Ventral Tegmental Area Regulation Of Stress-Induced Reinstatement Of Cocaine-Seeking Behavior

Jordan Michael Blacktop
Marquette University

VENTRAL TEGMENTAL AREA REGULATION OF STRESS-INDUCED REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR

by

Jordan M. Blacktop, B.S.

A Dissertation submitted to the Faculty of the Graduate School,
Marquette University,
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

Milwaukee, Wisconsin
May 2014

ABSTRACT

Ventral Tegmental Area Regulation of Stress-Induced Reinstatement of Cocaine-Seeking Behavior

Jordan M. Blacktop, B.S.

Marquette University, 2013

No FDA approved medications currently exist for the prevention of drug craving, drug seeking, and relapse to cocaine use. Stress is a major factor in causing relapse in cocaine dependent individuals. Cocaine use is positively correlated with stress-induced craving and relapse outcomes. Corticotropin-releasing factor (CRF) is a 41-amino acid neuropeptide that plays an important role in the stress response and in the reinstatement rodent model of stress-induced relapse. CRF is released during stress in brain regions associated with the effects of drugs of abuse, notably the ventral tegmental area (VTA). This dissertation addresses key unknown mechanisms behind drug-induced neuroplasticity and how that neuroplasticity gates the ability of stress to cause relapse. Chapter two reports that stress and intra-VTA CRF administration produces robust reinstatement in animals allowed extended long-access (LgA) but not short-access (ShA) cocaine self-administration. Moreover, LgA cocaine use increases susceptibility to stressor-induced relapse in part by augmenting CRF receptor 1 (CRF-R1) dependent regulation of VTA neurocircuitry. Chapter three characterizes VTA dopamine neuron activation under conditions where stress reinstates cocaine seeking. Dopamine neuron activation was significantly increased in ShA but not LgA rats. However, when examined across groups only in rats that display relapse in response to stress is a significant increase in dopamine neuron activation observed. This suggests that stress-induced reinstatement is associated with increased activation of VTA dopamine neurons. Lastly, chapter 4 addresses the necessity of VTA glutamate and GABA receptors in footshock and intra-VTA CRF dependent reinstatement of cocaine seeking. Intra-VTA administration of NMDA, AMPA, and $GABA_A$ receptor antagonists fail to block reinstatement. In contrast, $GABA_B$ receptor antagonism blocked reinstatement by both footshock and intra-VTA CRF suggesting $GABA_B$ activation is necessary for CRF actions in the VTA. The findings from this dissertation provide much needed insight into the neuroadaptations that occur in the VTA to regulate later stressor induced relapse in cocaine addicts. The hope is that these findings will help with the understanding and eventual long-term management of stressor-induced relapse in abstinent cocaine addicts.

ACKNOWLEDGEMENTS

Jordan M. Blacktop, B.S.

In no particular order, this dissertation is dedicated to my paternal grandparents Harry A. and Leona M. Blacktop, maternal grandparents Rose M. and Richard L. Rombach, parents Ann M. and Barry A. Blacktop, and my graduate advisor John R. Mantsch. Without these individuals this dissertation would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

CHAPTER 1

BACKGROUND AND SIGNIFICANCE

ADDICTION AND SOCIETY

The World Drug Report estimates that 5% of the world's population uses illegal drugs, with the economic burden exceeding several hundred billion dollars annually (UNODC, 2010). Moreover, the National Drug Intelligence Center (NDIC) estimates that drug abuse costs the United States approximately 120 billion dollars in lost productivity, 61 billion in drug-related crime, and 11 billion in drug addiction related healthcare (NDIC, 2011). Combining medical, criminal, economic, and social impact, addiction costs Americans upwards to half a trillion dollars a year (Jacobs, 2012). Importantly, drug addiction treatment has been shown to significantly decrease addiction associated-health and social costs by a far greater margin than the cost of implementing the treatment itself (Jacobs, 2012).

Treatment is much less expensive than its alternative, such as incarceration of these addicted persons (Jacobs, 2012). According to several conservative estimates, every $1 invested in addiction treatment programs yields a return of between $4 to $7 in reduced drug-related crime, criminal justice costs, and theft (Jacobs, 2012). Even more astonishing, when the savings related to health care are included, the total savings can exceed the costs by a ratio of 12 to 1 (Jacobs, 2012). Thus, it makes humane, economical sense to invest in developing better

treatments for addiction. Not only will this help the addicted individual, it will also give back to society through less interpersonal conflicts, increased workplace productivity, and fewer drug-related accidents (Jacobs, 2012). In summary, more effective treatment can help reduce the costs of addiction on society while also rehabilitating the addicted individual.

ADDICTION

Addiction is chronic relapsing neuropathy of the brain (O'Brien and McLellan, 1996) that results from long-term or even permanent changes to the circuitry of motivated behavior. The long-term or permanent changes in the circuitry of motivated behavior is referred to as drug-induced neuroplasticity. Drug-induced neuroplasticity in the circuitry of motivated behavior produces loss of control over drug intake, decreased drive for natural rewards, and increased relapse vulnerability even following periods of prolonged drug abstinence. Although, the initial decision to take the drug is a voluntary decision, as the motivation to use the drug takes over and the drug changes brain function, the person’s ability to exert control over drug intake is impaired (Volkow and Fowler, 2000, Kalivas and Volkow, 2005, Jacobs, 2012).

Human addicts do not maintain a steady state of use but instead increase the amount of drug used over time (Edwards, 1986, Gawin and Kleber, 1988, Gawin, 1991). As addiction develops there is transition to higher doses with longer bingeing duration (Ellinwood, 1977, Gawin and Kleber, 1986, Jekel et al., 1986, Gawin and Ellinwood, 1988). Therefore, the transition from recreational

drug use to drug addiction involves gradual escalation of drug intake over time (Edwards, 1986, O'Brien, 1986, Marlatt et al., 1988, Gawin, 1991). This is referred to as loss of control over drug intake. Additionally, addicts report generalized anhedonia with decreased reward perception to normal everyday life experiences (food, sex, hobbies, etc.) (Hall, 1988). Perception of reward is now primarily oriented around use of the drug. As addiction progresses addicts use the drug to feel “normal” and to avoid the dysphoric effects of withdrawal that come with abstinence.

WITHDRAWAL AND ABSTINENCE

Following cessation of drug use by human addicts a state of withdrawal can occur consisting of irritability, anxiety, anhedonia, and depression lasting from several hours to days (Gawin and Kleber, 1986). Recurrent cycles of drug binges followed by drug cessation with consequent severe dysphoria fuel the vicious cyclic nature of addiction. Following cessation of drug use there are three main stages that occur: (1) acute withdrawal, (2) early abstinence, (3) and protracted abstinence (Heilig et al., 2010). Acute withdrawal occurs 48-72 hours in humans a timecourse thought to correspond to 24-48 hours after cessation of drug use in rats (Heilig et al., 2010). Acute withdrawal is characterized by disruptions in sleep, increased anxiety, irritability, and crashing (Walsh et al., 2009). Early abstinence occurs 3-6 weeks in humans a timecourse thought to correspond to 1-2 weeks after cessation of drug use in rats (Heilig et al., 2010). Early abstinence is characterized by craving, anxiety, uncertainty (Dudish-

Poulsen and Hatsukami, 2000), severe depressive-like symptoms, and irritability (Gawin and Kleber, 1986). Protracted abstinence consists of abstinence past 3 months in humans and greater than two weeks in animals (Kuhar and Pilotte, 1996, Heilig et al., 2010). During protracted abstinence there is a decrease or absence of reward perception to normal pleasurable events (Gawin et al., 1986, Gawin, 1991, Heilig et al., 2010).

PROTRACTED ABSTINENCE

During protracted abstinence, normally insignificant and/or stressful challenges can provoke dysphoria, craving, and relapse (Sinha et al., 1999, Heilig et al., 2010). Human clinical observations reveal that addicts experience anhedonia, depression, and dysphoria during prolonged periods of abstinence from drugs (Dole et al., 1966, Martin and Jasinski, 1969). Unlike the acute physical withdrawal symptoms that occur during the first 24-48 hours of drug abstinence, dysphoria can persist for extended periods of time during the protracted abstinence phase (Gawin, 1991, Ahmed and Koob, 1998, Aston-Jones and Harris, 2004). Protracted abstinence from drug abuse has also been defined as a syndrome with “the persistence of a dysregulated reward system long after acute withdrawal” (Koob, 2010). Protracted abstinence is further characterized by low-level dysphoric symptoms due to drug abstinence with a persistent high relapse vulnerability.

TREATMENT DURING PROTRACTED ABSTINENCE

Non-pharmacological treatment for abstinent addicts involves teaching skills for adaptive coping to cravings triggered by cues, stress and other stimuli (Marlatt, 1990, Marlatt, 1996). Initial studies did attempt to use cue exposure therapy (Drummond, 1995) to treat human addicts. Initial studies reported cue exposure therapy unsuccessful (Marlatt, 1996), however, this line of research is ongoing. The current treatment standard consists of a combination of pharmacological intervention, depending on the drug of abuse, and cognitive behavioral therapy. Cognitive behavioral therapy (CBT) is thought to function by suppressing drug seeking through strengthening inhibitory control circuits, increasing non-drug reinforced incentive salience, and strengthen executive function, especially at times of high relapse risk (Marlatt, 1985). At all stages of drug abstinence in addicts there is persistent craving and high relapse susceptibility.

DSM-V CRITERIA: SUBSTANCE USE DISORDER AND CRAVING

The Diagnostic and Statstical Manual of Mental disorders (DSM) is the American Psychological Association (APA) classification and diagnostic resource used to diagnose psychiatric disorders. As understanding of psychiatric disorders evolves so must the DSM. The DSM-V substance-related disorders has combined the DSM-IV abuse and dependence categories into one substance use disorder category. Within the criteria legal problems is removed and importantly

replaced with craving. Total criteria include: (1) hazardous use, (2) social/interpersonal problems related to use, 3) neglected major roles to use, (4) withdrawal, (5) tolerance, (6) use larger amounts/longer, (7) repeated attempts to quit/control use, (8) much time spent using, (9) physical problems related to use, (10) activities given up to use, (11) and craving (DSM-V, 2013). To qualify for a substance use disorder, two or more abuse criteria within 12-month period must occur (Hasin et al., 2013). Drug craving is a hallmark in the diagnosis of substance us disorders (O'Brien et al., 1998, Miller and Goldsmith, 2001, Waters et al., 2004, Weiss, 2005, Heinz et al., 2009).

CRAVING

Craving is a strong desire or urge. Drug craving can be defined as an urgent abnormal/obsessive desire, longing, or yearning for the drug. Drug craving is associated with an unpleasant feeling that can be alleviated by use of the drug. This often is often observed as the chasing of drug-induced euphoria, comparable to that experienced upon initial drug use, which can be spurred upon by stimuli that induce craving for the drug. Anti-craving medications are currently being investigated with the hope that they will help in the long-term management of addiction (O'Brien, 2005). Repeated cycles of drug-induced euphoria, drug abstinent-induced dysphoria, and drug craving makes addiction cyclic in nature. Craving facilitates the cyclic nature to the addiction process by facilitating relapse. Addicts currently abstinent from drug use are still at risk for relapse due to drug-induced neuroplasticity within the reward system. In essence, drugs of

abuse hijack the endogenous reward/motivation systems, providing a neurochemical framework for high relapse susceptibility.

RELAPSE

Addiction is hard to treat because of compulsive drug seeking and high relapse vulnerability following periods of prolonged abstinence (Mendelson and Mello, 1996, O'Brien and McLellan, 1996, O'Brien, 1997). The high rate of relapse following abstinence from drug use is considered to be one of the fundamental obstacles to the long-term management of drug addiction (O'Brien, 2005, Kalivas and O'Brien, 2008). Drug craving and relapse to both drug seeking and drug use can be triggered by: (1) exposure to the previously self-administered drug (drug-induced relapse) (Meyer, 1979, Jaffe et al., 1989, de Wit, 1996), (2) stimuli associated with drug taking (cue-induced relapse) (Childress, 1992, Carter and Tiffany, 1999), and (3) exposure to stressors (stress-induced relapse).

THEORETICAL ASPECTS OF ADDICTION

Common to all drugs of abuse is activation of the midbrain dopamine system (Snyder, 1986, Cooper, 1991) which have been implicated in reward, euphoria, satisfaction, cognition, alertness, and motivation (Wise and Bozarth, 1982, Robbins et al., 1989, Di Chiara, 1995). Drugs of abuse are not only rewarding but also reinforcing (White, 1989), increasing the likelihood that the behavior of using the drug will be repeated. These findings provided the

framework behind the dopamine reward hypothesis. The dopamine reward hypothesis states that dopamine plays an essential role in motivated behavior and the rewarding effects of drugs of abuse (Wise and Rompre, 1989). However, increases in dopamine are not only associated with reward but also prediction of reward and salience of reward related stimuli (Robinson and Berridge, 1993, Schultz, 1998).

Drug-induced neuroplasticity has been posited to attribute incentive salience or wanting to drug-related stimuli that would otherwise be normally ambiguous (Robinson and Berridge, 1993). Drug use in essence changes the function of the reward system so that stimuli associated with drug use have increased incentive salience (Robinson and Berridge, 1993). Salience refers to the characteristics of stimuli that arouse and/or elicit an attentional response (Horvitz, 2000). Salience applies to reward, aversion, and new and unexpected stimuli that can affect the motivation to seek an anticipated reward, resulting in the facilitation of conditioned learning (Schultz, 2001, McClure et al., 2003). Importantly, incentive salience can transform drug-associated stimuli into an intense craving for the drug (Robinson and Berridge, 1993).

Repeated drug exposure can also lead to adaptations in the reward circuitry that oppose or neutralize the effects of the drug. In this way, addiction has been hypothesized to involve neuroadaptations that combat the effects of the drug on the brain (Wikler, 1973, Solomon and Corbit, 1974).The opponent process theory of addiction states that the rewarding effects of drugs are automatically opposed by “anti-reward” signaling such that removal of withdrawal

and craving now drives drug use instead of the addition of euphoria (Solomon and Corbit, 1974, Koob and Le Moal, 2008). In other words, there is a shift from positive reinforcement to negative reinforcement. This theory of addiction centers around physiological homeostasis that has been disrupted upon continued drug use. Allostasis is the concept of changes in physiology that occur due to environmental-induced deviations from a normal homeostatic set point (Sterling, 1988, McEwen and Stellar, 1993). The opponent process theory of addiction defines addiction as hedonic homeostatic dysregulation (Koob and Le Moal, 1997). This is very similar to Newton's third law of motion stating that for every action there is an equal and opposite reaction (Newton, 1686) but simply applied to the effects of the drug on neurophysiology.

