, *Annual Review of Pharmacology & Toxicology, 37*, 20-237.

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L.-J., Shaham, Y., Marinelli, M., et al. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118–U9. doi:10.1038/nature06995

Cooper, S. J., Al-Naser, H. A., & Clifton, P. G. (2006b). The anorectic effect of the selective dopamine D1-receptor agonist A-77636 determined by meal pattern analysis in free-feeding rats. *European Journal of Pharmacology*, *532*(3), 253–257. doi:10.1016/j.ejphar.2005.11.046

Cooper, D. C., Klipec, W. D., Fowler, M. A., & Ozkan, E. D. (2006a). A role for the subiculum in the brain motivation/reward circuitry. *Behavioural Brain Research*, *174*(2), 225–231. doi:10.1016/j.bbr.2006.05.036

Cooper, D. C., Moore, S. J., Staff, N. P., & Spruston, N. (2003). Psychostimulant-induced plasticity of intrinsic neuronal excitability in ventral subiculum. *The Journal of Neuroscience, 23*(30), 9937–9946.

Cornish, J. L., Duffy, P., & Kalivas, P. W. (1999). A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. *Neuroscience*, *93*(4), 1359–1367.

Cornish, J. L., & Kalivas, P. W. (2000). Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *The Journal of Neuroscience 20*(15), RC89.

Corrigall, W. A., & Coen, K. M. (1991). Cocaine self-administration is increased by both D1 and D2 dopamine antagonists. *Pharmacology, Biochemistry, and Behavior*, *39*(3), 799–802.

Crombag, H. S., Grimm, J. W., & Shaham, Y. (2002). Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. *Neuropsychopharmacology*, *27*(6), 1006–1015. doi:10.1016/S0893-133X(02)00356-1

Cruz, F. C., Marin, M. T., & Planeta, C. S. (2008). The reinstatement of amphetamine-induced place preference is long-lasting and related to decreased expression of AMPA receptors in the nucleus accumbens. *Neuroscience*, *151*(2), 313–319. doi:10.1016/j.neuroscience.2007.10.019

Cruz, F. C., Leão, R. M., Marin, M. T., & Planeta, C. S. (2010). Stress-induced reinstatement of amphetamine-conditioned place preference and changes in tyrosine hydroxylase in the nucleus accumbens in adolescent rats. *Pharmacology, Biochemistry, and Behavior*, *96*(2), 160–165. doi:10.1016/j.pbb.2010.05.001

Culpepper-Morgan, J. A., & Kreek, M. J. (1997). Hypothalamic-pituitary-adrenal axis hypersensitivity to naloxone in opioid dependence: a case of naloxone-induced withdrawal. *Metabolism: Clinical and Experimental*, *46*(2), 130–134.

Cullinan, W. E., Herman, J. P., & Watson, S. J. (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. *The Journal of Comparative Neurology*, *332*(1), 1–20. doi:10.1002/cne.903320102

Davis, M. (1992). The role of the amygdala in fear and anxiety. Annual review of

neuroscience, 15, 353–375. doi:10.1146/annurev.ne.15.030192.002033

de Kloet, E. R., Karst, H., & Joëls, M. (2008). Corticosteroid hormones in the central stress response: Quick-and-slow. *Frontiers in neuroendocrinology*, *29*(2), 268–272. doi:10.1016/j.yfrne.2007.10.002

Dembo, R., Dertke, M., Borders, S., Washburn, M., & Schmeidler, J. (1988). The Relationship Between Physical and Sexual Abuse and Tobacco, Alcohol, and Illicit Drug Use Among Youths in a Juvenile Detention Center. *The Internal Journal of the Addictions, 23* (4), 351-378.

Deminiere, J. M., Piazza, P. V., Le Moal, M., & Simon, H. (1989). Experimental approach to individual vulnerability to psychostimulant addiction. *Neuroscience and Biobehavioral Reviews*, *13*(2-3), 141–147.

Der-Avakian, A., Will, M. J., Bland, S. T., Deak, T., Nguyen, K. T., Schmid, M. J., Spencer, R. L., et al. (2005). Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats. *Psychopharmacology*, *179*(2), 409–417. doi:10.1007/s00213-004-2041-1

Derkach, V. A., Oh, M. C., Guire, E. S., & Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature Reviews Neuroscience*, *8*(2), 101–113. doi:10.1038/nrn2055

Deroche, V., Piazza, P. V., Casolini, P., Maccari, S., Le Moal, M., & Simon, H. (1992a). Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. *Brain Research*, *598*(1-2), 343–348.

Deroche, V., Piazza, P. V., Le Moal, M., & Simon, H. (1993). Individual differences in the psychomotor effects of morphine are predicted by reactivity to novelty and influenced by corticosterone secretion. *Brain Research*, *623*(2), 341–344.

Deroche, V., Piazza, P. V., Le Moal, M., & Simon, H. (1994). Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. *Brain Research*, *640*(1-2), 136–139.

Deroche, V., Piazza, P. V., Maccari, S., Le Moal, M., & Simon, H. (1992b). Repeated corticosterone administration sensitizes the locomotor response to amphetamine. *Brain Research*, *584*(1-2), 309–313.

de Quervain, D. J. F., Roozendaal, B., & McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Letters to Nature*, *394*(6695), 787–790. doi:10.1038/29542

de Vries, T. J., Schoffelmeer, A. N., Binnekade, R., Mulder, A. H., & Vanderschuren, L. J. (1998). Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. *The European Journal of Neuroscience*, *10*(11), 3565–3571.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, *75*(2), 134–143.

de Wit, H., & Stewart, J. (1983). Drug reinstatement of heroin-reinforced responding in the rat. *Psychopharmacology*, *79*(1), 29–31.

Diamond, D. M., Bennett, M. C., Fleshner, M., & Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus*, *2*(4), 421–430.

Diamond, D. M., Park, C. R., & Woodson, J. C. (2004). Stress generates emotional memories and retrograde amnesia by inducing an endogenous form of hippocampal LTP. *Hippocampus*, *14*(3), 281–291. doi:10.1002/hipo.10186

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behavioural Brain Research, 137*, 75-114.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274–5278.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M. Spina, L., Cadoni, C., et al. (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology, 47*, 227-241. doi: 10.1016/j.neuropharm.2004.06.032

Di Ciano, P., & Everitt, B. J. (2004). Direct Interactions between the Basolateral Amygdala and Nucleus Accumbens Core Underlie Cocaine-Seeking Behavior by Rats. *The Journal of Neuroscience*, *24*(32), 7167–7173. doi:10.1523/JNEUROSCI.1581-04.2004

Dickinson, B. A., Jo, J., Seok, H., Son, G., Whitcomb, D. J., Davies, C. H., Sheng, M., et al. (2009). A novel mechanism of hippocampal LTD involving muscarinic receptor-triggered interactions between AMPARs, GRIP and liprin-α. *Molecular Brain*, *2*(18), doi:10.1186/1756-6606-2-18

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews*, *51*(1), 7–62.

Doherty, M., & Gratton, A. (1997). NMDA receptors in nucleus accumbens modulate stress-induced dopamine release in nucleus accumbens and ventral tegmental area .

Synapse, (26), 225–234.

Dong, Z., Cao, J., & Xu, L. (2007). Opiate withdrawal modifies synaptic plasticity in subicular-nucleus accumbens pathway in vivo. *Neuroscience*, *144*(3), 845–854. doi:10.1016/j.neuroscience.2006.10.018

Donny, E. C., Bigelow, G. E., & Walsh, S. L. (2006). Comparing the physiological and subjective effects of self-administered vs yoked cocaine in humans. *Psychopharmacology*, *186*(4), 544–552. doi:10.1007/s00213-006-0312-8

Dube, S. R., Felitti, V. J., Dong, M., Chapman, D. P., Giles, W. H., & Anda, R. F. (2003). Childhood Abuse, Neglect, and Household Dysfunction and the Risk of Illicit Drug Use: The Adverse Childhood Experiences Study. *Pediatrics*, *111*(3), 564–572. doi:10.1542/peds.111.3.564

Dunn, A. J., & Berridge, C. W. (1990). Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Research Reviews*, *15*(2), 71–100.

Dworkin, S. I., Goeders, N. E., & Smith, J. E. (1985). The reinforcing and rate effects of intracranial dopamine administration. *Problems of Drug Dependence*, 242–248.

Dzung Lê, A., Funk, D., Harding, S., Juzytsch, W., & Fletcher, P. J. (2009). The role of noradrenaline and 5-hydroxytryptamine in yohimbine-induced increases in alcohol-seeking in rats. *Psychopharmacology*, *204*(3), 477–488. doi:10.1007/s00213-009-1481-z

Edwards, S., Graham, D. L., Whisler, K. N., & Self, D. W. (2009). Phosphorylation of GluR1, ERK, and CREB during spontaneous withdrawal from chronic heroin self-administration. *Synapse*, *63*(3), 224–235. doi:10.1002/syn.20601

Ehrenreich, H., Schuck, J., Stender, N., Pilz, J., Gefeller, O., Schilling, L., Poser, W., et al. (1997). Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. *Alcoholism, Clinical and Experimental Research*, *21*(7), 1285–1293.

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology*, *189*(1), 1–16. doi:10.1007/s00213-006-0529-6

Epstein, D. H., Willner-Reid, J., Vahabzadeh, M., Mezghanni, M., Lin, J. L., & Preston, K. L. (2009). Real-time electronic diary reports of cue exposure and mood in the hours before cocaine and heroin craving and use. *Archives of General Psychiatry*, *66*(1), 88-94.

Erb, S., & Stewart, J. (1999). A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. *Journal of Neuroscience, 99*, RC35.

Erb, S., Petrovic, A., Yi, D., & Kayyali, H. (2006). Central injections of CRF reinstate cocaine seeking in rats after postinjection delays of up to 3 h: an influence of time and environmental context. *Psychopharmacology*, *187*(1), 112–120. doi:10.1007/s00213-006-0392-5

Erb, S., Shaham, Y., & Stewart, J. (1998). The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. *The Journal of Neuroscience*, *18*(14), 5529–5536.

Esteban, J. A., Shi, S.-H., Wilson, C., Nuriya, M., Huganir, R. L., & Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking

underlying plasticity. *Nature Neuroscience*, *6*(2), 136–143. doi:10.1038/nn997

Ettenberg, A. (2009). The runway model of drug self-administration. *Pharmacology, Biochemistry, and Behavior*, *91*(3), 271–277. doi:10.1016/j.pbb.2008.11.003

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8*(11), 1481–1489. doi:10.1038/nn1579

Facchinetti, F., Volpe, A., Farci, G., Petraglia, F., Porro, C. A., Barbieri, G., Cioni, A., et al. (1985). Hypothalamus-pituitary-adrenal axis of heroin addicts. *Drug and Alcohol Dependence*, *15*(4), 361–366.

Feltenstein, M. W., & See, R. E. (2006). Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. *Behavioural Brain Research*, *174*(1), 1–8. doi:10.1016/j.bbr.2006.06.039

Fiorentini, C. C., Gardoni, F., Spano, P., De Luca, M., & Missale, C. (2003). Regulation of Dopamine D1 Receptor Trafficking and Desensitization by Oligomerization with Glutamate N-Methyl-D-aspartate Receptors. *Journal of Biological Chemistry*, *278*(22), 20196–20202. doi:10.1074/jbc.M213140200

Fitzgerald, L. W., Ortiz, J., Hamedani, A. G., & Nestler, E. J. (1996). Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *The Journal of Neuroscience*, *16*(1), 274–282.

Fitzjohn, S. M., Palmer, M. J., May, J. E. R., Neeson, A., Morris, S. A. C., & Collingridge, G. L. (2001). A characterisation of long-term depression induced by metabotropic glutamate receptor activation in the rat hippocampus in vitro. *The*

Journal of physiology, *537*(2), 421–430.

Floresco, S. B., West, A. R., Ash, B., Moore, H., & Grace, A. A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature Neuroscience*, *6*(9), 968–973. doi:10.1038/nn1103

Floresco, S. B., Yang, C. R., Phillips, A. G., & Blaha, C. D. (1998). Association Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. *The European Journal of Neuroscience*, *10*(4), 1241–1251.

Fonnum, F. (1984). Glutamate: a neurotransmitter in mammalian brain. *Journal of Neurochemistry*, *42*(1), 1–11.

Fourgeaud, L. (2004). A Single In Vivo Exposure to Cocaine Abolishes Endocannabinoid-Mediated Long-Term Depression in the Nucleus Accumbens. *The Journal of Neuroscience*, *24*(31), 6939–6945. doi:10.1523/JNEUROSCI.0671-04.2004

Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behavioral and Neural Biology*, *48*(1), 138–149.

French, S. J., & Totterdell, S. (2002). Hippocampal and prefrontal cortical inputs monosynaptically converge with individual projection neurons of the nucleus accumbens. *The Journal of Comparative Neurology*, *446*(2), 151–165. doi:10.1002/cne.10191

French, S. J., Hailstone, J. C., & Totterdell, S. (2003). Basolateral amygdala efferents to the ventral subiculum preferentially innervate pyramidal cell dendritic spines. *Brain*

Research, *981*(1-2), 160–167.

Fuchs, R. A., Branham, R. K., & See, R. E. (2006). Different Neural Substrates Mediate Cocaine Seeking after Abstinence versus Extinction Training: A Critical Role for the Dorsolateral Caudate-Putamen. *The Journal of Neuroscience*, *26*(13), 3584–3588. doi:10.1523/JNEUROSCI.5146-05.2006

Fuchs, R. A., Evans, K. A., Parker, M. P., & See, R. E. (2004). Differential involvement of orbitofrontal cortex subregions in conditioned cue-induced and cocaine-primed reinstatement of cocaine seeking in rats. The Journal of Neuroscience, 24(29), 6600–6610. doi:10.1523/JNEUROSCI.1924-04.2004

Fuchs, R. A., & See, R. E. (2002). Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. *Psychopharmacology, 160*, 425–433. doi: 10.1007/s00213-001-0997-7

Fulton, S. Woodside, B, Shizgal, P. (2000). Modulation of Brain Reward Circuitry by Leptin. *Science*, *287*(5450), 125–128. doi:10.1126/science.287.5450.125

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., Maratos-Flier, E., et al. (2006). Leptin Regulation of the Mesoaccumbens Dopamine Pathway. *Neuron*, *51*(6), 811–822. doi:10.1016/j.neuron.2006.09.006

Gass, J. T., & Olive, M. F. (2008). Glutamatergic substrates of drug addiction and alcoholism. *Biochemical Pharmacology*, *75*(1), 218–265. doi:10.1016/j.bcp.2007.06.039

Geisler, S., Derst, C., Veh, R. W., & Zahm, D. S. (2007). Glutamatergic afferents of the ventral tegmental area in the rat. *The Journal of Neuroscience*, *27*(21), 5730–5743.

Ghasemzadeh, M. B., Nelson, L. C., Lu, X. Y., & Kalivas, P. W. (1999).

Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. *Journal of Neurochemistry*, *72*(1), 157–165.

Goeders, N., & Smith, J. E. (1986). Reinforcing Properties of Cocaine in the Medial Prefrontal Cortex: Primary Action on Presynaptic Dopaminergic Terminals. *Pharmacology Biochemistry & Behavior, 25*, 191-195.

Gossop, M., Stewart, D., Browne, N., & Marsden, J. (2002). Factors associated with abstinence, lapse or relapse to heroin use after residential treatment: protective effect of coping responses. *Addiction*, *97*(10), 1259–1267.

Goto, Y., & Grace, A. A. (2005a). Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nature Neuroscience*, *8*(6), 805–812. doi:10.1038/nn1471

Goto, Y., & Grace, A. A. (2005b). Dopamine-Dependent Interactions between Limbic and Prefrontal Cortical Plasticity in the Nucleus Accumbens: Disruption by Cocaine Sensitization. *Neuron*, *47*(2), 255–266. doi:10.1016/j.neuron.2005.06.017

Goto, Y., & Grace, A. A. (2008). Limbic and cortical information processing in the nucleus accumbens. *Trends in Neurosciences*, *31*(11), 552–558. doi:10.1016/j.tins.2008.08.002

Goto, Y., & O'Donnell, P. (2001). Synchronous activity in the hippocampus and nucleus accumbens in vivo. *The Journal of Neuroscience, 21*, RC21.

Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, *41*(1), 1–24.

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of

dopaminergic neurons and control of goal-directed behaviors. *Trends in Neurosciences*, *30*(5), 220–227. doi:10.1016/j.tins.2007.03.003

Groc, L., Choquet, D., & Chaouloff, F. (2008). The stress hormone corticosterone conditions AMPAR surface trafficking and synaptic potentiation. *Nature Neuroscience*, *11*(8), 868–870. doi:10.1038/nn.2150

Groenewegen, H. J., Zee, der, E., & Kortschot, Te, A. (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience, 23*(1), 103–120.

Grüsser, S. M., Mörsen, C. P., Wölfling, K., & Flor, H. (2007). The Relationship of Stress, Coping, Effect Expectancies and Craving. *European Addiction Research*, *13*(1), 31–38.