Since there is substantial evidence for the dopamine reward, the incentive salience, and the opponent process theories of addiction, it is likely that addiction itself would best be described as a powerful combination of all three theories. Euphoric vivid memories are made during the uncontrolled binges of drug use that can increase the incentive salience of drug associated cues (Gawin and Kleber, 1986, Gawin and Ellinwood, 1988). These cues can produce drug craving during abstinence facilitating relapse. During abstinence an addict is in a state of anhedonia that is exacerbated when coupled with drug craving produced by drug associated stimuli in the environment of the user. In essence, dopamine regulates the euphoric effects of the drug and incentive salience of drug related stimuli, while the opponent process produces the anhedonic state, which interact to trigger overwhelming drug craving induced by cues, drug, or stress. This

overwhelming drug craving provides the neurochemical framework for both loss of control over drug seeking/use and high relapse susceptibility.

COCAINE AND SOCIETY

One drug of abuse that has a particularly high rate of developing addicts following recreational use is the euphoric psychostimulant cocaine. More than 23 million Americans have used cocaine at some time, and the number of frequent users, at least weekly, has remained steady since 1991 at ~ 600,000 (O'Brien, 2011). Approximately 16% of the United States general populace has used cocaine at least once in their life while 17% of those that try cocaine become addicted (Wagner and Anthony, 2002). These statistics are consistent with other studies reporting that 10 to 15% of those who try cocaine develop addiction within the first 10 years of first trying the drug (Wagner and Anthony, 2002). Crack cocaine has a substantially higher dependence/addiction and relapse rate compared to powdered intra-nasal cocaine. It has been estimated that approximately 63% of crack cocaine users develop dependence within 8 years of first trying the drug (Falck et al., 2008). These statistics suggest that approximately 1.6 to 2.7 percent of the United States general populace is addicted to cocaine (Wagner and Anthony, 2002).

The number cocaine initiates has declined (SAMHSA, 2012). However, there are still 1,800 new initiates per day (SAMHSA, 2012). Moreover, the percentage of individuals that try cocaine and become addicted hasn’t changed, and the percent of cocaine addicts that are unemployed has increased

(SAMHSA, 2012). Cocaine is the primary abused substance reported for 13% of all admissions into substance abuse programs in the United States (SAMHSA, 2009). The decrease in initiates but stable number of cocaine addicts could be explained by the fact that federal agencies are seizing 30% less cocaine in the continental United States (NDIC, 2011) allowing for those addicted to continue to have access to cocaine. This is even more problematic when combined with recent reports that relapse rates following treatment for cocaine dependence typically exceed 45% within 6 to 12 months of treatment (Hall et al., 1991, McKay et al., 1998, McKay et al., 1999). Most importantly, there still remains no food and drug administration (FDA) approved pharmacotherapy for the treatment of cocaine addiction (de Lima et al., 2002, Lima et al., 2003, Vocci and Elkashef, 2005). In summary, it is clear there is great need of improvement in treatment strategies to address the public health concern of cocaine addiction.

MECHANISM OF ACTION OF COCAINE

Research in human subjects has demonstrated that cocaine elicits a profound feeling of euphoria producing a reinforcing positive mood state. This has been described as a “rush” or “high” that is directly tied to a dramatic increase in dopamine concentrations in the nucleus accumbens (Koob and Bloom, 1988, Volkow et al., 1999a, Di Chiara, 2002). This rush can further be characterized by augmentation of confidence, well-being, alertness, emotion, hedonic drive (sex), communication, satiation, and disinhibition (Ellinwood, 1977, Van Dyke et al., 1982, Gawin and Kleber, 1985, Johanson and Fischman, 1989).

The reinforcing effects of cocaine correlate with its ability to block the dopamine transporter (DAT) at the synapse increasing concentrations of dopamine at critical brain sites (Ritz et al., 1987). Cocaine also blocks norepinephrine (NE) and serotonin (5-HT) reuptake (Ritz et al., 1990, O'Brien, 2011) having dramatic effects on mood and arousal states (Gawin and Kleber, 1984).

COCAINE CRAVING AND THE HUMAN BRAIN

Functional imaging studies on human addicts have given insight into the areas of the brain that are dysfunctional and correlated with intense cocaine craving. Structural abnormalities indicative of decreased function have been found in the prefrontal cortex (PFC) of human cocaine addicts. These abnormalities include decreased perfusion, glucose metabolism, and gray matter (Volkow et al., 1991, Volkow et al., 1992, Volkow and Fowler, 2000, Franklin et al., 2002, Matochik et al., 2003, Volkow et al., 2004). The PFC is involved in executive function, decision making, risk/reward assessment, and impulse control. Therefore, it has been hypothesized that irresistible drug cravings and decreased impulse control that ultimately lead to relapse of drug use are the result of decreased prefrontal cortical activity (Kaufman et al., 2003, Hester and Garavan, 2004, Volkow et al., 2004, Ersche et al., 2008, Li et al., 2008). This has been collectively referred to as drug-induced hypofrontality. In addition to hypofrontality, cocaine craving is also associated with dysfunctional striatal-limbic responses (Sinha et al., 2005, Wong et al., 2006). These are two main downstream targets of the midbrain dopamine system and of glutamatergic

projections from the PFC. This indicates that aberrant dopamine and or corticostriatal signaling is a phenotype of addicts that is associated with drug craving (Sinha, 2013).

Drug seeking is facilitated by impairment of prefrontal cortical mechanisms that in a healthy non-addicted individual, would inhibit harmful behaviors (Hyman et al., 2006). The medial prefrontal cortex (mPFC) is involved in both activation and suppression of drug-seeking behavior. Moreover, there appears to be aberrant communication between this brain region and midbrain dopamine neurons (Gu et al., 2010), and the nucleus accumbens (McFarland et al., 2003). This is thought to reflect devaluation of non-drug goals within the PFC (Montague et al., 2004), with drug-related goals undermining proper PFC function in the control of behavior (Paulus et al., 2005). Perhaps this streamlines drug-related motivation at the expense of natural motivation. In summary, drug craving is an unpleasant feeling and, when combined with aberrant dopamine signaling the ability to suppress drug use, is hindered, which facilitates relapse. This may be one mechanism involved in loss of control in drug seeking/intake seen in human addicts.

STRESS AND COCAINE CRAVING IN HUMAN ADDICTS

One cause of intesnse cocaine craving that precipitates relapse in human addicts is stress exposure. Stress is unpredictable and unavoidable in today's society for virtually everyone. For this reason, stress is a significant obstacle in maintaining abstinence because it facilitates the vicious cycle of addiction

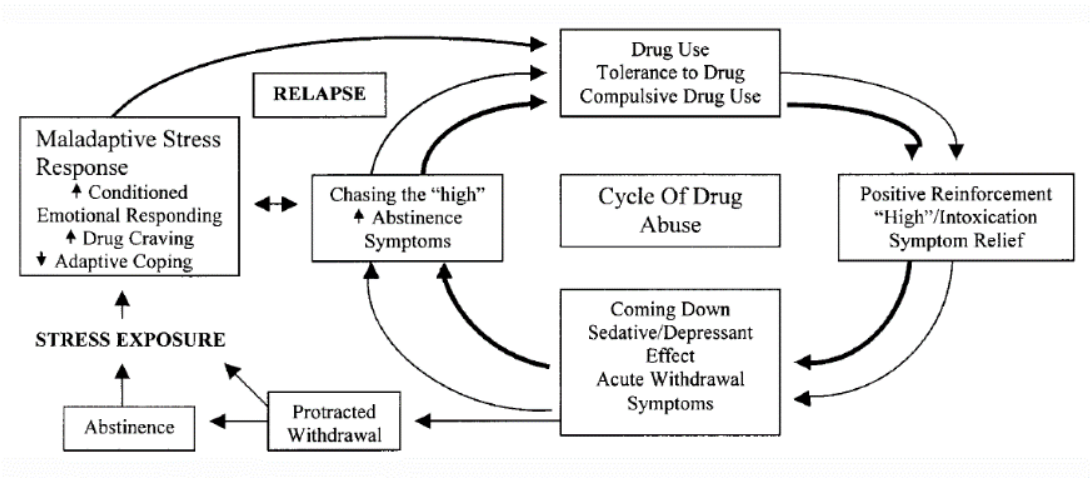
(Ludwig and Wikler, 1974, Littman, 1977, Marlatt, 1978, Bradley et al., 1989, Wallace, 1989, Sinha et al., 1999, Sinha, 2001). Stress can take the form of a perceived threat (anxiety), loss of a family member, a divorce, job dissatisfaction, amongst other things. Stress is embedded throughout the entire process of addiction. Stress appears to be a core factor in the initial decision to try a drug, continued drug use, development of addiction, and relapse to drug use following abstinence.

States of stress and stressor exposure have long been positively associated with relapse to drug seeking and drug use (Marlatt, 1980, Kreek and Koob, 1998, Koob and Kreek, 2007). Stressful stimuli increase cocaine craving in human addicts (Sinha et al., 1999, Back et al., 2010). Specifically, human cocaine users in a drug free state exposed to stressful imagery scripts display increased craving for drug, anxiety, heart rate, and even stress hormone levels (Sinha et al., 2000). Stress-induced cocaine craving is characterized by decreased activity in areas associated with control and regulation of emotion/distress (anterior cingulate cortex, hippocampus) (Sinha et al., 2005) with increased activity in areas associated with reward (caudate, dorsal striatum) (Sinha et al., 2005). Importantly, the magnitude of drug craving is positively correlated with the amount of cocaine previously used (Fox et al., 2005).

A prior history of higher frequency cocaine use is associated with augmented stress-induced craving measured in a laboratory setting in cocaine-dependent individuals (Fox et al., 2005, Back et al., 2010). This augmented stress-induced cocaine craving is correlated with higher relapse rates in

abstinent cocaine addicts (Sinha et al., 1999, Sinha et al., 2000, Sinha, 2001, Sinha et al., 2006, Sinha, 2008, 2009, Back et al., 2010). Stress perceived in the environment of a cocaine addict can cause relapse through craving because of an atypical stress response (Kreek, 1987). The addiction phenotype (Figure 1) involves a maladaptive stress response characterized by enhanced emotional responsiveness, drug craving, and decreased adaptive coping (Sinha, 2001).

Figure 1: Addiction stress and relapse; neuroadaptations in brain stress and reward circuits results in a maladaptive stress response in human addicts (Sinha, 2001).



COCAINE ADDICTION AND THE SELF-ADMINISTRATION ANIMAL MODEL OF RELAPSE

Although, human brain imaging and anecdotal reports of craving have been useful in determining a key role of stress in the addiction process it is very difficult to determine what is exactly happening at those brain regions beyond

changes in receptor binding (PET) and cerebral blood flow (fMRI) (Malonek and Grinvald, 1996, Volkow et al., 2001). In order to study the intricacies of addiction, animal models have been developed. In humans, craving is based on anecdotal reports from human addicts themselves. In laboratory animals craving is inferred from the behavioral response in the form of drug seeking (lever pressing) following abstinence or extinction.

As the duration of abstinence/withdrawal increases there are also increases in cue-induced craving in both subjective reports in humans and drug seeking in laboratory animals (Grimm et al., 2001, Bedi et al., 2011). This phenomenon is referred to as an incubation of drug craving (Grimm et al., 2001, Pickens et al., 2011). In contrast to cues, studies investigating incubation in response to both drug and stress have produced mixed results (Tran-Nguyen et al., 1998, Shalev et al., 2001, Deroche-Gamonet et al., 2003, Marinelli et al., 2003, Lu et al., 2004, Shepard et al., 2004, Sorge et al., 2005).

The reinstatement rodent model of relapse has provided a strong framework to investigate the neuromechanisms of relapse induced by cues, drug, and stress (Shaham et al., 2003). Reinstatement is simply a restoration of drug-seeking behavior (Stewart, 1987a, Bouton, 1991, Catania, 1992). The reinstatement model allows researchers to characterize the processes in the brain that contribute to the maladaptive behavior of relapse in a way that cannot be studied in people. This dissertation primarily utilizes the rodent reinstatement model of relapse to further characterize the neuromechanisms of stress-induced

relapse to cocaine-seeking behavior. This model is described in further detail in the following section.

The same stimuli that can provoke craving and relapse in human addicts can reinstate cocaine-seeking behavior in laboratory animals, even after periods of prolonged abstinence. In abstinent human cocaine addicts augmented drug craving and relapse probability can be triggered by exposure to: (1) the previously self-administered drug (Meyer, 1979, Jaffe et al., 1989, Preston et al., 1992), (2) stimuli previously associated with drug use (Childress, 1992, Carter and Tiffany, 1999), and (3) exposure to acute stress (Sinha, 2001, Fox et al., 2005). In the rodent reinstatement model of relapse vigorous active drug-seeking behavior can be induced by (1) priming injections of the drug (Gerber and Stretch, 1975), (2) cocaine predictive cues (Weiss et al., 2000), and (notable for this dissertation) (3) acute stress exposure (Erb et al., 1996, Ahmed and Koob, 1997, 1998, Shaham et al., 1998, Shaham et al., 2000, Mantsch et al., 2008a, Mantsch et al., 2008b, Shalev et al., 2010).

SELF-ADMINISTRATION MODEL

Drugs abused by humans will be self-administered by laboratory animals (Schuster and Thompson, 1969). Cocaine appears to have similar effects on brain function in both humans and rodents. As mentioned earlier, drugs of abuse exert their reinforcing effects through increased dopamine signaling (Snyder, 1986, Koob and Bloom, 1988, Wise and Rompre, 1989, Cooper, 1991). When humans or laboratory animals self-administer cocaine there are large and fast

increases in nucleus accumbens (striatal) dopamine signaling (Hurd et al., 1989, Pettit and Justice, 1989, 1991, Laruelle et al., 1995, Wise et al., 1995, Breiter et al., 1997, Volkow et al., 1999b, Volkow et al., 2002), an effect that has long been associated with reward. Moreover, when dopamine terminals are depleted in the nucleus accumbens cocaine self-administration drops significantly (Pettit et al., 1984), suggesting that increasing dopamine neurotransmission in the nucleus accumbens is essential for cocaine self-administration.

The drug-self-administration model involves the laboratory animal learning to perform an operant task, such as lever pressing, to receive intravenous infusions of drug (Weeks, 1962). In this model the reinforcing properties of drugs can be measured in animals, such as rats, equipped with intravenous catheters connected to lever-regulated pumps, which provide direct systemic administration of drug in response to operant lever pressing (O'Brien, 2011). These animals will work to obtain injections of the same drugs that are abused by humans in roughly the same order of potency (O'Brien, 2011). Moreover, evidence suggests that contingent drug-induced neuroplasticity, as seen in the self-administration model, can be distinct from noncontingent drug-induced neuroplasticity (Stewart, 1987b, Goudie, 1989, Stewart, 1992, Dworkin et al., 1995, Hemby et al., 1997, Badiani et al., 1998, Robinson et al., 1998, Markou et al., 1999).