Guo, Y., Wang, H.-L., Xiang, X.-H., & Zhao, Y. (2009). The role of glutamate and its receptors in mesocorticolimbic dopaminergic regions in opioid addiction. *Neuroscience and Biobehavioral Reviews*, *33*(6), 864–873. doi:10.1016/j.neubiorev.2009.02.005

Gulley, J. M., & Stanis, J. J. (2010). Adaptations in medial prefrontal cortex function associated with amphetamine-induced behavioral sensitization. Neuroscience, 166(2), 615–624. doi:10.1016/j.neuroscience.2009.12.044

Hagan, M. M., Havel, P. J., Seeley, R. J., Woods, S. C., Ekhator, N. N., Baker, D. G., Hill, K. K., et al. (1999). Cerebrospinal fluid and plasma leptin measurements: covariability with dopamine and cortisol in fasting humans. *Journal of Clinical Endocrinology & Metabolism*, *84*(10), 3579–3585.

Haney, M., Maccari, S., Le Moal, M., Simon, H., & Piazza, P. V. (1995). Social stress increases the acquisition of cocaine self-administration in male and female rats. *Brain Research*, *698*(1-2), 46–52.

Hansel, C., & Linden, D. (2001). Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nature Neuroscience, 4*(5), 467-475.

Harris, G. C., & Aston-Jones, G. (2003). Critical Role for Ventral Tegmental Glutamate in Preference for a Cocaine-Conditioned Environment. *Neuropsychopharmacology*, *28*(1), 73–76. doi:10.1038/sj.npp.1300011

Harris, G. C., Wimmer, M., Byrne, R., & Aston-Jones, G. (2004). Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neuroscience*, *129*(3), 841–847.

Hayashi, Y., Shi, S.-H., Esteban, J., Picini, A., Poncer, J.-C., & Malinow, R. (2000). Driving AMPA Receptors into Synapses by LTP and CaMKII: Requirement for GluR1 and PDZ Domain Interaction. *Science*, *287*(5461), 2262–2267. doi:10.1126/science.287.5461.2262

Hayes, R. J., Vorel, S. R., Spector, J., Liu, X., & Gardner, E. L. (2003). Electrical and chemical stimulation of the basolateral complex of the amygdala reinstates cocaine-seeking behavior in the rat. *Psychopharmacology, 168*(1-2), 75–83. doi:10.1007/s00213-002-1328-3

Headley, P. M., & Grillner, S. (1990). Excitatory amino acids and synaptic transmission: the evidence for a physiological function. *Trends in Pharmacological Sciences*, *11*(5), 205–211.

Health Canada (2005). Canadian Addiction Survey (CAS) Detailed Report, 1–101.

Hemby, S. E., Co, C., Koves, T. R., Smith, J. E., & Dworkin, S. I. (1997). Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. *Psychopharmacology*, *133*(1), 7–16.

Hemby, S. E., Tang, W., Muly, E. C., Kuhar, M. J., Howell, L., & Mash, D. C. (2005). Cocaine-induced alterations in nucleus accumbens ionotropic glutamate receptor subunits in human and non-human primates. *Journal of neurochemistry*, *95*(6), 1785–1793. doi:10.1111/j.1471-4159.2005.03517.x

Herman, J. P., & Mueller, N. K. (2006). Role of the ventral subiculum in stress integration. *Behavioural Brain Research*, *174*(2), 215–224. doi:10.1016/j.bbr.2006.05.035

Herman, J. P., Cullinan, W. E., Morano, M. I., Akil, H., & Watson, S. J. (1995). Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *Journal of Neuroendocrinology*, *7*(6), 475–482.

Herman, J. P., Dolgas, C. M., & Carlson, S. L. (1998). Ventral subiculum regulates hypothalamo-pituitary-adrenocortical and behavioural responses to cognitive stressors. *Neuroscience*, *86*(2), 449–459.

Ho, W. K., Wen, H. L., Fung, K. P., Ng, Y. H., Au, K. K., & Ma, L. (1977). Comparison of plasma hormonal levels between heroin-addicted and normal subjects. *Clinica Chimica acta; International Journal of Clinical Chemistry*, *75*(3), 415–419.

Hollmann, M., Hartley, M., & Heinemann, S. (1991). Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. *Science*, *252*(5007), 851–853.

Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience*, *10*(9), 647–658. doi:10.1038/nrn2699

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z.-W., Gao, X.-B., Thurmon, J. J., et al. (2006). Leptin Receptor Signaling in Midbrain Dopamine Neurons Regulates Feeding. *Neuron*, *51*(6), 801–810. doi:10.1016/j.neuron.2006.08.023

Hser, Y.-I. (2007). Predicting Long-Term Stable Recovery from Heroin Addiction. *Journal of Addictive Diseases*, *26*(1), 51–60. doi:10.1300/J069v26n01_07

Hunt, W. A., & Bespalec, D. A. (1974). Relapse rates after treatment for heroin addiction. *Journal of Community Psychology, 2*(1), 85-87.

Ikemoto, S. (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain Research Reviews*, *56*(1), 27–78. doi:10.1016/j.brainresrev.2007.05.004

Ikemoto, S., Glazier, B. S., Murphy, J. M., & McBride, W. J. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *The Journal of Neuroscience, 17*(21), 8550-8557.

Inglis, F., & Moghaddam, B. (1999). Dopaminergic innervation of the amygdala is highly responsive to stress. *Journal of Neurochemistry, 72*, 1088-1094.

Jaffe, J. H. (1990). Trivializing dependence. *British Journal of Addiction*, *85*, 1425–1427.

Joëls, M., Krugers, H., & Karst, H. (2007). Stress-induced changes in hippocampal function. *Progress in Brain Research*, *167*, 3–15. doi:10.1016/S0079-6123(07)67001-0.3d

Joëls, M., & Krugers, H. J. (2007). LTP after Stress: Up or Down? *Neural plasticity*, *2007*, 1–6. doi:10.1155/2007/93202

Johnson, S. W., & North, R. A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *Journal of Physiology*, *450*, 455–468.

Kalivas, P. W. (2004). Glutamate systems in cocaine addiction. *Current Opinion in Pharmacology*, *4*(1), 23–29. doi:10.1016/j.coph.2003.11.002

Kalivas, P. W. (1995). Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug and Alcohol Dependence*, *37*(2), 95–100.

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience*, *10*(8), 561–572. doi:10.1038/nrn2515

Kalivas, P. W., & Duffy, P. (1997). Dopamine regulation of extracellular glutamate in the nucleus accumbens. *Brain Research*, *761*(1), 173–177.

Kalivas, P. W., & Duffy, P. (1995). Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Research, 675*, 325-328.

Kalivas, P. W., Pierce, R. C., Cornish, J. L., & Sorg, B. A. (1998). A role for sensitization in craving and relapse in cocaine addiction. *Journal of Psychopharmacology, 12*(1), 49–53. doi:10.1177/026988119801200107

Kalivas, P. W., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology*, *168*(1-2), 44–56. doi:10.1007/s00213-003-1393-2

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: a pathology of motivation and choice. *The American Journal of Psychiatry*, *162*(8), 1403–1413.

doi:10.1176/appi.ajp.162.8.1403

Kalivas, P. W., LaLumiere, R. T., Knackstedt, L. A., & Shen, H. (2009). Glutamate transmission in addiction. *Neuropharmacology*, *56 Suppl 1*, 169–173. doi:10.1016/j.neuropharm.2008.07.011

Kanarek, R. B., & Marks-Kaufman, R. (1988). Animal models of appetitive behavior: interaction of nutritional factors and drug seeking behavior. *Current concepts in nutrition*, *16*, 1–25.

Karler, R., Calder, L. D., Chaudhry, I. A., & Turkanis, S. A. (1989). Blockade of "Reverse Tolerance" to cocaine and amphetamine by MK-801. *Life sciences*, *45*, 599–606.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schütz, G., & Joëls, M. (2005). Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(52), 19204.

Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nature Reviews Neuroscience*, *8*(11), 844–858. doi:10.1038/nrn2234

Kenny, P. J. (2011). Common cellular and molecular mechanisms in obesity and drug addiction. *Nature Reviews Neuroscience*, *12*(11), 638–651. doi:10.1038/nrn3105

Kenny, P. J., Chartoff, E., Roberto, M., Carlezon, W. A., & Markou, A. (2009). NMDA Receptors Regulate Nicotine-Enhanced Brain Reward Function and Intravenous Nicotine Self-Administration: Role of the Ventral Tegmental Area and Central Nucleus of the Amygdala. *Neuropsychopharmacology*, *34*, 266–281. doi:10.1038/npp.2008.58

Kerrigan, T., Daniel, J. W., Regan, P. L., & Cho, K. (2012). The role of neuronal calcium sensors in balancing synaptic plasticity and synaptic dysfunction. *Frontiers in Molecular Neuroscience,* 5 (57), 1-4. doi:10.3389/fnmol.2012.00057/

Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Neuroscience*, *3*, 453–462.

Kim, J. J., Foy, M. R., & Thompson, R. F. (1996). Behavioral stress modifies hippocampal plasticity through N-methyl-D-aspartate receptor activation. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(10), 4750–4753.

Kiyatkin, E. A., & Gratton, A. (1994). Electrochemical monitoring of extracellular dopamine in nucleus accumbens of rats lever-pressing for food. *Brain Research*, *652*(2), 225–234.

Khantzian, E. J. (1985). Psychotherapeutic interventions with substance abusers--the clinical context. *Journal of Substance Abuse Treatment*, *2*(2), 83–88.

Knackstedt, L. A., Moussawi, K., LaLumiere, R. T., Schwendt, M., Klugmann, M., & Kalivas, P. W. (2010). Extinction Training after Cocaine Self-Administration Induces Glutamatergic Plasticity to Inhibit Cocaine Seeking. *The Journal of Neuroscience*, *30*(23), 7984–7992. doi:10.1523/JNEUROSCI.1244-10.2010

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends in pharmacological sciences*, *13*, 177–184.

Koob, G. F. & Le Moal, M. (1997). Drug Abuse: Hedonic Homeostatic Dysregulation. *Science*, *278*(5335), 52–58. doi:10.1126/science.278.5335.52

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. *Neuron*,

21(3), 467–476.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–238. doi:10.1038/npp.2009.110

Kosten, T. A., & Ambrosio, E. (2002). HPA axis function and drug addictive behaviors: insights from studies with Lewis and Fischer 344 inbred rats. *Psychoneuroendocrinology*, *27*(1-2), 35–69.

Kourrich, S., Rothwell, P. E., Klug, J. R., & Thomas, M. J. (2007). Cocaine Experience Controls Bidirectional Synaptic Plasticity in the Nucleus Accumbens. *The Journal of Neuroscience*, *27*(30), 7921–7928. doi:10.1523/JNEUROSCI.1859-07.2007

Kiyatkin, E. A., & Gratton, A. (1994). Electrochemical monitoring of extracellular dopamine in nucleus accumbens of rats lever-pressing for food. *Brain Research*, *652*(2), 225–234.

Kreek, M. J., & Koob, G. F. (1998). Drug dependence: stress and dysregulation of brain reward pathways. *Drug and Alcohol Dependence*, *51*(1-2), 23–47.

Kupferschmidt, D. A., Tribe, E., & Erb, S. (2009). Effects of repeated yohimbine on the extinction and reinstatement of cocaine seeking. *Pharmacology, Biochemistry, and Behavior, 91*(3), 473–480. doi:10.1016/j.pbb.2008.08.026

LaLumiere, R. T., & Kalivas, P. W. (2008). Glutamate release in the nucleus accumbens core is necessary for heroin seeking. *The Journal of Neuroscience*, *28*(12), 3170–3177. doi:10.1523/JNEUROSCI.5129-07.2008

LaLumiere, R. T., Niehoff, K. E., & Kalivas, P. W. (2010). The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. *Learning & Memory*, *17*(4), 168–175. doi:10.1101/lm.1576810

Lazarus, R. & Folkman, S. (1984). Stress, Appraisal, and Coping. New York, NY: Springer Publishing Company, New York.

Leão, R. M., Cruz, F. C., & Planeta, C. S. (2009). Exposure to acute restraint stress reinstates nicotine-induced place preference in rats. *Behavioural pharmacology*, *20*(1), 109–113. doi:10.1097/FBP.0b013e3283242f41

Lecca, D., Cacciapaglia, F., Valentini, V., Acquas, E., & Chiara, G. (2007a). Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat. *Psychopharmacology*, *191*(3), 653–667. doi:10.1007/s00213-006-0496-y

Lecca, D., Valentini, V., Cacciapaglia, F., Acquas, E., & Di Chiara, G. (2007b). Reciprocal effects of response contingent and noncontingent intravenous heroin on in vivo nucleus accumbens shell versus core dopamine in the rat: a repeated sampling microdialysis study. *Psychopharmacology*, *194*(1), 103–116. doi:10.1007/s00213-007-0815-y

Ledford, C. C., Fuchs, R. A., & See, R. E. (2003). Potentiated Reinstatement of Cocaine-Seeking Behavior Following D-amphetamine Infusion into the Basolateral Amygdala. *Neuropsychopharmacology, 28*(10), 1721–1729. doi:10.1038/sj.npp.1300249

Lee, H. K., Takamiya, K., Han, J. S., Man, H., Kim, C. H., Rumbaugh, G., Yu, S., et al. (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell*, *112*(5), 631–643.

Lee, B., Tiefenbacher, S., Platt, D. M., & Spealman, R. D. (2004). Pharmacological Blockade of α2-Adrenoceptors Induces Reinstatement of Cocaine-Seeking Behavior

in Squirrel Monkeys. *Neuropsychopharmacology*, *29*(4), 686–693. doi:10.1038/sj.npp.1300391

LeDoux, J. E. (1993). Emotional memory systems in the brain. Behavioural brain research, 58(1-2), 69–79.

Legault, M., & Wise, R. A. (2001). Novelty-evoked elevations of nucleus accumbens dopamine: dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental. *European Journal of Neuroscience* 13, 819-828.

LeSage, M., Burroughs, D., Dufek, M., Keyler, D., & Pentel, P. (2004). Reinstatement of nicotine self-administration in rats by presentation of nicotine-paired stimuli, but not nicotine priming. *Pharmacology, Biochemistry, and Behavior*, *79*(3), 507–513. doi:10.1016/j.pbb.2004.09.002

Levine, M. S., Altemus, K. L., Cepeda, C., Cromwell, H. C., Crawford, C., Ariano, M. A., Drago, J., et al. (1996). Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. *The Journal of Neuroscience*, *16*(18), 5870–5882.

Levine, A. A., Guan, Z., Barco, A., Xu, S., Kandel, E. R., & Schwartz, J. H. (2005). CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(52), 19186.

Liechti, M. E., Lhuillier, L., Kaupmann, K., & Markou, A. (2007). Metabotropic Glutamate 2/3 Receptors in the Ventral Tegmental Area and the Nucleus Accumbens Shell Are Involved in Behaviors Relating to Nicotine Dependence. *The Journal of*

Neuroscience, *27*(34), 9077–9085. doi:10.1523/JNEUROSCI.1766-07.2007

Litman, G. K., Eiser, J. R., Rawson, N. S., & Oppenheim, A. N. (1977). Towards a typology of relapse: a preliminary report. *Drug and Alcohol Dependence*, *2*(3), 157–162.

Litman, G. K., Stapleton, J., Oppenheim, A. N., Peleg, M., & Jackson, P. (1984). The relationship between coping behaviours, their effectiveness and alcoholism relapse and survival. *British Journal of Addiction*, *79*(3), 283–291.

Liu, X., Jernigen, C., Gharib, M., Booth, S., Caggiula, A. R., & Sved, A. F. (2010). Effects of dopamine antagonists on drug cue-induced reinstatement of nicotine-seeking behavior in rats. *Behavioural pharmacology*, *21*(2), 153–160. doi:10.1097/FBP.0b013e328337be95

Liu, T.-L., & Liang, K. C. (2009). Posttraining infusion of cholinergic drugs into the ventral subiculum modulated memory in an inhibitory avoidance task: Interaction with the bed nucleus of the stria terminalis. *Neurobiology of learning and memory*, *91*(3), 235–242. doi:10.1016/j.nlm.2008.11.001

Liu, S. J., & Zukin, R. S. (2007). Ca2+-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends in Neurosciences*, *30*(3), 126–134. doi:10.1016/j.tins.2007.01.006

Lu, L., Ceng, X., & Huang, M (2000). Corticotropin-releasing factor receptor type 1 mediates stress-induced relapse to opiate dependence in rats. *Neuroreport*, *11*(11), 2373-2378.

Lu, L., Chen, H., Su, W., Ge, X., Yue, W., Su, F., & Ma, L. (2005). Role of withdrawal in reinstatement of morphine-conditioned place preference. *Psychopharmacology*,

181(1), 90–100. doi:10.1007/s00213-005-2207-5

Lu, L., Dempsey, J., Shaham, Y., & Hope, B. T. (2005). Differential long-term neuroadaptations of glutamate receptors in the basolateral and central amygdala after withdrawal from cocaine self-administration in rats. *Journal of Neurochemistry*, *94*(1), 161–168. doi:10.1111/j.1471-4159.2005.03178.x

Lynch, W. J., & Taylor, J. R. (2005). Persistent changes in motivation to self-administer cocaine following modulation of cyclic AMP-dependent protein kinase A (PKA) activity in the nucleus accumbens. *European Journal of Neuroscience*, *22*(5), 1214–1220. doi:10.1111/j.1460-9568.2005.04305.x

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *Annual Review of Neuroscience*, *14*(1), 379–397.