Human addicts do not maintain a steady state of use but instead increase the amount of drug used over time (Edwards, 1986, Gawin and Kleber, 1988, Gawin, 1991), thus resulting in a gradual escalation of drug intake (Edwards,

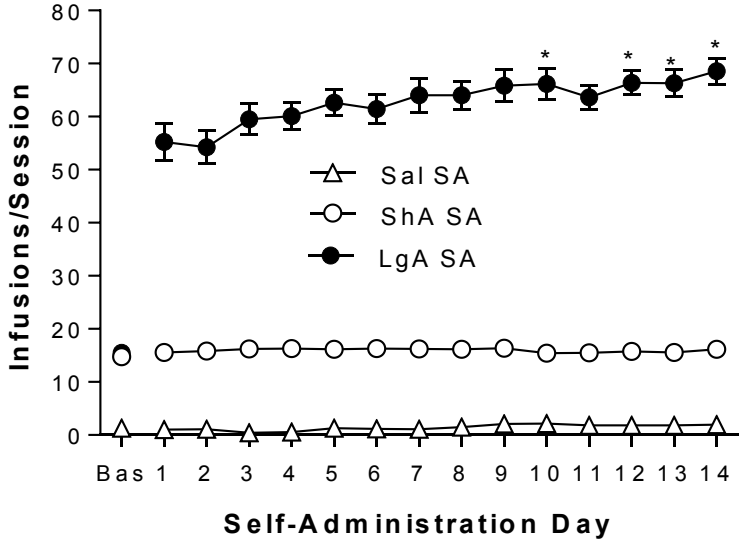
1986, O'Brien, 1986, Marlatt et al., 1988, Gawin, 1991). This escalation of drug use is also closely associated with loss of control over drug intake (DSM-V, 2013). Escalation is thought to represent evidence of drug-induced neuroplasticity. Loss of control over drug intake, as seen in the human condition (DSM-V, 2013), can be established, in the form of escalation, in animals allowed extended daily access to cocaine for self-administration (Ahmed and Koob, 1998, Mantsch et al., 2004, Mantsch et al., 2008a, Mantsch et al., 2008b). Extended cocaine self-administration access has been termed long-access (LgA) self-administration.

SHORT-ACCESS AND LONG-ACCESS MODELS OF COCAINE SELF-ADMINISTRATION

Researchers have utilized short-access (ShA), 1-2 hours of cocaine self-administration access per day, and long-access (LgA), 6-10 hours of cocaine self-administration access per day, models to study cocaine abuse/addiction. Long-access self-administration (14 x 6 hrs) results in very high levels of drug intake (> 70 mg/kg/day), which are substantially higher than the total intake in short access animals (~ 15 mg/kg/day) (Mantsch et al., 2008a). Under both ShA and LgA conditions, rats learn—often on the first day of exposure—to lever-press for intravenous drug, such as cocaine. Short-access self-administration sessions produce stable, controlled, and regulated responding for drug (Ahmed and Koob, 1999, Mantsch et al., 2008a). On the other hand, long-access (LgA) self-administration produces a progressive escalation in cocaine self-administration (Figure 2) (Ahmed and Koob, 1997, 1998, 1999, Ahmed et al., 2003, Ahmed and

Koob, 2004, Mantsch et al., 2008a, Blacktop et al., 2011). Progressive escalation is simply a gradual increase in drug taking across consecutive self-administration sessions. Escalation of self-administration in laboratory animals thought to be analogous to the loss of control of drug taking seen in human addicts (Ahmed and Koob, 1998).

Figure 2: Escalation of drug SA in long-access rats (LgA/closed circles) but not in short-access (ShA/open circle) or saline (Sal/closed triangle). The asterisk (*) indicates significant escalation from the previous SA session (Blacktop et al., 2011). These findings are reproducing those originally reported by Ahmed and Koob (1998).

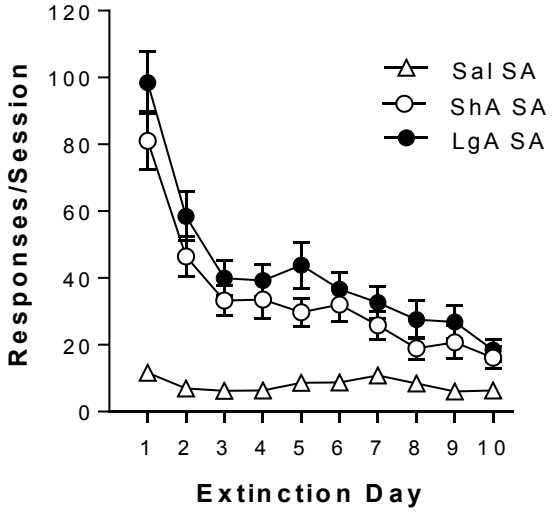


EXTINCTION

The next step in the preclinical animal model of relapse after self-administration is commonly extinction. In order to study relapse using the SA approach, it is first necessary to reduce drug-seeking behavior which can be accomplished by extinction. Extinction permits a decrease of drug-reinforced

behavior to pre-training baseline levels allowing the researcher to test for reinstatement of drug seeking (Stretch et al., 1971, Stewart, 1987a). Simply, extinction is decreased self-administration behavior when the drug and its pharmacological effects have been removed (Yap and Miczek, 2008); i.e. replacing cocaine with saline (Figure 3) (Shalev et al., 2002). Importantly, during extinction the animal is presented with previously drug associated stimuli (self-administration chamber with levers, lights, etc.) in the absence of the drug (Sorg, 2012). Following 14 days of cocaine self-administration training, it takes about 10 days of daily 2 hour extinction sessions before response rates drop back to their infrequent pre-training level (Mantsch et al., 2008a, Blacktop et al., 2011). Moreover, there are no differences in extinction rates between LgA and ShA animals.

Figure 3: Extinction of drug-seeking behavior in short-access (ShA/open circle) and long-access rats (LgA/closed circles) compared to saline controls (Sal/closed triangle). Refer to chapter two figure 1B. (Blacktop et al., 2011).



Extinction is defined as a reduction in drug-seeking when the contingency is broken between the drug-seeking behavior or drug-predicting stimuli and drug reward (Millan et al., 2011). Extinction is an active learning process. During extinction, the animal is not unlearning that the active lever results in drug reinforcement, but learning that the previously active lever no longer results in drug reinforcement. This produces conflicting associations between the original drug-seeking associations and the extinction associations (Millan et al., 2011). Therefore, extinction doesn't erase drug seeking but rather counteracts it.

The major caveat to the self-administration model of relapse is that human addicts do not typically go through extinction. However, extinction may represent an important component of cognitive behavioral therapy. Cognitive behavioral therapy is thought to suppress drug seeking by strengthening inhibitory control circuits, increasing non-drug reinforced incentive salience, and strengthening executive function at times of high relapse risk (Marlatt, 1985). Although extinction and cognitive behavioral therapy are not the same they both are thought to function by strengthening neural circuits that inhibit drug seeking.

REINSTATEMENT

Reinstatement refers to the restoration of a previously drug-reinforced behavior following its suppression, typically through extinction (Stewart, 1987a, Bouton, 1991, Catania, 1992). The dependent variables that are measured during a reinstatement session are non-reinforced responses on the lever that previously delivered drug (active lever) and responses on a second lever not

previously associated with drug infusions (inactive lever). Reinstatement is a significant increase in active lever responses representative of drug seeking, usually without a significant increase in inactive responses representative of nonspecific or generalized response activity (Catania, 1992, Shalev et al., 2002, Shaham et al., 2003).

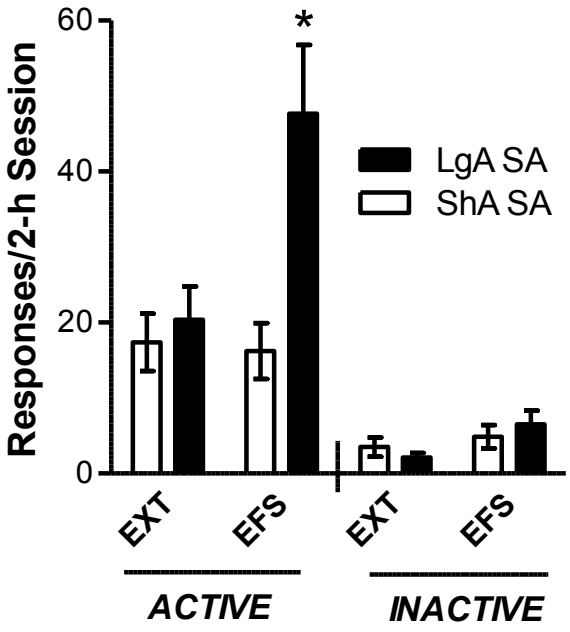
Reinstatement of drug seeking in the rodent model has been hypothesized to be the acute failure of extinction training to inhibit drug seeking (de Wit and Stewart, 1981, Crombag and Shaham, 2002). The important balance between driving and inhibiting drug seeking appears to involve different inputs from the medial prefrontal cortex to different regions of the nucleus accumbens (Cornish et al., 1999, McFarland and Kalivas, 2001, McFarland et al., 2003, McFarland et al., 2004, Fuchs et al., 2008, Peters et al., 2008, Van den Oever et al., 2010). This will be discussed in further detail in the following mesocorticolimbic dopamine system section and in chapter five. In summary, the reinstatement model is not perfect but is still a very powerful tool for addiction research. The same drugs abused by humans will be administered by the rodent, and the same stimuli that precipitate relapse in humans reinstate drug seeking in the rodent.

STRESS-INDUCED REINSTATEMENT

During stress-induced reinstatement a stressful stimulus is administered prior to an otherwise normal extinction session, usually within the self-administration chamber. The most common external stimuli to induce stress-induced reinstatement of drug seeking is unpredictable, uncontrollable, intermittent electric footshock delivered through the grid of the self-administration

chamber. Intermittent footshock reinstates drug seeking in rats previously trained to self-administer cocaine (Erb et al., 1996, Ahmed and Koob, 1997, Mantsch and Goeders, 1999, Sutton et al., 2000). Intermittent unpredictable footshock stress not only reinstates cocaine seeking but also reinstates drug seeking for most drugs of abuse, including heroin, nicotine, and alcohol (Erb et al., 1998, Shaham et al., 1998, Le et al., 2000, Bruijnzeel et al., 2009).

Figure 4: Stress-induced reinstatement of extinguished cocaine-seeking behavior in LgA but not ShA rats, which his specific to the active lever (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011).



Importantly, our laboratory reports that footshock stress only reliably reinstates drug seeking in rodents with a history of LgA cocaine self-administration (Figure 4) (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011), supporting other studies reporting inconsistent reinstatement in ShA

animals (Shelton, 2005). It should be noted that others do report stress-induced reinstatement in ShA animals (Erb et al., 1996, Shaham et al., 1998, Shaham et al., 2000). However, our findings that stress-induced reinstatement occurs more reliably in animals with long-access cocaine self-administration history is consistent with intake dependent stress reactivity in humans. Human studies report that higher frequency cocaine user's exhibit augmented cocaine craving in response to stress, increasing the probability of relapse (Fox et al., 2005, Sinha et al., 2006).

Although, escalation is observed in LgA animals and not ShA animals (Ahmed and Koob, 1998, Mantsch et al., 2008a, Blacktop et al., 2011), there is no evidence reporting escalation as the cause of stress-induced reinstatement to cocaine seeking. However, both escalation of drug intake and augmented reinstatement are intake-dependent and characterize human addiction. LgA animals also show augmented reinstatement by cocaine priming (Mantsch et al., 2004, Madayag et al., 2011) and cocaine cues (Loweth et al., 2013) as compared to ShA animals. This suggests an intake-dependent neuroplastic change in the circuitry of motivated behavior that augments the ability of cocaine priming, cocaine cues, and footshock stress to reinstate cocaine in LgA relative to ShA animals. Intake dependent drug-induced changes in the circuitry of motivated behavior, as seen in the human condition, can be further characterized in the laboratory by comparing the neurological differences between drug naïve, ShA, and LgA access animals. Therefore, this dissertation characterizes the neuromechanisms regulating augmented stress-induced reinstatement of

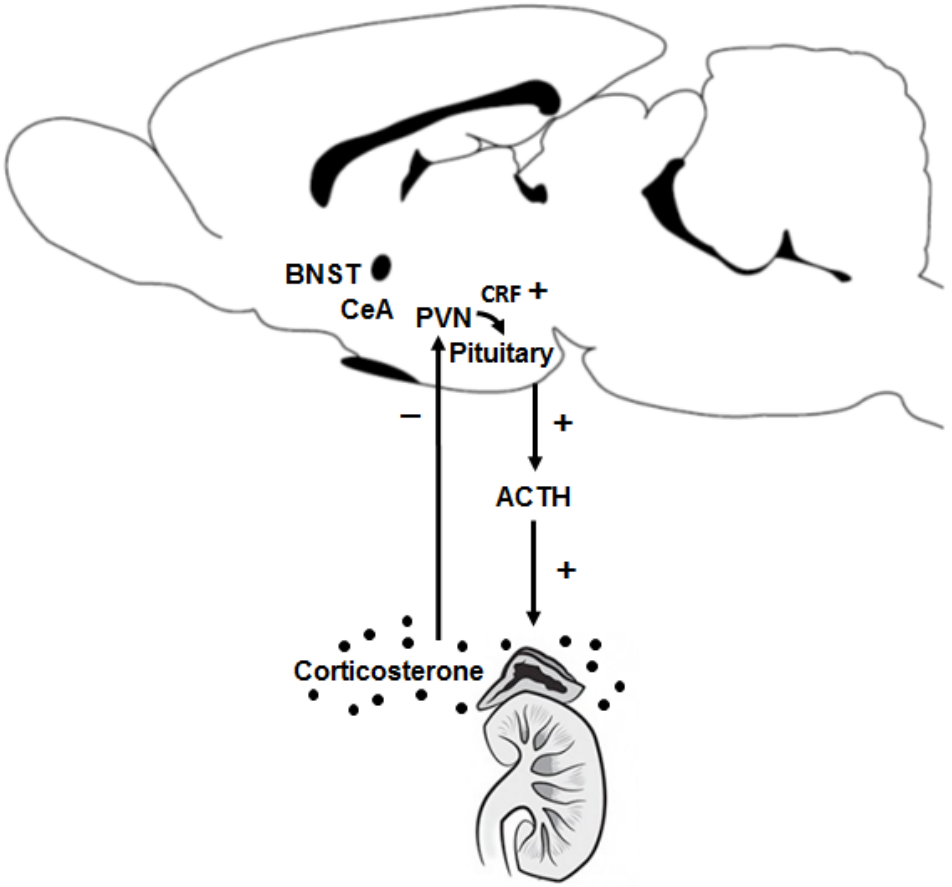
extinguished cocaine-seeking behavior observed following LgA self-administration with the goal of understanding the neuroplasticity responsible for the transition to addiction.