Maldonado-Irizarry, C. S., Swanson, C. J., & Kelley, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *The Journal of Neuroscience*, *15*(10), 6779–6788.

Malenka, R. (1994). Synaptic plasticity in the hippocampus: LTP and LTD. *Cell*, *78*, 535-538.

Malenka, R. C., & Nicoll, R. (1999). Long-term potentiation--a decade of progress? *Science*, *285*(5435), 1870.

Malinow, R., & Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience*, *25*, 103–126. doi:10.1146/annurev.neuro.25.112701.142758

Mammen, A. L., Kameyama, K., Roche, K. W., & Huganir, R. L. (1997).

Phosphorylation of the α-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *Journal of Biological Chemistry*, *272*(51), 32528–32533.

Manzardo, A. M., Del Rio, J. A., Stein, L., & Belluzzi, J. D. (2001). Rats choose cocaine over dopamine agonists in a two-lever self-administration preference test. *Pharmacology, Biochemistry, and Behavior*, *70*(2-3), 257–265.

Maren, S., & Baudry, M. (1995). Properties and mechanisms of long-term synaptic plasticity in the mammalian brain: relationships to learning and memory. *Neurobiology of learning and memory*, *63*(1), 1–18. doi:10.1006/nlme.1995.1001

Marinelli, M., Le Moal, M., & Piazza, P. V. (1996). Acute pharmacological blockade of corticosterone secretion reverses food restriction-induced sensitization of the locomotor response to cocaine. *Brain Research*, *724*(2), 251–255.

Marinelli, M., Piazza, P. V., Deroche, V., Maccari, S., Le Moal, M., & Simon, H. (1994). Corticosterone circadian secretion differentially facilitates dopamine-mediated psychomotor effect of cocaine and morphine. *The Journal of Neuroscience*, *14*(5), 2724–2731.

Martin, M., Chen, B. T., Hopf, F. W., Bowers, M. S., & Bonci, A. (2006). Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nature Neuroscience*, *9*(7), 868–869. doi:10.1038/nn1713

Martin, S. J., Grimwood, P. D., & Morris, R. G. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annual Review of Neuroscience*, *23*, 649–711. doi:10.1146/annurev.neuro.23.1.649

Matheny, K. B., & Weatherman, K. E. (1998). Predictors of smoking cessation and

maintenance. *Journal of Clinical Psychology*, *54*(2), 223–235.

McEntee, W. J., & Crook, T. H. (1993). Glutamate: its role in learning, memory, and the aging brain. *Psychopharmacology*, *111*(4), 391–401.

McCullough, L. D., & Salamone, J. D. (1992). Involvement of nucleus accumbens dopamine in the motor activity induced by periodic food presentation: a microdialysis and behavioral study. *Brain Research*, *592*(1), 29–36.

McDougle, C. J., Black, J. E., Malison, R. T., Zimmermann, R. C., Kosten, T. R., Heninger, G. R., & Price, L. H. (1994). Noradrenergic dysregulation during discontinuation of cocaine use in addicts. *Archives of General Psychiatry*, *51*(9), 713.

McFarland, K. & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *The Journal of Neuroscience*.

McFarland, K., Davidge, S. B., Lapish, C. C., & Kalivas, P. W. (2004). Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *The Journal of Neuroscience*, *24*(7), 1551–1560. doi:10.1523/JNEUROSCI.4177-03.2004

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *The Journal of Neuroscience, 23*(8), 3531–3537.

McLaughlin, J., & See, R. E. (2003). Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology*, *168*(1-2), 57–65. doi:10.1007/s00213-002-1196-x

McKay, J., Franklin, T., Patapis, N., & Lynch, K. (2006). Conceptual, methodological,

and analytical issues in the study of relapse. *Clinical Psychology Review*, *26*(2), 109–127. doi:10.1016/j.cpr.2005.11.002

McKay, J., Rutherford, M. J., Alterman, A. I., & Cacciola, J. S. (1996). Development of the cocaine relapse interview: an initial report. *Addiction*, *91*(4), 535–548.

Mead, A. N., Morris, H. V., Dixon, C. I., Rulten, S. L., Mayne, L. V., Zamanillo, D., & Stephens, D. N. (2006). AMPA receptor GluR2, but not GluR1, subunit deletion impairs emotional response conditioning in mice. *Behavioral Neuroscience*, *120*(2), 241–248. doi:10.1037/0735-7044.120.2.241

Meil, W. M., & See, R. E. (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behavioural Brain Research, 87*, 139-148.

Mello, N. K., & Mendelson, J. H. (1997). Cocaine's effects on neuroendocrine systems: clinical and preclinical studies. *Pharmacology, Biochemistry, and Behavior*, *57*(3), 571–599.

Miczek, K. A., & Mutschler, N. H. (1996). Activational effects of social stress on IV cocaine self-administration in rats. *Psychopharmacology*, *128*(3), 256–264.

Miller, W. R., Westerberg, V. S., Harris, R. J., & Tonigan, J. S. (1996). What predicts relapse? Prospective testing of antecedent models. *Addiction*, *91 Suppl*, S155–72.

Miller, L. (2010). Analyzing gels and western blots with ImageJ. Retrieved from: http://lukemiller.org/index.php/2010/11/analyzing-gels-and-western-blots-with-image-j/

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Progress in*

neurobiology.

Moult, P. R., Milojkovic, B., & Harvey, J. (2009). Leptin reverses long-term potentiation at hippocampal CA1 synapses. *Journal of Neurochemistry*, *108*(3), 685–696. doi:10.1111/j.1471-4159.2008.05810.x

Mueller, D., & Stewart, J. (2000). Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behavioural Brain Research*, *115*(1), 39–47.

Murray, F., Harrison, N. J., Grimwood, S., Bristow, L. J., & Hutson, P. H. (2007). Nucleus accumbens NMDA receptor subunit expression and function is enhanced in morphine-dependent rats. *European Journal of Pharmacology*, *562*(3), 191–197. doi:10.1016/j.ejphar.2007.01.027

Nair, S. G., Navarre, B. M., Cifani, C., Pickens, C. L., Bossert, J. M., & Shaham, Y. (2011). Role of Dorsal Medial Prefrontal Cortex Dopamine D1-Family Receptors in Relapse to High-Fat Food Seeking Induced by the Anxiogenic Drug Yohimbine. *Neuropsychopharmacology*, *36*, 497–510. doi:10.1038/npp.2010.181

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience*, *2*(2), 119–128. doi:10.1038/35053570

Nettles, K. W., Pesold, C., & Goldman, M. B. (2000). Influence of the ventral hippocampal formation on plasma vasopressin, hypothalamic-pituitary-adrenal axis, and behavioral responses to novel acoustic stress. *Brain Research*, *858*(1), 181–190.

Nicola, S. M., Surmeier, J., & Malenka, R. C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annual Review of Neuroscience*, *23*, 185–215. doi:10.1146/annurev.neuro.23.1.185

O'Brien, C. P. (2005). Anticraving medications for relapse prevention: a possible new class of psychoactive medications. *American Journal of Psychiatry*, *162*(8), 1423–1431.

O'Brien, C. P., & McLellan, A. T. (1996). Myths about the treatment of addiction. *Lancet*, *347*(8996), 237–240.

O'Dell, L., Sussman, A. N., Meyer, K. L., & Neisewander, J. L. (1999). Behavioral effects of psychomotor stimulant infusions into amygdaloid nuclei. *Neuropsychopharmacology*, 20(6), 591–602.

Omelchenko, N., & Sesack, S. R. (2007). Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. *Neuroscience*, *146*(3), 1259–1274.

Oomura, Y., Hori, N., Shiraishi, T., Fukunaga, K., Takeda, H., Tsuji, M., Matsumiya, T., et al. (2006). Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. *Peptides*, *27*(11), 2738–2749. doi:10.1016/j.peptides.2006.07.001

Park, W.-K., Bari, A. A., Jey, A. R., Anderson, S. M., Spealman, R. D., Rowlett, J. K., & Pierce, R. C. (2002). Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *Journal of Neuroscience*, *22*(7), 2916–2925.

Parliament of Canada. Library of Parliament. Research Branch. Substance Abuse and Public Policy by Nancy Miller Chenier. Rev. Ed. Ottawa: Supply and Services Canada. 2001. Current Issue Review 94-2E. Retrieved Feb 1, 2011 from:

http://www.parl.gc.ca/Content/LOP/researchpublications/942-e.htm

Patterson, T. A., Brot, M. D., Zavosh, A., Schenk, J. O., Szot, P., & Figlewicz, D. P. (1998). Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology*, *68*(1), 11–20.

Paxios, G., & Watson, C. (2005). The Rat Brain in Stereotaxic Coordinates. Burlington, MA: Elsevier Academic Press.

Perrine, S. A., Miller, J. S., & Unterwald, E. M. (2008). Cocaine regulates protein kinase B and glycogen synthase kinase-3 activity in selective regions of rat brain. *Journal of Neurochemistry*, *107*(2), 570–577. doi:10.1111/j.1471-4159.2008.05632.x

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning & Memory*, *16*(5), 279–288. doi:10.1101/lm.1041309

Peters, J., LaLumiere, R. T., & Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of Neuroscience*, *28*(23), 6046–6053. doi:10.1523/JNEUROSCI.1045-08.2008

Piazza, P. V., & Le Moal, M. (1998). The role of stress in drug self-administration. *Trends in Pharmacological sciences*, *19*(2), 67–74.

Piazza, P. V., & Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annual Review of Pharmacology and Toxicology*, *36*, 359–378. doi:10.1146/annurev.pa.36.040196.002043

Piazza, P. V., Maccari, S., Deminière, J. M., Le Moal, M., Mormède, P., & Simon, H. (1991). Corticosterone levels determine individual vulnerability to amphetamine self-

administration. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(6), 2088–2092.

Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Biobehavioral Reviews*, *30*(2), 215–238. doi:10.1016/j.neubiorev.2005.04.016

Pierce, R. C., Bell, K., Duffy, P., & Kalivas, P. W. (1996). Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *The Journal of Neuroscience*, *16*(4), 1550–1560.

Ping, A., Xi, J., Prasad, B. M., Wang, M.-H., & Kruzich, P. J. (2008). Contributions of nucleus accumbens core and shell GluR1 containing AMPA receptors in AMPA- and cocaine-primed reinstatement of cocaine-seeking behavior. *Brain Research*, *1215*, 173–182. doi:10.1016/j.brainres.2008.03.088

Pinheiro, P., & Mulle, C. (2006). Kainate receptors. *Cell and Tissue Research*, *326*(2), 457–482. doi:10.1007/s00441-006-0265-6

Plant, K., Pelkey, K. A., Bortolotto, Z. A., Morita, D., Terashima, A., McBain, C. J., Collingridge, G. L., et al. (2006). Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nature Neuroscience*, *9*(5), 602–604. doi:10.1038/nn1678

Pulvirenti, L., Maldonado-Lopez, R., & Koob, G. F. (1992). NMDA receptors in the nucleus accumbens modulate intravenous cocaine but not heroin self-administration in the rat. *Brain Research*, *594*(2), 327–330.

Redila, V. A., & Chavkin, C. (2008). Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology*, *200*(1), 59–70.

doi:10.1007/s00213-008-1122-y

Rehm, J., Ballunas, D., Brochu, Fisher, B., Gnam, W., Patra, J., Popova, S., et al. (2006). The Costs of Substance Abuse in Canada 2002. Ottawa: Canadian Centre on Substance Abuse. Available online: http://www.ccsa.ca

Reissner, K. J., Uys, J. D., Schwacke, J. H., Comte-Walters, S., Rutherford-Bethard, J. L., Dunn, T. E., Blumer, J. B., et al. (2011). AKAP Signaling in Reinstated Cocaine Seeking Revealed by iTRAQ Proteomic Analysis. *The Journal of Neuroscience*, *31*(15), 5648–5658. doi:10.1523/JNEUROSCI.3452-10.2011\

Reynolds, J. L., Mahajan, S. D., Sykes, D., & Nair, M. P. N. (2006). Heroin-induces differential protein expression by normal human astrocytes (NHA). *American journal of infectious diseases*, *2*(2), 49.

Riegert, C., Galani, R., Heilig, S., Lazarus, C., Cosquer, B., & Cassel, J.-C. (2004). Electrolytic lesions of the ventral subiculum weakly alter spatial memory but potentiate amphetamine-induced locomotion. *Behavioural Brain Research*, *152*(1), 23–34. doi:10.1016/j.bbr.2003.09.011

Rivier, C. L., & Plotsky, P. M. (1986). Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. *Annual Review of Physiology*, *48*, 475–494. doi:10.1146/annurev.ph.48.030186.002355

Rizos, Z., Ovari, J., & Leri, F. (2005). Reconditioning of heroin place preference requires the basolateral amygdala. *Pharmacology, Biochemistry and Behahvior, 82*, 300-305.

Robinson, T. E., & Berridge, K. C. (2003). Addiction. *Annual Review of Psychology*, *54*(1), 25–53. doi:10.1146/annurev.psych.54.101601.145237

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an

incentive-sensitization theory of addiction. *Brain Research Reviews*, *18*(3), 247–291.

Robinson, T. E., & Berridge, K. C. (2000). The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction*, *95*(8s2), 91–117. doi:10.1046/j.1360-0443.95.8s2.19.x

Robinson, T. E., Gorny, G., Savage, V. R., & Kolb, B. (2002). Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. *Synapse*, *46*(4), 271–279. doi:10.1002/syn.10146

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology*, *47*, 33–46. doi:10.1016/j.neuropharm.2004.06.025

Rogers, J. L., Ghee, S., & See, R. E. (2008). The neural circuitry underlying reinstatement of heroin-seeking behavior in an animal model of relapse. *Neuroscience, 151*(2), 579–588. doi:10.1016/j.neuroscience.2007.10.012

Ronald, G., Connoly, D. & Bédard, H. (2004). 2002 Canadian Community Health Survey: Mental Health and Well-being (Catalogue number 82-617-XIE). Retrieved February 1, 2011 from Statistics Canada: http://www5.statcan.gc.ca/bsolc/olc-cel/olc-cel?catno=82-617-XIE&lang=eng

Rothwell, P. E., Kourrich, S., & Thomas, M. J. (2011). Environmental novelty causes stress-like adaptations at nucleus accumbens synapses: implications for studying addiction-related plasticity. *Neuropharmacology*, *61*(7), 1152–1159. doi:10.1016/j.neuropharm.2011.01.038

Saal, D., Dong, Y., & Bonci, A. (2003). Drugs of abuse and stress trigger a common

synaptic adaptation in dopamine neurons. *Neuron*, 37, 577-582.

Sanchez, C. J., Bailie, T. M., Wu, W.-R., Li, N., & Sorg, B. A. (2003). Manipulation of dopamine d1-like receptor activation in the rat medial prefrontal cortex alters stress- and cocaine-induced reinstatement of conditioned place preference behavior. *Neuroscience*, *119*(2), 497–505.

Sandi, C., & Pinelo-Nava, M. T. (2007). Stress and Memory: Behavioral Effects and Neurobiological Mechanisms. Neural plasticity, 2007, 1–20. doi:10.1155/2007/78970

Santos, S. D., Carvalho, A. L., Caldeira, M. V., & Duarte, C. B. (2009). Regulation of AMPA receptors and synaptic plasticity. *Neuroscience*, *158*(1), 105–125. doi:10.1016/j.neuroscience.2008.02.037

Sastre, M., Ventayol, P., & García-Sevilla, J. A. (1996). Decreased density of I2-imidazoline receptors in the postmortem brains of heroin addicts. *Neuroreport*, *7*(2), 509.

Scott, L., Kruse, M. S., Forssberg, H., Brismar, H., Greengard, P., & Aperia, A. (2002). Selective up-regulation of dopamine D1 receptors in dendritic spines by NMDA receptor activation. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(3), 1661–1664. doi:10.1073/pnas.032654599

Schluger, J. H., Ho, A., Borg, L., Porter, M., Maniar, S., Gunduz, M., Perret, G., et al. (1998). Nalmefene causes greater hypothalamic-pituitary-adrenal axis activation than naloxone in normal volunteers: implications for the treatment of alcoholism. *Alcoholism, clinical and experimental research*, *22*(7), 1430–1436.

Schmidt, H. D., & Pierce, R. C. (2006). Cooperative activation of D1-like and D2-like

dopamine receptors in the nucleus accumbens shell is required for the reinstatement of cocaine-seeking behavior in the rat. *Neuroscience, 142*, 451–461. doi:10.1016/j.neuroscience.2006.06.004

Schultz, W. (2001). Reward Signaling by Dopamine Neurons. *The Neuroscientist*, *7*(4), 293–302. doi:10.1177/107385840100700406

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science*, *275*(5306), 1593–1599.

See, R. E. (2009). Dopamine D1 receptor antagonism in the prelimbic cortex blocks the reinstatement of heroin-seeking in an animal model of relapse. *The International Journal of Neuropsychopharmacology, 12*(3), 431–436. doi:10.1017/S1461145709000054

Self, D. W. (2004). Extinction Training Regulates Neuroadaptive Responses to Withdrawal from Chronic Cocaine Self-Administration. *Learning & Memory*, *11*(5), 648–657. doi:10.1101/lm.81404

Self, D. W., & Stein, L. (1992). The D1 agonists SKF 82958 and SKF 77434 are self-administered by rats. *Brain Research*, *582*(2), 349–352.