STRESS

Stress is a general response to demands on the body (Selye, 1936) involving alterations in physiological homeostasis (Burchfield, 1979). Stress has been simplistically and controversially defined as a nonspecific response of the body to a demand that is characterized by the production of glucocorticoids and therefore hypothalamic-pituitary-adrenal (HPA) axis activation (Selye, 1936, 1937). The HPA axis is comprised of the hypothalamus, anterior pituitary gland, and the adrenal cortex. A stressor causes the initial cascade of the HPA axis in the form of release of corticotropin releasing factor (CRF) from neurons in the paraventricular nucleus (PVN) of the hypothalamus which project to the external zone of the median eminence. These neurons release CRF into the adenohypophyseal portal circulation (Whitnall, 1993), where CRF activation of CRF receptors on anterior pituitary corticotrophs results in the synthesis, processing, and release of proopiomelanocortin (POMC). POMC is a large precursor protein which is proteolytically cleaved to produce several biologically active peptides including β-endorphin and adrenocorticotrophin hormone (ACTH) (Turnbull and Rivier, 1997, Goeders, 2007). POMC-derived ACTH then circulates throughout general circulation until it reaches the adrenal glands stimulating the biosynthesis of adrenocorticosteroids to be released from the adrenal gland (Figure 5). These adrenocorticosteroids are notably referred to as glucocorticoids

which consist of cortisol in humans and corticosterone in rodents (Vale et al., 1981).

Figure 5: Schematic illustrating simplified HPA axis signaling. Stress results in release of CRF from the PVN of the hypothalamus to the anterior pituitary activating CRF receptors resulting in ACTH release throughout the general circulation. ACTH stimulates the release of adrenocorticosteroids from the adrenal gland, notably corticosterone in rodents and cortisol in humans. Corticosterone provides negative feedback to the PVN. The bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA) are two key anatomical regions enriched in CRF containing neurons with afferents throughout the brain that can function in conjunction with or independently from HPA axis activity.



Two adrenocorticosteroid receptors have been identified, both of which bind corticosterone and are expressed in the brain (Joels and de Kloet, 1994). One is normally fully occupied at basal levels of corticosterone due to its high affinity (Reul and de Kloet, 1985), and is referred to as the type I mineralocorticoid receptor (MR). The other glucocorticoid receptor has lower affinity for corticosterone and is more likely to be occupied during times of stress when the plasma levels of corticosterone are elevated (Reul and de Kloet, 1985). This is referred to as the GR type II receptor (glucocorticoid receptor).

Circulating glucocorticoids released by the adrenal glands promote the mobilization of energy stores and augment sympathetic nervous system effects (Ulrich-Lai and Herman, 2009), alerting and maintaining homeostasis of an organism challenged by either environmental or physiological changes (Herman and Cullinan, 1997). Circulating glucocorticoids exert prominent negative feedback through activation of GR type II receptors at the PVN which inhibit further CRF release to maintain tolerable limits of glucocorticoid secretion (Herman and Cullinan, 1997). This appears not to be the only negative feedback mechanism, with others occurring from GR activation of neuronal inhibitory pathways that work in parallel with negative steroidal feedback (Dobrakovova et al., 1982, Jacobson et al., 1988, Jacobson and Sapolsky, 1991, Bradbury et al., 1993, Diorio et al., 1993, Herman, 1993).

The neurobiological stress response involves physiological homeostatic imbalances implicating both the HPA axis and the autonomic sympathetic nervous system (Selye, 1937, 1951, Korf et al., 1973, Dallman et al., 1987,

Abercrombie et al., 1988, Valentino et al., 1993, Herman and Cullinan, 1997). As first elegantly described by Axelrod and Reisine (1984) homeostatic demands (stress) involve release of ACTH from the anterior pituitary, glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from sympathetic nerves (Axelrod and Reisine, 1984). Therefore, stressful stimuli can be conveyed to the brain activating both the HPA axis and autonomic neural systems with the goal of minimizing net cost while maintaining physiological homeostasis (Ulrich-Lai and Herman, 2009).

The maintenance or re-establishment of homeostasis in response to stress also involves both autonomic and neuroendocrine stress signaling (Ulrich-Lai and Herman, 2009). This signaling involves the limbic forebrain, hypothalamus, and the brainstem allowing for the integration of a prior stress response to memory (Ulrich-Lai and Herman, 2009). This allows not only for the appropriate physiological response to homeostatic challenges, but also the ability to have a stress system that responds according to prior experience. In this light, stress is adaptive and good for the organism. Therefore, it is not surprising there are different categories of stress. Stress itself has been viewed as a variation in physiological homeostasis whereby hyperstress (overstress), hypostress (understress), distress (damaging stress), or eustress (good stress) have been characterized (Selye, 1983).

In summary, stress can be defined broadly as actual or anticipated homeostasis disruption to an organism's physiology involving the HPA axis, the autonomic nervous system, and key brain regions regulating these systems. In

addition to the study of acute stressors and their regulation of cocaine seeking, this dissertation will refer to stress as increased distress produced by prolonged variation in physiological homeostasis (e.g., long-access cocaine use) that results in a maladaptive physiological response (e.g., drug seeking in response to stress), and not simply activation of the HPA axis.

STRESS AND COCAINE SELF-ADMINISTRATION

Stress is embedded in the entire process of cocaine addiction from the first decision to try the drug to repetitive relapses following extended periods of abstinence. Acute cocaine use is, in fact, physiologically stressful to an organism by way of activation of the HPA axis and autonomic nervous system (Rivier and Vale, 1987, Saphier et al., 1993, Goeders, 2002, Koob and Kreek, 2007). Cocaine self-administration stimulates the release of corticosterone and epinephrine from the adrenals in rats (Chiueh and Kopin, 1978, Moldow and Fischman, 1987) consistent with changes in physiological homeostasis and increased arousal (Axelrod and Reisine, 1984). Much of this section will focus on corticosterone and not epinephrine interactions with cocaine use because it is better studied and characterized.

Corticosterone (Cort) is regulated by cocaine self-administration and cocaine self-administration is regulated by corticosterone. Daily administration of Cort facilitates the acquisition of stable cocaine self-administration (Mantsch et al., 1998). Cocaine self-administration increases plasma Cort levels, an effect blocked by GR type II receptor agonists (Mantsch et al., 1998) likely through

negative feedback. Cort levels at the end of a self-administration session are positively correlated with the amount of drug administered (Mantsch and Goeders, 2000). Moreover, cocaine self-administration and its escalation can be significantly attenuated by inhibiting corticosterone synthesis or surgically removing adrenal glands, the primary physiological source of Cort (Piazza et al., 1994, Goeders and Guerin, 1996a, Goeders et al., 1998, Graf et al., 2011). Chronic stress during cocaine self-administration can produce Cort-dependent escalating patterns of intake in ShA animals, comparable to what is seen without chronic stress in LgA animals (Mantsch and Katz, 2007). Altogether, these findings suggest that corticosterone regulates cocaine-induced neuroplasticity which can in turn regulate cocaine self-administration.

Stress-induced corticosterone secretion can regulate dopamine in the nucleus accumbens (Piazza and Le Moal, 1996), and rats will self-administer corticosterone under certain conditions (Deroche et al., 1993, Piazza et al., 1993). This suggests that the mesocorticolimbic dopamine system and Cort signaling can interact to regulate motivated behavior. This interaction can become dysregulated as a result of extended-access cocaine self-administration. Rats that self-administer cocaine under LgA conditions display decreased corticosterone levels basally and in response to cocaine (Mantsch et al., 2003), augmented corticosterone levels in response to stress, and impaired negative feedback of the HPA axis (Mantsch et al., 2007). These changes may contribute to the augmented ability for footshock stress to induce reinstatement of extinguished LgA cocaine seeking (Mantsch et al., 2004).

Increased corticosterone (Cort) signaling has been implicated in both cocaine self-administration and footshock-induced reinstatement of cocaine-seeking behavior. Early reports suggested that blockade of corticosterone synthesis and/or activation of type II glucocorticoid receptors attenuates cocaine self-administration and blocks footshock-induced reinstatement of extinguished cocaine seeking behavior (Sonino, 1987, Goeders et al., 1998, Mantsch and Goeders, 1999). These findings suggested that increased corticosterone signaling is necessary for both stable cocaine self-administration and later footshock stress-induced reinstatement of cocaine-seeking behavior (Goeders et al., 1998, Mantsch and Goeders, 1999). A more recent study found that corticosterone is not necessary for cocaine self-administration (Graf et al., 2011). However, Cort signaling appears to be necessary for the drug-induced neuroplasticity that is put in place at the time of LgA cocaine self-administration that contributes to escalation and augmented reinstatement (Mantsch et al., 2008b), but is not necessary at the time of later footshock exposure for stress-induced reinstatement (Graf et al., 2011). This suggests that the neuroplastic effects we see in the LgA model are dependent on glucocorticoid signaling during self-administration, and that another stress signal is acutely regulating later footshock-induced reinstatement. The key candidate for this is the corticotropin releasing factor (CRF) signaling system.

CORTICOTROPIN-RELEASING FACTOR SYSTEM SIGNALING

Corticotropin releasing factor (CRF) is an important regulator of the stress response and plays a central role in stress-induced reinstatement of drug-seeking behavior (Shaham et al., 1997, Erb et al., 1998, Stewart, 2003, Spealman et al., 2004). Initial studies found CRF to have many different roles, but most were implicated in adapting the body's behavioral, autonomic, and immune responses to stress (Beyermann, 1997). CRF system signaling is not only an integral regulator of hypothalamic-pituitary-adrenal (HPA) axis activity but there are numerous CRF pathways outside of the hypothalamus (Lymangrover and Brodish, 1973) where CRF acts as a central neuromodulator (Swanson et al., 1983, Nemeroff, 1997). The CRF signaling system is comprised of 4 ligands, a binding protein, and two receptors (Vale et al., 1981, Behan et al., 1995, Steckler and Holsboer, 1999, Ryabinin et al., 2002, Fekete and Zorrilla, 2007).

Corticotropin releasing factor (CRF) is a 41-amino acid neuropeptide involved in hypothalamic-pituitary release of ACTH from the pituitary gland (Vale et al., 1981) and stress related neurotransmission throughout the brain (Swanson et al., 1983). CRF was first characterized in 1981 (Vale et al., 1981, Beyermann, 1997) and shown to stimulate the secretion of ACTH-like and β-endorphin-like immunoreactivities *in vitro* and *in vivo* (Vale et al., 1981, Beyermann, 1997). In 1983 CRF was successfully cloned and sequenced from the human CRF gene from which the human precursor was characterized (Shibahara et al., 1983, Beyermann, 1997). Isolation and sequence analysis of rat CRF was found to be

identical to the human peptide (Rivier et al., 1983). CRF is released at synaptic terminals due to depolarization (Smith et al., 1986) and is characterized by saturable, reversible, specific binding to its receptors (De Souza et al., 1985). The distribution of CRF neurons in the brain is consistent with its role in endocrine, physiological, and behavioral responses to stress. Although CRF is widely expressed throughout the brain, the highest concentrations of CRF containing cell bodies are found in the paraventricular nucleus of the hypothalamus (PVN), bed nucleus of the stria terminalis (BNST), and the central nucleus of the amygdala (CeA) (Merchenthaler et al., 1982, Olschowka et al., 1982, Swanson et al., 1983, Petrusz, 1992).

The largest concentration of CRF cell bodies outside of the PVN is in the BNST, followed by the CeA (Figure 7) (Swanson et al., 1983). The BNST, in addition to having influences on HPA axis function (Herman et al., 1994), plays a critical role in anxiety involving interpretation of threatening or aversive stimuli (Walker et al., 2003). The central nucleus of the amygdala (CeA) plays a critical role in anxiety and conditioned fear (Davis, 1992b, a). The BNST and CeA may communicate with one another through reciprocal CRF inputs. Moreover, a CRF projection from the CeA to the BNST has been identified (Sakanaka et al., 1986) suggesting that increases in CRF found in the BNST following stress exposure (Lee and Davis, 1997) can be regulated by CeA activation (Erb and Stewart, 1999). The BNST and CeA send CRF projections throughout the brain allowing for complex integration of stress specific CRF signaling.

CORTICOTROPIN RELEASING FACTOR

Corticotropin releasing factor (CRF) signaling is activated during times of stress and found to have many different roles. These roles include the integration of endocrine, autonomic, emotional, and behavioral responses of the organism (Brown et al., 1982b, Bilezikjian and Vale, 1987, Baldwin, 1990, Dunn and Berridge, 1990, Beyermann, 1997, Moreau, 1997). Central administration of corticotropin releasing factor (CRF) produces increases in: 1) ACTH release from the pituitary (Rivier et al., 1984), 2) glucocorticoid secretion (Rivest et al., 1989, Korte et al., 1993, Linthorst et al., 1997), 3) epinephrine and norepinephrine release (Brown et al., 1982a), 4) glucagon levels (Brown et al., 1982a), 5) and even dopamine and norepinephrine turnover rates in discrete brain regions (Matsuzaki et al., 1989). In summary, central CRF signaling is specifically involved in increases in both the physiological and the behavioral manifestation of a state of stress.

CRF-induced stress-related behavior includes increased locomotion (Sutton et al., 1982, Veldhuis and De Wied, 1984, Eaves et al., 1985, Sherman and Kalin, 1986, Ehlers and Chaplin, 1987, Sherman and Kalin, 1987), hypophagia (Britton et al., 1982, Gosnell et al., 1983, Britton et al., 1986a, Ruckebusch and Malbert, 1986), and anxiogenic behavior (Britton et al., 1982). Anxiogenic behavior consists of decreased rearing, food pellet approaches, and exploratory behavior (Berridge and Dunn, 1986, 1987) along with increased grooming and freezing behavior (Britton et al., 1982, Berridge and Dunn, 1986,

1987). Ventricular CRF administration also augments the acoustic startle reflex, (Swerdlow et al., 1989) and diminishes social interaction (Dunn and File, 1987).

In addition to producing pro-stress effects by itself, CRF can also exacerbate behavior in the presence of stress. In support, ventricular CRF administration augments freezing (Sherman and Kalin, 1988) and fighting (Tazi et al., 1987) behavior induced by inescapable footshock. On the other hand, ventricular administration CRF receptor antagonists block these effects of stress (Heinrichs et al., 1994, Menzaghi et al., 1994, Spina et al., 2000). In summary, global CRF administration to the brain of rodents mimics the behavioral response during stress (Dunn and Berridge, 1990) while global antagonism of CRF receptor signaling blocks stress-related behavioral responses (Heinrichs et al., 1994, Menzaghi et al., 1994, Spina et al., 2000).

UROCORTINS

Importantly CRF is not the only stress-related neuropeptide in the brain. Additional members of the CRF peptide family have been identified, including urocortins1-3 (Figure 6), which differ in their tissue distribution and receptor pharmacology (Vaughan et al., 1995, Hsu and Hsueh, 2001, Lewis et al., 2001, Reyes et al., 2001). The identification of CRF receptor agonists found in fish (urotensin), frog skin (sauvagine), and additional non-mammalian members (teleosts and amphibians) led to the discovery of additional mammalian CRF-related peptides (Beyermann, 1997). The first stress related neuropeptide other than CRF to be discovered was a novel 40-amino acid peptide termed urocortin

one (Ucn1) (Beyermann, 1997). Ucn1 was named after its sequence similarity to both carp urotensin (63%, “uro”) and mammalian CRF (45% “cort”) (Koob, 2010). Ucn1 projection distribution both overlaps and has differential distribution with CRF throughout the brain (Zorrilla, 2005).

Ucn1 is most heavily expressed in the edinger westphal nucleus (Vaughan et al., 1995). Two additional urocortin cloned ligand family members were discovered, termed Urocortin two (Ucn2) and Urocortin three (Ucn3). Ucn2, also called stresscopin-related peptide, is expressed in the hypothalamus, brain stem, and spinal cord (Hsu and Hsueh, 2001, Reyes et al., 2001, Yamauchi et al., 2005, Fekete and Zorrilla, 2007). Ucn3, also called stresscopin, is expressed in the hypothalamus and amygdala (Hsu and Hsueh, 2001, Lewis et al., 2001, Li et al., 2002, Fekete and Zorrilla, 2007).

Although, urocortins (1-3) share 20-45% sequence homology with CRF (Vaughan et al., 1995), the physiological functions of these stress related neuropeptides vary significantly. In contrast to CRF, urocortins do not regulate activation of the HPA-axis (Kageyama et al., 2003, Nemoto et al., 2009). Moreover, urocortin signaling has been shown to produce both a reduction in anxiety-related behavior and recovery from the effects of stress (Coste et al., 2000, Valdez et al., 2003, Todorovic et al., 2007, Tanaka and Telegdy, 2008). These effects have been compared and found to be the exact opposite to those of CRF signaling (Schank et al., 2012). Paradoxically, urocortin signaling has additionally been reported to also produce pro-stress-like effects (Henry et al., 2006, Land et al., 2008, Fekete et al., 2009, Pastor et al., 2011). With this in

mind, it is important to note that the most consistent finding when comparing CRF function to urocortin function is that CRF appears to regulate the initial reactions to stress while urocortin appears to regulate later stress adaptation (Weninger et al., 2000, Kozicz et al., 2001, Gaszner et al., 2004, Kozicz, 2007, 2009, Neufeld-Cohen et al., 2010a, Neufeld-Cohen et al., 2010b, Kozicz et al., 2011).

Figure 6: Corticotropin releasing factor peptide family ligand amino acid sequences. CRF-R1 binds CRF and Ucn1 but not Ucn2 and Ucn3. CRF-R2 binds all urocortins with higher affinity that CRF. Red are proline residues at position 11 and alanine residues at position 35 and 39 that are specific to CRF-R2 specific ligands Ucn2 and Ucn3. Blue are nonspecific CRF receptor invariant arginine at position 35 and 39 (Grammatopoulos, 2012).

		Binding Affinity	
		CRF-R1	CRF-R2
CRF	S-E-E-P-P-I-S-L-D-L-T-F-H-L-L-R-E-V-L-E-M-A-R-A-E-Q-L-A-Q-Q-A-H-S-N-R-K-L-M-E-I-I	++	+
Ucn1	D-N-P-S-L-S-I-D-L-T-F-H-L-L-R-T-L-L-E-L-A-R-T-Q-S-Q-R-E-R-A-E-Q-N--R-I--I--F--D-S-V	++	++
Ucn2	I-V-L-S-L-D-V-P-I-G-L-L-Q-I-L-L-E-Q-A-R-A-R-A-A-R-E-Q-A-T-T-N---------A-R-I-L---A-R-V-G-H-C	–	++
Ucn3	F-T-L-S-L-D-V-P-T-N-I-M-N-L-L-F-N-I-A-K-A-K-N-L-R-A-Q-A-A-A-N---------A-H-L-M--A-Q-I	–	++

NEUROPEPTIDES ARE NEUROMODULATORS

Neuropeptides, such as CRF and Urocortins, are characterized by volume neurotransmission. Volume neurotransmission is distinct from other forms of neurotransmission because the transmitter can diffuse outside of the synaptic cleft at biologically relevant concentrations (Garris et al., 1994, Zoli and Agnati, 1996, Barbour and Hausser, 1997, Gonon, 1997) a mode of signaling referred to as an ‘open’ synapse (Fuxe, 1991, Agnati et al., 1995, Zoli and Agnati, 1996).

Neuropeptides can act at longer distances from their release sites than classical neurotransmitters. The large dense core peptide-containing vesicles can be close to the neuropeptide receptors (Herkenham, 1987, Nusbaum, 2002) but most of these receptors are extrasynaptic (Zoli et al., 1999). Peptide release occurs in the general vicinity of axon terminals (Lysakowski et al., 1999, Karhunen et al., 2001, Nusbaum, 2002, Salio et al., 2006). Neuropeptides lack reuptake mechanisms (Zoli and Agnati, 1996) and the regulators of extracellular neuropeptide concentrations are enzymes which either degrade or convert the neuropeptide. These enzymes are located in the extracellular space or on nonsynaptic cell membranes (Burbach, 1993, Davis and Konings, 1993, Konkoy and Davis, 1996). Therefore the distance of neuropeptide action in the brain is determined by membrane bound peptidases (Nassel, 2009).

Neuropeptides and classical neurotransmitters are often produced by the same neurons (Jan and Jan, 1983, Chan-Palay, 1984, Hokfelt et al., 1987, Zupanc, 1996, Merighi, 2002, Salio et al., 2006). Neuropeptides such as CRF can also be co-packaged and co-released with classical neurotransmitters (Waselus and Van Bockstaele, 2007, Reyes et al., 2011). However, neuropeptides are often released only at higher stimulus frequencies than classical neurotransmitters (Nassel, 2009). Therefore, neuropeptides such as CRF and Ucns are released in a less precise manner than their co-released classical neurotransmitter counterpart (Nusbaum, 2002).

Peptidergic receptors, usually G-protein-coupled receptors (GPCRs), are commonly located perisynaptically on post- and pre-synaptic neurons.

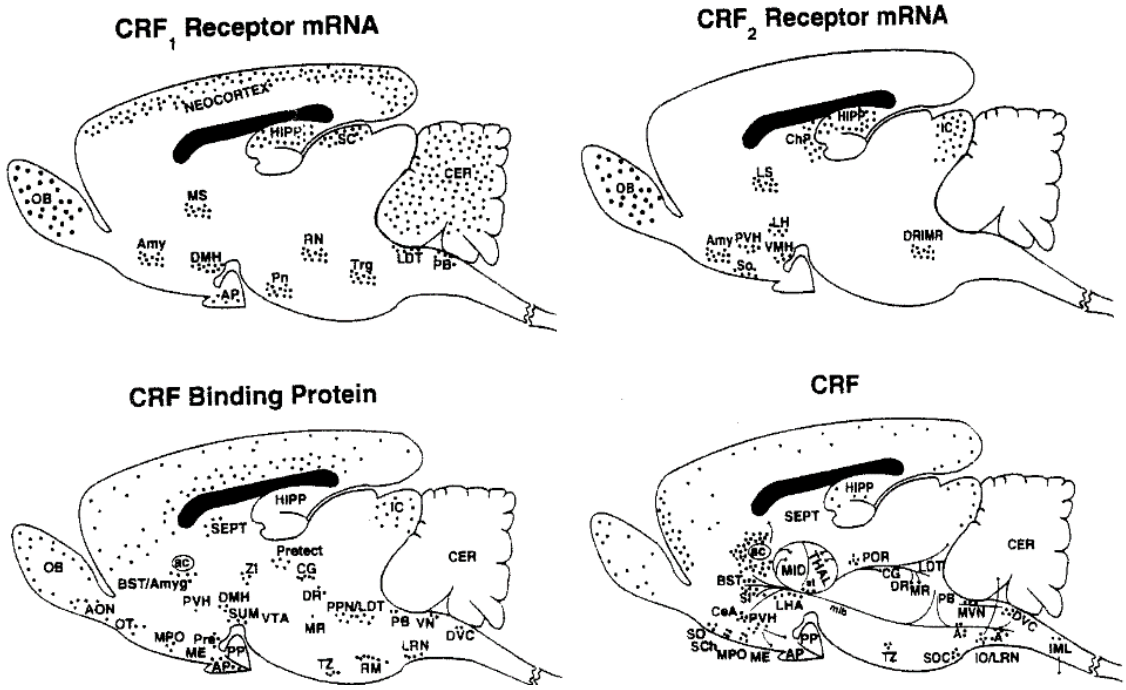
Neuropeptides can activate their GPCR receptor presynaptically resulting in autoregulation, postsynaptically on the same target neuron, or even different target neurons than their co-packaged, co-released classical neurotransmitter partners (Nassel, 2009). In this way, the GPCR signaling cascades of neuropeptide receptor activation modulate the response to classical neurotransmitters (Nassel, 2009). For these reasons neuropeptides, such as CRF and Ucns, are considered neuromodulators of classical neurotransmitters.

CORTICOTROPIN RELEASING FACTOR RECEPTORS

CRF and Ucns produce their effect through the coordinated action of two CRF receptor subtypes (Dautzenberg and Hauger, 2002). CRF receptors belong to the class B subtype of G protein-coupled receptors (GPCR) (Kehne and De Lombaert, 2002, Hartz et al., 2004). Class B receptors bind moderate-sized peptides involved in both endocrine and neuroendocrine functions (Ulrich et al., 1998, Harmar, 2001). The extracellular domain of CRF receptors primarily interacts with C-terminal residues of CRF (Rijkers et al., 2004, Yamada et al., 2004, Grace et al., 2007b). The N-terminal residues of CRF are required for both activation and the conformational changes of the receptors enabling their activation (Grace et al., 2004). Binding of a CRF receptor ligand agonist induces a conformational change and receptor activation where the G-protein (Gα-subunit) undergoes an exchange of GDP (inactive state) for GTP (active state) (Hamm and Gilchrist, 1996, Bohm et al., 1997). Once GTP is bound to the Gα-subunit, it dissociates from the Gβγ dimer, allowing for a variety of downstream signaling (Hillhouse and Grammatopoulos, 2006).

CRF receptors are seven transmembrane, G protein-coupled, and predominantly link to adenylate cyclase activation through $G\alpha_s$. However, CRF receptors are highly promiscuous, having the ability to activate multiple Gα subunits, including $G\alpha_s$, $G\alpha_o$, $G\alpha_{q/11}$, $G\alpha_{i1/2}$, and $G\alpha_z$ (Grammatopoulos et al., 1999, Grammatopoulos et al., 2001, Blank et al., 2003). The ability of GPCRs, such as CRF receptors, to couple to multiple G protein heterotrimers with different Gα-subunits results in diverse downstream signaling cascades and cellular responses (Hillhouse and Grammatopoulos, 2006). However, most physiological functions of CRF in the CNS involve the coupling of CRF to $G\alpha_s$-proteins (Grammatopoulos, 2012).

Figure 7: Anatomical expression of CRF-R1, CRF-R2, CRF binding protein, and CRF throughout the rat brain (Behan et al., 1996a).



The $G\alpha_s$ pathway initiates intracellular events both in the cytoplasm, resulting in acute post-translational modification of target proteins by protein kinase A (PKA), and in the nucleus at the level of gene transcription regulation by cyclic adenosine monophosphate (cAMP) response element-binding proteins (Shaywitz and Greenberg, 1999, Tasken and Aandahl, 2004). CRF-induced phosphorylation/activation of CREB leads to downstream regulation of genes containing the Ca^{2+}/cAMP response element (Rossant et al., 1999) such as *c-fos* (Boutillier et al., 1991). Interestingly, which Gα subunit is activated also depends on the ligand that is binding to that receptor (CRF vs. Ucns) (Grammatopoulos et al., 2000).

CORTICOTROPIN RELEASING FACTOR RECEPTOR 1

The first CRF receptor identified encoded a 415-amino acid comprised of seven putative membrane-spanning domains characteristic of G_s-coupled receptors, and was designated CRF receptor 1 (CRF-R1) (Chen et al., 1993). Species homologs for CRF-R1 have been isolated from the brains of both rat (Chang et al., 1993, Perrin et al., 1993) and mouse (Vita et al., 1993) which are 98% identical over their full length of 415 amino acids (De Souza, 1997). CRF-R1 shows reversible, saturable, high-affinity binding to CRF (K_D ~ 150 pM) (De Souza, 1997). CRF-R1 is coupled to a G-protein and when incubated in the presence of CRF, stimulates the production of cAMP with an EC_{50} of ~ 1 nM (De Souza, 1997). The CRF-R1 driven cAMP/PKA pathway can diverge and activate multiple downstream signaling molecules (Grammatopoulos, 2012).

Desensitization occurs through $G\alpha_s$/PKA induced phosphorylation of the CRF-R1 receptor, selectively impairing CRF-R1/$G\alpha_q$ coupling (Papadopoulou et al., 2004) and, indicative of yet even another level of complexity to CRF-R1 signaling. CRF-R1 has α and β isoforms in addition to subtypes designated c-h, which have been detected in both human and rodent tissue (Bale and Vale, 2004). However, many of these isoforms have been found to be nonfunctional (Chen et al., 1993, Ross et al., 1994, Grammatopoulos et al., 1999, Pisarchik and Slominski, 2001). cAMP produced by CRF activation of CRF-R1 receptor is competitively inhibited by CRF-R1 antagonists antalarmin and CP-376395 (Webster et al., 1996, De Souza, 1997, Chen et al., 2004, Guo et al., 2005, Di Fabio et al., 2008).

CRF-R1 is widely expressed throughout the brain with very high expression in the neocortex, cerebellum, and sensory relay structures (Figure 7) (Chalmers et al., 1995, Primus et al., 1997, Van Pett et al., 2000) such as the cortex, cerebellum, hippocampus, amygdala, olfactory bulb, lateral septum, thalamus, basal ganglia, the raphe nuclei, pituitary, brain stem, and spinal cord (De Souza, 1997, Van Pett et al., 2000, Korosi et al., 2006, Korosi et al., 2007, Justice et al., 2008). Expression of the CRF-R1 overlaps with the distribution of CRF and Ucn 1 (Behan et al., 1996a, Skelton et al., 2000, Koob, 2010).

CRF and urocortin release can also regulate CRF-R1 gene expression through changes in transcription activity (Luo et al., 1995, Mansi et al., 1996, Brunson et al., 2002, Kasagi et al., 2002, Parham et al., 2004, Herringa et al., 2006). CRF-R1 mRNA expression has been well characterized in the rodent

brain (Van Pett et al., 2000), however, due to insufficient commercially available CRF receptor antibodies (Refojo et al., 2011) CRF-R1 protein expression in specific brain regions have not been accurately assessed. This is also true for the CRF-R2 receptor.