Self, D. W., Belluzzi, J. D., Kossuth, S., & Stein, L. (1996). Self-administration of the D1 agonist SKF 82958 is mediated by D1, not D2, receptors. *Psychopharmacology*, *123*(4), 303–306.

Sepehrizadeh, Z., Bahrololoumi Shapourabadi, M., Ahmadi, S., Hashemi Bozchlou, S., Zarrindast, M. R., & Sahebgharani, M. (2008a). Decreased AMPA GLuR2, but not GLuR3, mRNA expression in rat amygdala and dorsal hippocampus following morphine-induced behavioural sensitization. *Clinical and Experimental*

Pharmacology and Physiology, *35*(11), 1321–1330. doi:10.1111/j.1440-1681.2008.05004.x

Sepehrizadeh, Z., Sahebgharani, M., Ahmadi, S., Shapourabadi, M. B., Bozchlou, S. H., & Zarrindast, M. R. (2008b). Morphine-Induced Behavioral Sensitization Increased the mRNA Expression of NMDA Receptor Subunits in the Rat Amygdala. *Pharmacology*, *81*(4), 333–343. doi:10.1159/000122959

Sesack, S. R., & Pickel, V. M. (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *Journal of Comparitive Neurology*, *320*, 145–160.

Sesack, S. R., Carr, D. B., Omelchenko, N., & Pinto, A. (2003). Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. *Annals of the New York Academy of Sciences*, *1003*, 36–52.

Sesack, S. R., & Grace, A. A. (2010). Cortico-Basal Ganglia Reward Network: Microcircuitry. *Neuropsychopharmacology*, *35*(1), 27–47. doi:10.1038/npp.2009.93

Setlow, B. (1997). The nucleus accumbens and learning and memory. *Journal of Neuroscience Research*, *49*(5), 515–521.

Shaham, Y., & Stewart, J. (1994). Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. *Psychopharmacology*, *114*(3), 523–527.

Shaham, Y., & Stewart, J. (1995). Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. *Psychopharmacology*, *119*(3), 334–341.

Shaham, Y., & Stewart, J. (1996). Effects of opioid and dopamine receptor antagonists on

relapse induced by stress and re-exposure to heroin in rats. *Psychopharmacology*, *125*, 385–391.

Shaham, Y., Funk, D., Erb, S., Brown, T. J., Walker, C. D., & Stewart, J. (1997). Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. *The Journal of Neuroscience*, *17*(7), 2605–2614.

Shaham, Y., Shalev, U., Lu, L., de Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*, *168*(1-2), 3–20. doi:10.1007/s00213-002-1224-x

Shalev, U., Erb, S., & Shaham, Y. (2010). Role of CRF and other neuropeptides in stress-induced reinstatement of drug seeking. *Brain Research*, *1314*, 15–28. doi:10.1016/j.brainres.2009.07.028

Shalev, U., Finnie, P. S., Quinn, T., Tobin, S., & Wahi, P. (2006). A role for corticotropin-releasing factor, but not corticosterone, in acute food-deprivation-induced reinstatement of heroin seeking in rats. *Psychopharmacology*, *187*(3), 376–384. doi:10.1007/s00213-006-0427-y

Shalev, U., Highfield, D., Yap, J., & Shaham, Y. (2000). Stress and relapse to drug seeking in rats: studies on the generality of the effect. *Psychopharmacology*, *150*(3), 337–346.

Shalev, U., Marinelli, M., Baumann, M. H., Piazza, P.-V., & Shaham, Y. (2003). The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. *Psychopharmacology*, *168*(1-2), 170–176. doi:10.1007/s00213-002-1200-5

Shalev, U., Yap, J., & Shaham, Y. (2001). Leptin Attenuates Acute Food Deprivation-Induced Relapse to Heroin Seeking. *The Journal of Neuroscience*, *21*(RC129).

Shabat-Simon, M., Levy, D., Amir, A., Rehavi, M., & Zangen, A. (2008). Dissociation between Rewarding and Psychomotor Effects of Opiates: Differential Roles for Glutamate Receptors within Anterior and Posterior Portions of the Ventral Tegmental Area. *The Journal of Neuroscience*, *28*(34), 8406–8416. doi:10.1523/JNEUROSCI.1958-08.2008

Shepard, J. D., Bossert, J. M., Liu, S. Y., & Shaham, Y. (2004). The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biological psychiatry*, *55*(11), 1082–1089. doi:10.1016/j.biopsych.2004.02.032

Shors, T. J., & Matzel, L. D. (1997). Long-term potentiation: what's learning got to do with it? *Behavioral and Brain Sciences*, *20*(4), 597–614.

Shors, T. J., Levine, S., & Thompson, R. F. (1990). Effect of Adrenalectomy and Demedullation on the Stress-Induced Impairment of Long-Term Potentiation. *Neuroendocrinology*, *51*(1), 70–75.

Shors, T. J., Seib, T. B., Levine, S., & Thompson, R. F. (1989). Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science*, *244*(4901), 224–226.

Sinha, R. (2001). How does stress increase risk of drug abuse and relapse? *Psychopharmacology*, *158*(4), 343–359.

Sinha, R. (2009). Modeling stress and drug craving in the laboratory: implications for addiction treatment development. *Addiction Biology*, *14*(1), 84–98. doi:10.1111/j.1369-1600.2008.00134.x

Sinha, R., Lacadie, C., Skudlarski, P., Fulbright, R. K., Rounsaville, B. J., Kosten, T. R., & Wexler, B. E. (2005). Neural activity associated with stress-induced cocaine

craving: a functional magnetic resonance imaging study. *Psychopharmacology*, *183*(2), 171–180. doi:10.1007/s00213-005-0147-8

Steketee, J. D., & Kalivas, P. W. (2011). Drug Wanting: Behavioral Sensitization and Relapse to Drug-Seeking Behavior. *Pharmacological Reviews*, *63*(2), 348–365. doi:10.1124/pr.109.001933

Sokoloff, P., & Schwartz, J. C. (1995). Novel dopamine receptors half a decade later., *16*(8), 270–275.

Spanagel, R., Herz, A., & Shippenberg, T. S. (1990). The Effects of Opioid Peptides on Dopamine Release in the Nucleus Accumbens: An In Vivo Microdialysis Study. *Journal of Neurochemistry*, *55*(5), 1734–1740. doi:10.1111/j.1471-4159.1990.tb04963.x

Spanagel, R., & Kiefer, F. (2008). Drugs for relapse prevention of alcoholism: ten years of progress. *Trends in Pharmacological Sciences*, *29*(3), 109–115. doi:10.1016/j.tips.2007.12.005

Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: past and current status. *Trends in Neurosciences*, *22*(11), 521–527.

Stinus, L., Cador, M., Zorrilla, E. P., & Koob, G. F. (2005). Buprenorphine and a CRF1 Antagonist Block the Acquisition of Opiate Withdrawal-Induced Conditioned Place Aversion in Rats. *Neuropsychopharmacology*, *30*(1), 90–98. doi:10.1038/sj.npp.1300487

Stevenson, C. W., & Gratton, A. (2004). Basolateral amygdala dopamine receptor

antagonism modulates initial reactivity to but not habituation of the acoustic startle response. *Behavioural Brain Research, 153*(2), 383–387. doi:10.1016/j.bbr.2003.12.014

Stewart, J., & Vezina, P. (1988). A comparison of the effects of intra-accumbens injections of amphetamine and morphine on reinstatement of heroin intravenous self-administration behavior. *Brain Research*, *457*(2), 287–294.

Stine, S. M., Southwick, S. M., Petrakis, I. L., Kosten, T. R., Charney, D. S., & Krystal, J. H. (2002). Yohimbine-induced withdrawal and anxiety symptoms in opioid-dependent patients. *Biological psychiatry*, *51*(8), 642–651.

Stratford, T., & Kelley, A. (1997). GABA in the Nucleus Accumbens Shell Participates in the Central Regulation of Feeding Behavior. *The Journal of Neuroscience*.

Suaud-Chagny, M. F., Chergui, K., Chouvet, G., & Gonon, F. (1992). Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neuroscience*, *49*(1), 63–72.

Sun, W., & Rebec, G. V. (2003). Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. *The Journal of Neuroscience*, *23*(32), 10258–10264.

Sweatt, J. D. (2001). The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry*, *76*(1), 1–10.

Sweet, K. L., & Neill, D. B. (1999). Amphetamine injections into the nucleus accumbens enhance the reward of stimulation of the subiculum. *Annals of the New York*

Academy of Sciences, *877*, 828–830.

Terry, P., & Katz, J. L. (1992). Differential antagonism of the effects of dopamine D1-receptor agonists on feeding behavior in the rat. *Psychopharmacology*, *109*(4), 403–409.

Tobin, S., Newman, A. H., Quinn, T., & Shalev, U. (2009). A role for dopamine D1-like receptors in acute food deprivation-induced reinstatement of heroin seeking in rats. *International Journal of Neuropsychopharmacology*, *12*, 217–226. doi:10.1017/S1461145708008778

Taepavarapruk, P., & Phillips, A. G. (2003). Neurochemical correlates of relapse to d-amphetamine self-administration by rats induced by stimulation of the ventral subiculum. *Psychopharmacology*, *168*(1-2), 99–108. doi:10.1007/s00213-002-1337-2

Thomas, M. J., Beurrier, C., Bonci, A., & Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nature Neuroscience*, *4*(12), 1217–1223.

Ungless, M., Whistler, J., Malenka, R., & Bonci, A. (2001). Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Letters to Nature*, *411*, 583–587.

Van den Oever, M. C., Goriounova, N. A., Wan Li, K., van der Schors, R. C., Binnekade, R., Schoffelmeer, A. N. M., Mansvelder, H. D., et al. (2008). Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. *Nature Neuroscience*. doi:10.1038/nn.2165

Vescovi, P. P., Coiro, V., Volpi, R., & Passeri, M. (1992). Diurnal variations in plasma ACTH, cortisol and beta-endorphin levels in cocaine addicts. *Hormone Research*,

37(6), 221–224.

Vandenberghe, W., Robberecht, W., & Brorson, J. R. (2000). AMPA receptor calcium permeability, GluR2 expression, and selective motoneuron vulnerability. *The Journal of Neuroscience*, *20*(1), 123–132.

Verty, A. N. A., McGregor, I. S., & Mallet, P. E. (2004). The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Δ9-tetrahydrocannabinol. *Brain Research*, *1020*(1-2), 188–195. doi:10.1016/j.brainres.2004.06.033

Vigh, J., Lenard, L., & Fekete, E. (1999). Bombesin microinjection into the basolateral amygdala influences feeding behavior in the rat. *Brain Research*, *847*(2), 253–261.

Vorel, S. R., Liu, X., Hayes, R. J., Spector, J., & Gardner, E. L. (2001). Relapse to Cocaine-Seeking After Hippocampal Theta Burst Stimulation. *Science, 292*(5519), 1175–1178. doi:10.1126/science.1058043

Wallace, B. C. (1989). Psychological and environmental determinants of relapse in crack cocaine smokers. *Journal of Substance Abuse Treatment*, *6*(2), 95–106.

Wang, B., Shaham, Y., Zitman, D., Azari, S., Wise, R. A., & You, Z.-B. (2005). Cocaine Experience Establishes Control of Midbrain Glutamate and Dopamine by Corticotropin-Releasing Factor: A Role in Stress-Induced Relapse to Drug Seeking. *The Journal of Neuroscience*, *25*(22), 5389–5396. doi:10.1523/JNEUROSCI.0955-05.2005

Wang, H. L., Wayner, M. J., Chai, C. Y., & Lee, E. H. (1998). Corticotrophin-releasing factor produces a long-lasting enhancement of synaptic efficacy in the hippocampus. *The European Journal of Neuroscience*, 10(11), 3428–3437.

Wang, Z., Xu, N.-L., Wu, C.-P., Duan, S., & Poo, M.-M. (2003). Bidirectional changes in

spatial dendritic integration accompanying long-term synaptic modifications. *Neuron*, *37*(3), 463–472.

Westerink, B. H., Kwint, H. F., & de Vries, J. B. (1996). The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *The Journal of Neuroscience 16*(8), 2605–2611.

Widom, C. S., Weiler, B. L., & Cottler, L. B. (1999). Childhood victimization and drug abuse: A comparison of prospective and retrospective findings. *Journal of Consulting and Clinical Psychology*, *67*(6), 867.

Will, M. J., Watkins, L. R., & Maier, S. F. (1998). Uncontrollable stress potentiates morphine's rewarding properties. *Pharmacology, Biochemistry, and Behavior*, *60*(3), 655–664.

Wills, T., McNamara, G., Vaccaro, D., & Hirky, A. E. (1996). Escalated substance use: A longitudinal grouping analysis from early to middle adolescence. *Journal of Abnormal Psychology*, *105*(2), 166–180.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, *5*(6), 483–494. doi:10.1038/nrn1406

Wise, R. A. (2009). Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and addiction. *Trends in Neurosciences*, *32*(10), 517–524. doi:10.1016/j.tins.2009.06.004

Wise, R. A. (2009). Ventral tegmental glutamate: A role in stress-, cue-, and cocaine-induced reinstatement of cocaine-seeking. *Neuropharmacology, 56*, 174-176.

Wise, R. A., Leone, P., Riverst, R., & Leeb, K. (1995). Elevations of nucleus accumbens

dopamine and DOPAC levels during intravenous heroin self-administration. *Synapse 21*, 140-148.

Wise, R. A., Wang, B., & You, Z.-B. (2008). Cocaine Serves as a Peripheral Interoceptive Conditioned Stimulus for Central Glutamate and Dopamine Release. *PLoS ONE*, *3*(8), e2846. doi:10.1371/journal.pone.0002846.g004

Wolf, M. E. (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in Neurobiology*, *54*(6), 679–720.

Wolf, M. E. (2002). Addiction: making the connection between behavioral changes and neuronal plasticity in specific pathways. *Molecular Interventions*, *2*(3), 146–157. doi:10.1124/mi.2.3.146

Xi, Z.-X., & Stein, E. A. (2002). Blockade of ionotropic glutamatergic transmission in the ventral tegmental area reduces heroin reinforcement in rat. *Psychopharmacology*, *164*(2), 144–150. doi:10.1007/s00213-002-1190-3

Xi, Z.-X., Gilbert, J., Campos, A. C., Kline, N., Ashby, C. R., Jr., Hagan, J. J., Heidbreder, C. A., et al. (2004). Blockade of mesolimbic dopamine D3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. *Psychopharmacology*, *176*(1), 57–65. doi:10.1007/s00213-004-1858-y

Yamaguchi, M., Suzuki, T., Abe, S., Hori, T., Kurita, H., Asada, T., Okado, N., et al. (2002). Repeated cocaine administration differentially affects NMDA receptor subunit (NR1, NR2A-C) mRNAs in rat brain. *Synapse, 46*(3), 157–169. doi:10.1002/syn.10132

Yang, C. R., & Mogenson, G. J. (1984). Electrophysiological responses of neurones in the nucleus accumbens to hippocampal stimulation and the attenuation of the

excitatory responses by the mesolimbic dopaminergic system. *Brain Research*, *324*(1), 69–84.

Yang, C. R., & Mogenson, G. J. (1985). An electrophysiological study of the neural projections from the hippocampus to the ventral pallidum and the subpallidal areas by way of the nucleus accumbens. *Neuroscience*, *15*(4), 1015–1024.

Yao, Y., Kelly, M. T., Sajikumar, S., Serrano, P., Tian, D., Bergold, P. J., Frey, J. U., et al. (2008). PKM Maintains Late Long-Term Potentiation by N-Ethylmaleimide-Sensitive Factor/GluR2-Dependent Trafficking of Postsynaptic AMPA Receptors. *The Journal of Neuroscience*, *28*(31), 7820–7827. doi:10.1523/JNEUROSCI.0223-08.2008

Zahm, D. (1999). Functional-anatomical Implications of the Nucleus Accumbens Core and Shell Subterritories. *Annals of the New York Academy of Sciences*, 877, 113-128. doi: 10.1111/j.1749-6632.1999.tb09264.x

Zarrindast, M. R., Owji, A. A., & Hosseini-Nia, T. (1991). Evaluation of dopamine receptor involvement in rat feeding behaviour. *General Pharmacology*, *22*(6), 1011–1016.

Zavala, A., Weber, S. M., Rice, H. J., Alleweireldt, A. T., & Neisewander, J. L. (2003). Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. Brain Research, 990(1-2), 157–164. doi:10.1016/S0006-8993(03)03452-8

Zijlstra, F., Booij, J., Van den Brink, W., & Franken, I. H. A. (2008). Striatal dopamine D2 receptor binding and dopamine release during cue-elicited craving in recently abstinent opiate-dependent males. *European Neuropsychopharmacology*, *18*(4),

262–270. doi:10.1016/j.euroneuro.2007.11.002

Zhou, W., Zhang, F., Liu, H., Tang, S., Lai, M., Zhu, H., & Kalivas, P. W. (2008). Effects of training and withdrawal periods on heroin seeking induced by conditioned cue in an animal of model of relapse. *Psychopharmacology*, *203*(4), 677–684. doi:10.1007/s00213-008-1414-2