CORTICOTROPIN RELEASING FACTOR RECEPTOR 2

A second CRF receptor exists and is referred to as CRF-R2 (Lovenberg et al., 1995b). CRF-R2 is a 397-437 amino acid protein also expressed in the brain (Kishimoto et al., 1995, Lovenberg et al., 1995b, Kostich et al., 1998, Palchaudhuri et al., 1999, Van Pett et al., 2000, Korosi et al., 2007). CRF-R2 activation in the presence of CRF stimulates the production of cAMP with an EC_{50} of 20 nM (Liaw et al., 1996). cAMP produced by CRF activation of CRF-R2 receptors is competitively inhibited by CRF-R2 antagonists (Ruhmann et al., 2002). However, CRF-R2 abundance is not as dense as that of CRF-R1 (Figure 7) (Van Pett et al., 2000). CRF-R2 is confined to subcortical structures, showing relative high expression levels in the pituitary, lateral septum, hypothalamus, dorsal and median raphe, extended amygdala, and spinal cord (Chalmers et al., 1995, Primus et al., 1997, Palchaudhuri et al., 1999, Bittencourt and Sawchenko, 2000, Van Pett et al., 2000, Korosi et al., 2006, Korosi et al., 2007, Lukkes et al., 2011). CRF-R2 has three functional isoforms α, β, and γ (Dautzenberg and Hauger, 2002) produced through alternative splicing of a single gene, resulting in different N-terminal domains (De Souza, 1997).

CRF-R2 isoforms differ in their N-terminal sequence and their distribution in different tissues and species. CRF-R2α and CRF-R2β are present in humans and rodents (Lovenberg et al., 1995a, Liaw et al., 1996). In contrast, CRF-R2γ has only been reported in humans (Kostich et al., 1998) and is predominantly expressed in the brain (Kostich et al., 1998). CRF-2α and CRF-2β are both expressed in the rodent brain (Lovenberg et al., 1995a, Kostich et al., 1998). However, CRF-2α is the predominant isoform in the brain (Kostich et al., 1998) and is potently and selectively antagonized by astressin-2B and antisauvagine-30 (Ruhmann et al., 1998, Rivier et al., 2002).

DIFFERENCES BETWEEN CRF-R1 AND CRF-R2

The different CRF receptors are produced from distinct genes with several splice variants (Bale and Vale, 2004). Different CRF receptor subtypes exhibit varying degrees of ligand affinities and sensitivity to G-protein coupling. There is 70% sequence homology between CRF-R1 and CRF-R2 at the amino acid level (Lovenberg et al., 1995b) with greater than 80% homology at the transmembrane and intracellular domains (Lovenberg et al., 1995b). The third intracellular loop is the receptor region that interacts with G proteins and is identical between both CRF receptors (Perrin and Vale, 1999, Arai et al., 2001). However, these receptors exhibit considerable differences at the N-terminal extracellular domain (~ 47%) (Dautzenberg and Hauger, 2002, Grammatopoulos, 2012). The juxtamembrane region of the N-terminus and the second and third extracellular domains are important ligand action sites that determine ligand binding and

receptor specificity (Assil et al., 2001, Hofmann et al., 2001, Perrin et al., 2001, Dautzenberg et al., 2002, Perrin et al., 2003).

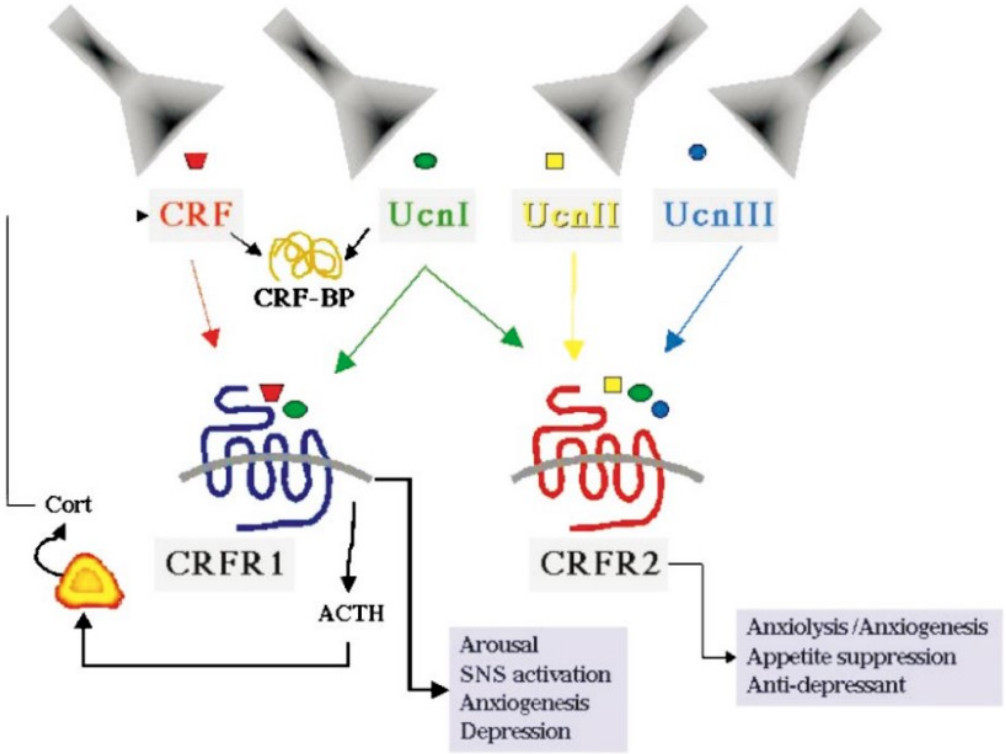
CRF has at least tenfold higher affinity for CRF-R1 over CRF-R2 (cAMP EC_{50} = 3 nM over 20 nM; Ki = 2 nM over 30 nM) (Chen et al., 1993, Lovenberg et al., 1995b, Behan et al., 1996a), while Ucn1 has equal affinities for both receptors (cAMP EC_{50} = 0.3 nM-R1 and .2 nM-R2; Ki = 0.3 nM-R1 and 0.6 nM-R2) (Perrin et al., 1995, Reyes et al., 2001). Ucn 2 and 3 bind and activate CRF-R1 and CRF-R2 receptors with varying affinities (Bale and Vale, 2004, Boorse et al., 2005, Fekete and Zorrilla, 2007). Although, Ucn1 binds to both CRF receptors with equal affinities (Reyes et al., 2001) Ucn2 and Ucn3 bind only to the CRF-R2 receptor at physiological levels (cAMP EC_{50} = 0.1 nM-Ucn2 and 0.07 nM-Ucn3; Ki = 2 nM-Ucn2 and 5 nM-Ucn3) (Lewis et al., 2001, Zorrilla et al., 2003).

In summary, CRF-R1 binds CRF as well as urocortin 1 with high affinity, but binds Ucn2 and Ucn3 with significantly lower affinity (Reyes et al., 2001). CRF-R2 binds all urocortins with significantly higher affinity that CRF (Chen et al., 1993, Lovenberg et al., 1995b, Vaughan et al., 1995, Hsu and Hsueh, 2001, Lewis et al., 2001, Reyes et al., 2001). In contrast to CRF-R1, CRF-R2 has higher affinity for urocortins 2 and 3 than for CRF suggesting that these urocortins and not CRF may be the endogenous ligand for the CRF-R2 receptor (De Souza, 1997).

Ucn2 and Ucn3 are endogenous CRF-R2 specific agonists while both CRF and Ucn1 are nonspecific agonists (Figure 8). Recently, cortagine, a CRF-

R1 agonist, was generated by the synthesis of chimeric peptides derived from human/rat CRF, ovine CRF, and sauvagine (Tezval et al., 2004). This chimeric peptide allows for further pharmacological characterization of CRF-R1 versus CRF-R2 function.

Figure 8: CRF ligand and receptor specificity with their physiological and behavioral affects. CRF increases stress responsiveness through activation of CRF-R1, while activation of CRF-R2 has been hypothesized to be involved in coping with stress. (Bale and Vale, 2004).



CORTICOTROPIN RELEASING FACTOR BINDING PROTEIN

CRF not only has high affinity for the CRF-R1 receptor but also for the corticotropin releasing factor-binding protein (CRF-BP). CRF-BP is a 37 kDa N-

linked secreted glycoprotein that binds extracellular CRF. CRF-BP prevents CRF from activating its receptors (Suda et al., 1988, Woods et al., 1994, Herringa et al., 2004) while also promoting CRF clearance and degradation (Burrows et al., 1998, Karolyi et al., 1999). CRF-BP is found throughout the rodent and primate brain, including in the hypothalamus, cortical regions, amygdala, bed nucleus of the stria terminalis (BNST), and raphe nuclei (Potter et al., 1991, Potter et al., 1992, Chen et al., 1993, Potter et al., 1994, Behan et al., 1995, Cortright et al., 1995). CRF-BP binds both CRF and urocortin 1 (Potter et al., 1991, Behan et al., 1996b) and is highly conserved between humans and rats (Denver, 2009).

CRF-BP binds both CRF and Ucn1 with high affinity (Ki = 0.2 nM-CRF and 0.9 nM-Ucn1) (Orth and Mount, 1987, Boorse et al., 2005), and Ucn2 with lower affinity (Ki = 12 nM-Ucn2), but does not bind Ucn3 (Huising et al., 2008). CRF-BP affinity for both CRF and Ucn1 is several-fold higher than that of either CRF receptor (Huising et al., 2008). When CRF-BP binds CRF or Ucn1, a functional dimer complex is formed which is hypothesized to have multiple functions including uptake and degradation of the CRF-BP/CRF complex (Potter et al., 1991, Behan et al., 1996b, Kemp et al., 1998, Sajdyk et al., 1999, Chan et al., 2000, Roseboom et al., 2007). CRF-BP is often considered a functional CRF receptor antagonist due to its ability to sequester ligands and prevent CRF receptors from becoming activated (Huising et al., 2008).

CRF-BP levels are ~ 10-fold higher than CRF levels in most brain regions and CRF-BP binds between 40-90% of endogenous CRF (Suda et al., 1988, Behan et al., 1996a). In this way, CRF-BP limits the concentrations of CRF and Ucn1

available to activate receptors by acting as a buffer for CRF system signaling (Behan et al., 1996b, Chan et al., 2000). Stress-induced CRF release can increase CRF-BP mRNA levels independently of corticosterone or CRF receptor function (Herringa et al., 2004, Herringa et al., 2006). Acute stress and ICV CRF administration both increase CRF-BP mRNA levels (Herringa et al., 2004, Herringa et al., 2006). This increased CRF-BP mRNA expression is not blocked by CRF receptor antagonists nor mimicked by administration of corticosterone (Herringa et al., 2006). These findings support the notion that when CRF is bound by CRF-BP there is an increase in CRF-BP transcription (Lombardo et al., 2001, Roseboom et al., 2007). This increased CRF-BP transcription likely represents an acute compensatory homeostatic mechanism in response to increased CRF levels.

In summary, CRF was originally discovered to have a central role in initiating the hypothalamic-pituitary-adrenal (HPA) axis response to stress, yet its role throughout the rest of the brain is very complex involving: CRF, Ucn1, Ucn2, Ucn3, CRF-BP, CRF-R1 and CRF-R2 (Vale et al., 1981, Behan et al., 1995, Steckler and Holsboer, 1999, Ryabinin et al., 2002, Fekete and Zorrilla, 2007).

CORTICOTROPIN RELEASING FACTOR RECEPTOR FUNCTION

CRF receptors participate in the regulation and maintenance of homeostasis in response to stress (Preil et al., 2001, Bale et al., 2002, Bale and Vale, 2004, Janssen and Kozicz, 2013). Ventricular administration of CRF-R1 antagonists inhibit stress-induced behaviors and decrease both basal and stress-

induced HPA axis activation (Swerdlow et al., 1989, Heinrichs et al., 1992, Kalin, 1998, Habib et al., 2000). Generally administration of CRF-R1 antagonists produces anxiolytic effects (Schulz et al., 1996, Deak et al., 1999, Okuyama et al., 1999, Risbrough et al., 2004). In contrast, CRF-R2 activation has been associated with an increase and a decrease in both stress responsiveness and stress-related behavior (Radulovic et al., 1999, Bale et al., 2000, Kishimoto et al., 2000, Pelleymounter et al., 2000, Cullen et al., 2001, Takahashi et al., 2001, Bakshi et al., 2002, Valdez et al., 2002, Risbrough et al., 2004, Kehne and Cain, 2010, Neufeld-Cohen et al., 2012).

A coordinated functional dualism, where each receptor regulates different aspects of the stress response has been proposed. Specifically, it has been suggested that CRF-R1 activation results in the initiation of a physiological stress response, while activation of the CRF-R2 receptor facilitates recovery from stress (Janssen and Kozicz, 2013). However, this simple dualism of CRF receptor subtype function is likely too simplistic (Janssen and Kozicz, 2013). The coordinated action of these receptors along with their differential anatomical distribution (Chalmers et al., 1995, Van Pett et al., 2000) suggests more complexity than a simple functional dualism.

Although there is conflicting evidence as to the role for CRF-R2 activation in the physiological and behavioral response to stress, there remains little question as to the function of the CRF-R1 receptor. CRF-R1 activation produces effects that cause and resemble the stress response (Koob and Thatcher-Britton, 1985, Britton et al., 1986b, Rassnick et al., 1993, Menzaghi et al., 1994,

Rodriguez de Fonseca et al., 1996, Koob and Heinrichs, 1999, Cullen et al., 2001, Spina et al., 2002).

CRF AND COCAINE-SEEKING BEHAVIOR

The effects of CRF system signaling has been implicated in cocaine-seeking behavior. Cocaine-intake dependent increases in CRF signaling have been reported in the extended amygdala (Sarnyai et al., 1995, Zhou et al., 1996, Gardi et al., 1997). The extended amygdala consists of basal forebrain regions that share cytoarchitecture and circuitry involved in feeding, reproduction, learning, cognition, punishment, and reward (Alheid, 2003). The extended amygdala is made up of the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), and the shell of the nucleus accumbens (Heimer and Alheid, 1991). These areas that make up the extended amygdala share similarities in immunohistochemistry, morphology, and connectivity (Alheid and Heimer, 1988). The extended amygdala plays a pivotal role in the behavioral, emotional, and physiological response of an organism to fear and anxiety (Duvarci et al., 2009, Regev et al., 2011, Park et al., 2012).

Following drug exposure the extended amygdala regulates dysphoria during both withdrawal and protracted abstinence (Koob, 1999a). This dysphoria is regulated by norepinephrine and CRF signaling throughout the extended amygdala and can be exacerbated with exposure to acute stress (Rassnick et al., 1993, Koob, 1994, Heinrichs et al., 1995, Rodriguez de Fonseca et al., 1997, Shaham et al., 1997, Erb et al., 1998, Shaham et al., 1998, Erb and Stewart,

1999, Richter and Weiss, 1999, Sinha et al., 1999, Erb et al., 2000, Stine et al., 2002, Sinha et al., 2003, McFarland et al., 2004, Feltenstein and See, 2006, Smith and Aston-Jones, 2008, Brown et al., 2009, Mantsch et al., 2010, Brown et al., 2011, Graf et al., 2011, Jobes et al., 2011, Vranjkovic et al., 2012).

LgA self-administration appears to recruit the CRF system. CRF immunoreactivity is increased in the extended amygdala following daily long-access to cocaine for self-administration (Zorrilla et al., 2012). Moreover, ventricular administration of a CRF-R1 antagonist only attenuates cocaine self-administration in rats tested under LgA conditions (Specio et al., 2008). This suggests that loss of control over drug intake is at least partly regulated by CRF signaling at the time of drug self-administration (Ahmed and Koob, 1998). In addition, withdrawal from cocaine produces increased CRF signaling and anxiogenic behavior which can be blocked by inhibiting CRF system signaling (Sarnyai et al., 1995). These findings suggest that recruitment of the CRF system plays an integral part in both escalating patterns of self-administration and withdrawal induced dysphoria.

Increases in CRF function induced by high intake cocaine self-administration are not transient but persist throughout protracted abstinence (Koob and Le Moal, 2001). This may contribute to the augmented ability of footshock stress to induce reinstatement of extinguished cocaine seeking following LgA self-administration (Mantsch et al., 2004, Blacktop et al., 2011, Graf et al., 2011). Thus, repeated cocaine self-administration changes CRF reactivity in the brain in a way that promotes later stress-induced reinstatement of extinguished cocaine

seeking (Erb et al., 1998, Shaham et al., 1998, Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011).

Ventricular CRF administration is sufficient to reinstate extinguished cocaine seeking; an effect blocked by systemic CRF-R1 specific antagonists or central ICV CRF-R1/R2 nonspecific antagonists (Erb et al., 1998, Shaham et al., 1998, Erb et al., 2006b, Mantsch et al., 2008a, Graf et al., 2011, Buffalari et al., 2012). Notably, CRF-R1 antagonists attenuate footshock-induced reinstatement of not only cocaine- but also heroin-, alcohol, and nicotine-seeking (Erb et al., 1998, Shaham et al., 1998, Le et al., 2000, Bruijnzeel et al., 2009). Thus, the CRF system represents a common mechanism for stress-induced relapse to multiple common drugs of abuse. For these reasons, the CRF system has been hypothesized to represent a promising target for the development of medications that prevent stress from facilitating relapse in abstinent human drug addicts (Webster et al., 1996, Deak et al., 1999, Koob and Zorrilla, 2010).

Stress-induced reinstatement of drug seeking can occur independently of acute stress-induced activation of the HPA axis at the time of reinstatement (Shaham et al., 1997, Erb et al., 1998, Le et al., 2000, Graf et al., 2011). Ventricular CRF administration is sufficient for reinstatement, while ICV delivery of a nonspecific CRF receptor antagonist blocks footshock-induced reinstatement of cocaine seeking. CRF- and stress-induced reinstatement is observed both in animals that have functioning or nonfunctioning corticosterone signaling (Erb et al., 1998, Lu et al., 2003b, Mantsch et al., 2008b, Graf et al., 2011). However, although glucocorticoids do not appear to be required for acute stress-induced

reinstatement, they are necessary for the neuroplastic changes to occur at the time of drug use (Mantsch et al., 2008b) hypothesized to gate the ability of later footshock stress to cause reinstatement (Graf et al., 2011). Corticosterone levels are increased as a result of cocaine self-administration (Goeders and Guerin, 1996b, Deroche et al., 1997, Mantsch et al., 2000, Mantsch et al., 2003). This increase in corticosterone is hypothesized to induce neuroplastic changes in areas of convergence between stress and motivational neurocircuitry. This neuroplasticity may change brain function to where the circuitry of motivated behavior is more responsive to acute stress-induced CRF release. In summary, stress-induced reinstatement of extinguished cocaine seeking following LgA self-administration is dependent on glucocorticoid secretion at the time of drug self-administration, and CRF signaling by an acute stressor (footshock) at the time of reinstatement.

CRF actions in both the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) are critical for footshock-induced reinstatement of extinguished cocaine seeking (Erb et al., 1996, Erb and Stewart, 1999, Erb et al., 2001). As mentioned previously, the two brain regions outside of the paraventricular nucleus of the hypothalamus (PVN) that have the highest concentrations of CRF-containing cell bodies are the BNST and the CeA (Merchenthaler et al., 1982, Olschowka et al., 1982, Swanson et al., 1983, Petrusz, 1992). The BNST and CeA both send CRF-containing projections to the ventral tegmental area (VTA) (Beckstead et al., 1979, Phillipson, 1979a, Wallace

et al., 1989, Rodaros et al., 2007). We hypothesize that this circuitry enables CRF to regulate the effects of stress on the dopamine system.

The VTA is the origin of midbrain dopamine neurons that make up the circuitry of motivated behavior. In addition to CRF inputs, CRF-R1 and CRF-R2 are also found in the VTA (De Souza, 1987, Perrin et al., 1993, Behan et al., 1996a, Van Pett et al., 2000, Ungless et al., 2003, Korotkova et al., 2006, Wang et al., 2007, Wanat et al., 2008, Beckstead et al., 2009, Blacktop et al., 2011, Wanat et al., 2013). This positions the ventral tegmental area as a potential key neuroanatomical region involved in the complex integration of stress and reward related signaling.

REINSTATEMENT NEUROCIRCUITRY

The mesocorticolimbic dopamine system consists of midbrain dopamine neurons in the ventral tegmental area (VTA; meso) and their terminal fields in the medial prefrontal cortex (mPFC; cortico), and nucleus accumbens (NA; limbic) (Figure 9) (Fields et al., 2007). The NA is involved in responding to rewarding and salient stimuli, while the PFC is involved in emotion, cognition, executive function, and inhibitory control processes (Everitt and Robbins, 2005). It is well established that central to addiction is neurotransmission within the mesocorticolimbic system (Di Chiara and Imperato, 1988, Wise and Rompre, 1989). This includes the motivation processes that underlie the reinstatement of drug seeking.

The upstream mechanisms of cocaine-, cue-, and stress-induced cocaine seeking can differ. For example, stress-induced reinstatement but not cocaine-induced reinstatement is blocked by CRF receptor antagonists (Graf et al., 2011) suggesting distinct neuromechanisms (Capriles et al., 2003). In contrast, cue-induced and stress-induced reinstatement are less easily differentiated because footshock stress-induced reinstatement only occurs when given in the drug-taking context (Shalev et al., 2000). This is indicative that drug-related cues may be critically involved in CRF-dependent stress-induced relapse. However, cocaine-, cue-, and stress-induced reinstatement of cocaine seeking all have been suggested to share a common downstream neurocircuit (Figure 10). This putative circuit involves dopaminergic input from the VTA to the medial prefrontal cortex which in turn provides glutamate input to the nucleus accumbens core (McFarland and Kalivas, 2001, McFarland et al., 2004).

Figure 9: Schematic illustrating simplified version of dopamine (**blue**), glutamate (**red**), and GABA (**green**) signaling in the mesocorticolimbic system.

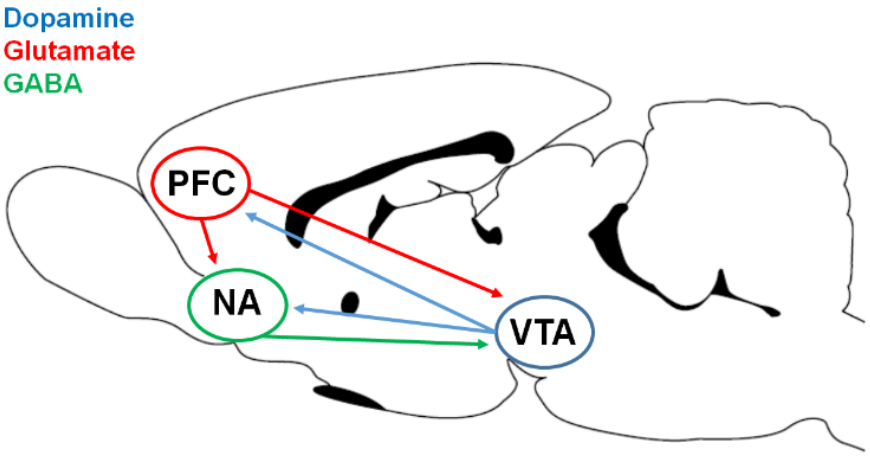
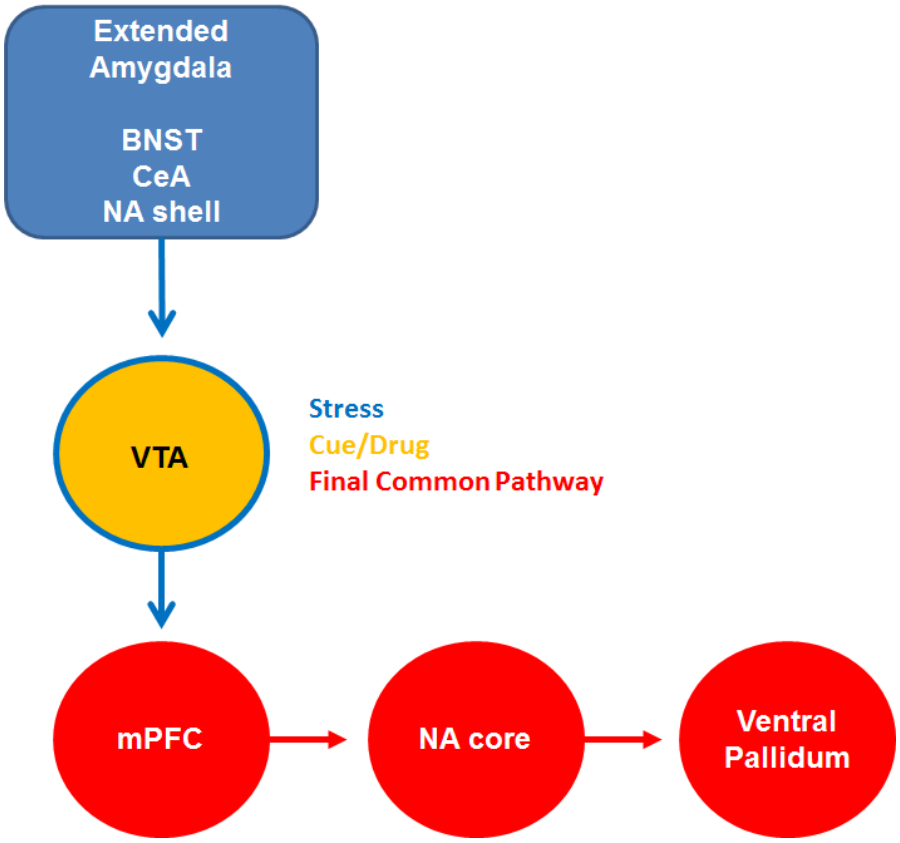


Figure 10: Schematic illustrating that the PFC functions as a final relay station in the reinstatement of drug seeking (Kalivas and Volkow, 2005). The blue is illustrating the upstream extended amygdala component of stress-induced reinstatement (**blue**), the conversion of both cue (**yellow**) and stress triggers upon the VTA, and the final common pathway for all three stimuli (**red**) (drug, cue, and stress) in reinstatement.



The medial prefrontal cortex (mPFC) functions as a main terminal field of the VTA as well as a final relay station in relapse evoked by drugs, cues, and stress (Kalivas et al., 2005, Kalivas and Volkow, 2005). In support, inactivation of this region blocks reinstatement of cocaine seeking by all three modalities (McFarland and Kalivas, 2001, McLaughlin and See, 2003, Fuchs et al., 2005). Drug seeking depends on activation (Ciccocioppo et al., 2001, Zavala et al.,

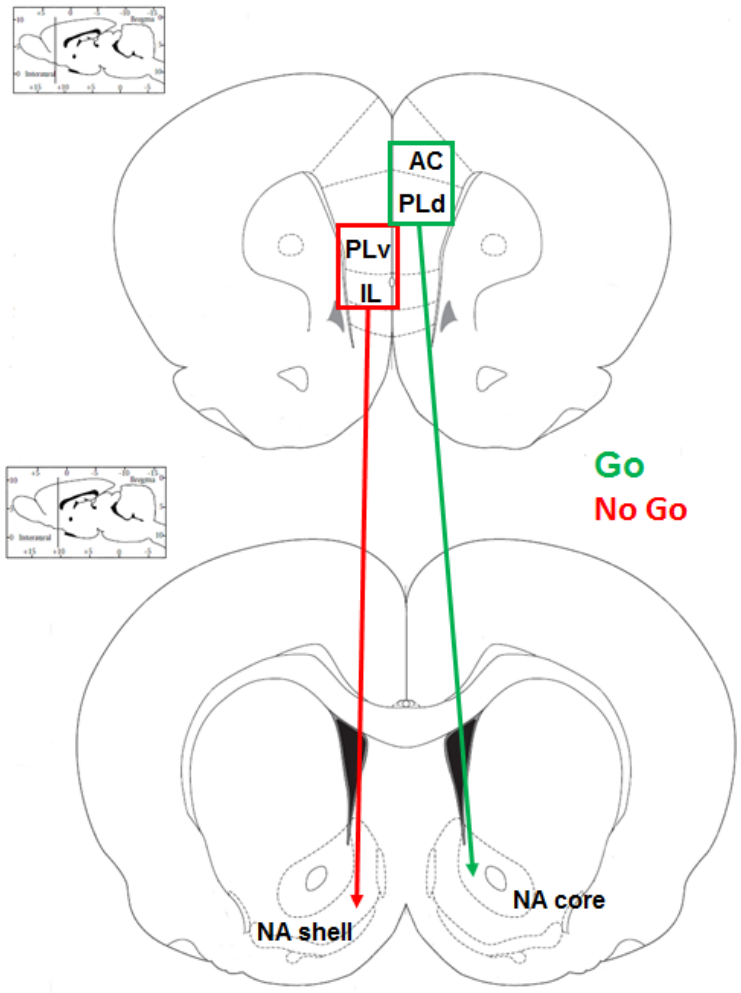
2008) of a glutamate projection from the PFC to the nucleus accumbens core (Kalivas et al., 2005). This regulates the NA core output to the ventral pallidum which completes the pallido-thalamo-cortical circuit involved in motivated behavior (Kalivas et al., 1999).

The nucleus accumbens (NA) is divided into two traditional subregions with differential functions. These regions are the nucleus accumbens (NA) core and the nucleus accumbens shell, which together form the ventral striatum. The NA core is often considered an extension of the dorsal striatum functioning in instrumental learning and cue-induced reinstatement (Ito et al., 2000, Cardinal and Everitt, 2004, Ito et al., 2004).The NA shell is characterized as a transitional zone between the striatum and the extended amygdala functioning in the reinforcing effects of drugs of abuse (Kalivas et al., 2005, Pierce and Kumaresan, 2006). The NA core receives glutamate inputs from the dorsomedial prefrontal cortex (Gabbott et al., 2005, Reynolds and Zahm, 2005) while NA shell receives glutamate input from the ventromedial PFC (Gabbott et al., 2005, Reynolds and Zahm, 2005).

The dmPFC is comprised of the anterior cingulate (AC) and dorsal prelimbic (dPL) cortices while the vmPFC is comprised of the ventral prelimbic (vPL) and infralimbic cortices (Van den Oever et al., 2010). In summary, the projections from mPFC to the NA are organized into a dorsal-ventral pattern, where the dorsal mPFC projects predominantly to the NA core while the vmPFC projects predominantly to the NA shell (Figure 11) (Heidbreder and Groenewegen, 2003,

Voorn et al., 2004). These two circuits have differential role in regulating drug-seeking behavior.

Figure 11: Schematic illustrating the mPFC subregions and their inputs to NA subregions producing the 'Go, No Go' circuit (Van den Oever et al.) involved in footshock-induced reinstatement (Capriles et al., 2003, Sanchez et al., 2003, McFarland et al., 2004). Glutamate inputs from the dorsal medial PFC (anterior cingulate [AC] + dorsal prelimbic cortex [PLd]; **green**) to the NA core is thought to be a final relay to induce drug seeking (McFarland et al., 2003). In contrast, glutamate projections from the ventral medial PFC (ventral prelimbic cortex [PLv], infralimbic cortex [IL]; **red**) to the NA shell are thought to suppress drug seeking (Peters et al., 2008). Together, these circuits make the "Go No Go" circuit involved in drug-seeking behavior (LaLumiere et al., 2012), both regulated through dopaminergic inputs from the VTA



The glutamate input from the dmPFC to the nucleus accumbens core has been proposed to be a final downstream circuit involved in the renewal of extinguished drug seeking induced by drugs, cues, and footshock stress (Cornish et al., 1999, Capriles et al., 2003, McFarland et al., 2003, McFarland et al., 2004, LaLumiere and Kalivas, 2008). A parallel circuit involves the glutamatergic input from the vmPFC to nucleus accumbens shell which suppresses drug seeking (LaLumiere et al., 2010, LaLumiere et al., 2012). This circuit appears to be involved in the extinction process.

Extinction is a form of learning (Bouton, 2002). It has been proposed that the ventral medial prefrontal cortex and its glutamate input into the nucleus accumbens shell regulates suppression of drug seeking (Peters et al., 2008) through the formation of new extinction memories (LaLumiere et al., 2010). This is further supported by the findings that inactivation of either the infralimbic cortex or the nucleus accumbens shell reinstates drug seeking without drug, cue, or stress triggers (Peters et al., 2008).

Following extinction of drug-seeking behavior, the glutamatergic neurons in the dorsal medial PFC that project to the NA core are highly responsive to reinstating stimuli (Van den Oever et al., 2010). This produces opposing regulation of drug seeking through two different circuits involving different subregions of the medial prefrontal cortex and nucleus accumbens (Cornish et al., 1999, McFarland and Kalivas, 2001, McFarland et al., 2003, McFarland et al., 2004, Fuchs et al., 2008, Peters et al., 2008, Van den Oever et al., 2010,

LaLumiere et al., 2012). Notably, stress-induced reinstatement of cocaine seeking requires the activity of the ventral tegmental area, dorsal medial prefrontal cortex, and the nucleus accumbens core (Di Ciano and Everitt, 2001, McFarland and Kalivas, 2001, McLaughlin and See, 2003). Therefore, footshock stress-induced reinstatement of extinguished LgA cocaine seeking likely involves activation of the prelimbic nucleus accumbens core pathway, inhibition of the infralimbic nucleus accumbens shell pathway, or both. Since the VTA projects to both these circuits and receives CRF input from the extended amygdala, it is ideally positioned to regulate drug seeking in response to stress.

THE VENTRAL TEGMENTAL AREA: CONVERGENCE OF STRESS AND MOTIVATIONAL NEUROCIRCUITRY

The VTA represents a convergence point where dopamine (Wise and Rompre, 1989, Kalivas, 1993, Wise et al., 1995), glutamate (Stuber et al., 2010, Tecuapetla et al., 2010), and GABA (Van Bockstaele and Pickel, 1995, Ikemoto et al., 1997a, Steffensen et al., 1998, Xi and Stein, 1998, Carr and Sesack, 2000a, Doherty and Gratton, 2007) neurons interact to control motivated behavior. VTA dopamine, CRF, GABA, and glutamate neurotransmission have all been implicated in drug addiction and drug seeking (Brebner et al., 2002, Wang et al., 2005, Wang et al., 2007, Koob and Volkow, 2010). This implication includes stress-induced reinstatement of cocaine seeking. Points of convergence between reward- and stress-related circuitry, as seen in the VTA, provide potential neurobiological substrates for the therapeutic intervention in stress-induced relapse.

VTA dopamine neurons have an integral role in reward- and motivationally-relevant behaviors (Wise and Rompre, 1989, Bjorklund and Dunnett, 2007, Schultz, 2007b, Matsumoto and Hikosaka, 2009b, Tsai et al., 2009, Bromberg-Martin et al., 2010, Cohen et al., 2012, Kim et al., 2012). To this end, VTA dopamine neuron firing conveys rewarding and motivationally relevant information (White, 1996)- initiating, promoting, maintaining, and driving reward-seeking behavior (Fields et al., 2007, Sun, 2011). In order to characterize the effects of intra-VTA drug-induced neuroplasticity on stress-induced relapse, it is important to first acknowledge the complexities of VTA neurocircuitry.

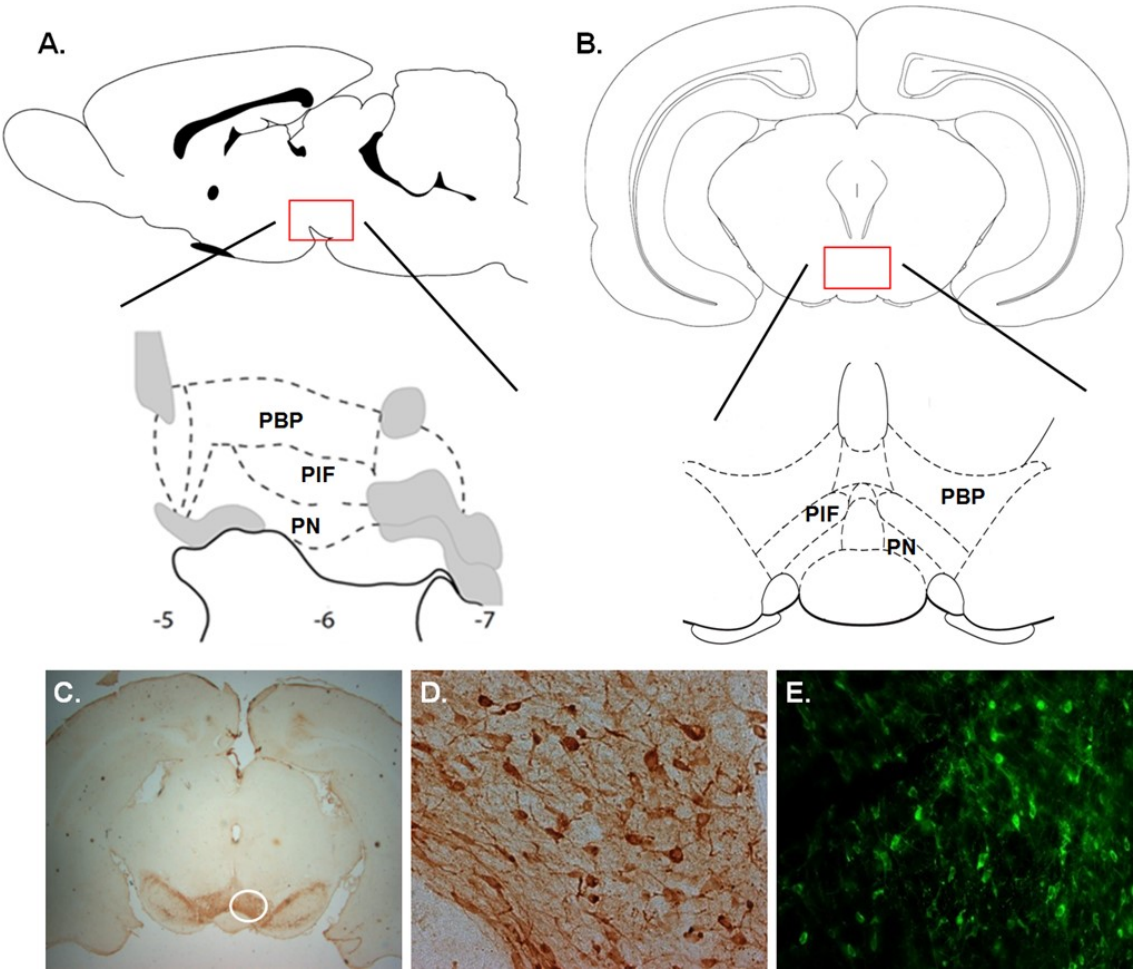
The ventral tegmental area (VTA) is synonymous with area A10 of grouped dopamine neurons (Dahlstrom, 1964, Fallon and Moore, 1978, Moore and Bloom, 1979). The VTA/A10 region is comprised of three major nuclei: (1) the parabrachial pigmented (PBP), (2) parainterfascicular (PIF), and (3) paranigral (PN) nuclei (Figure 12) (Swanson, 1982). These nuclei are particularly rich in dopaminergic cell body concentrations (Dahlstrom, 1964, Fallon et al., 1978, Moore and Bloom, 1978, Phillipson, 1979b, Halliday and Tork, 1986, Paxinos, 2007). The VTA is a midbrain structure that consists of approximately 14,000 neurons comprised of a mixture of dopamine neurons (~65%) and GABA neurons (~35%) (Swanson, 1982, Oades and Halliday, 1987, Johnson and North, 1992b). Subpopulations of these VTA dopamine and GABA neurons can subserve different functions (Guarraci and Kapp, 1999, Bjorklund and Dunnett, 2007, Lammel et al., 2008, Margolis et al., 2008, Berridge et al., 2009, Matsumoto and Hikosaka, 2009b, Bromberg-Martin et al., 2010, Sesack and

Grace, 2010, Lammel et al., 2011, Cohen et al., 2012, Kim et al., 2012, Tan et al., 2012, van Zessen et al., 2012).

VENTRAL TEGMENTAL AREA NEUROCIRCUITRY IS NOT HOMOGENOUS

The ventral tegmental area (VTA) should not be viewed as a homogenous structure in function, anatomy, or physiology. VTA dopamine neurons have unique subpopulations with different inputs, axonal projections, and unique neurochemical and electrophysiological properties (Bannon and Roth, 1983, White, 1996, Tzschentke, 2001, Margolis et al., 2006, Ikemoto, 2007, Lammel et al., 2008, Margolis et al., 2008, Lammel et al., 2011, Lammel et al., 2012). The heterogeneous nature of the VTA provides different neuronal populations that are essential for the expression of motivated behaviors and actions related to addiction (Wise, 2004, Fields et al., 2007, Ikemoto, 2007, Lammel et al., 2008, Lammel et al., 2011, Luscher and Malenka, 2011, Lammel et al., 2012). The functional heterogeneity of the VTA was first demonstrated along its rostral caudal axis in behavioral studies via local intracranial self-administration procedures (Carlezon et al., 2000, Rodd-Henricks et al., 2000, Ikemoto and Wise, 2002, Zangen et al., 2002, Bolanos et al., 2003, Olson et al., 2005, Rodd et al., 2005a, Rodd et al., 2005b, Ikemoto et al., 2006).

Figure 12: Schematic illustrating the area of interest for this dissertation, the posterior VTA. 12A & 12B are illustrating sagittal and coronal views of the VTA, respectively, with a close up illustrating PBP, PIF, and PN nuclei. 12C-E are illustrating high dopamine neuron expression in the area of interest using (12C) immunohistochemistry (IHC) for tyrosine hydroxylase (TH; marker for dopamine neurons in the VTA) in low mag (12D) high mag, and (12E) high mag using immunofluorescence for TH (Paxinos, 2004, Brischoux et al., 2009).



The VTA can be divided into rostral and caudal subregions constituting functionally distinct areas in their ability to regulate rewarding effects of drugs of abuse (Ikemoto et al., 1997a, Ikemoto et al., 1997b, 1998, Carlezon et al., 2000, Ikemoto and Wise, 2002, Zangen et al., 2002, Bolanos et al., 2003, Ikemoto et al., 2003, Olson et al., 2005, Rodd et al., 2005a). These distinct subregions are referred to as anterior, posterior, and tail regions of the VTA (Ikemoto et al., 1998, Zangen et al., 2002, Olson et al., 2005, Perrotti et al., 2005, Ikemoto, 2007, Shabat-Simon et al., 2008, Kaufling et al., 2009).

The anterior VTA (aVTA) is the region dorsal to the medial mamillary nucleus and medial to the substantia nigra pars compacta (SNC) (Zhao-Shea et al., 2011). The aVTA contains the ventral tegmental area rostral (VTAR) and the parabrachial pigmented area (PBP), but not midline nuclei (interfascicular nucleus [IF], rostral linear [RLi] nucleus) or the A10 dopamine neurons of supramamillary nucleus (Hokfelt et al., 1984a, Hokfelt et al., 1984b, Zhao-Shea et al., 2011). The posterior VTA (pVTA) is dorsal to the interpeduncular nucleus, medial to the SNC, and ventral to the red nucleus (Zhao-Shea et al., 2011). The pVTA is comprised of the parabrachial pigmented nucleus (PBP), parainterfascicular (PIF), and the paranigral nucleus (PN) (Fig. 12). The pVTA does not include the midline nuclei (IF, RLi), the caudal linear nucleus (CLi), or A10 dopamine neurons of the dorsal raphe nucleus (Hokfelt et al., 1984a, Hokfelt et al., 1984b, Zhao-Shea et al., 2011). The pVTA is the area of interest for this dissertation. The pVTA has a higher percentage of dopamine neurons than both the aVTA or tail of the VTA (tVTA) (Zhao-Shea et al., 2011). Moreover, the aVTA

and pVTA subregions respond differently to drugs of abuse (Boehm et al., 2002, Rodd et al., 2004, Rodd et al., 2005b, Ericson et al., 2008, Shabat-Simon et al., 2008). Dopamine neurons of the pVTA are more responsive and critical for reinforcement as compared to the aVTA (Zhao-Shea et al., 2011).

The tail of the VTA (tVTA), also called the rostromedial tegmental nucleus (RMTn), is the most caudal extent of the VTA limited to a subregion posterior to the paranigral nucleus and dorsolateral to the interpeduncular nucleus. The tVTA shifts dorsally and slightly laterally more caudally to become embedded within the superior cerebellar peduncle decussation (SCP) (Zhao-Shea et al., 2011). The tVTA has a low density of dopamine neurons, a high density of GABAergic neurons, and does not include the midline nuclei (Zhao-Shea et al., 2011). The tail of VTA has GABAergic output to the anterior and posterior VTA (Kaufling et al., 2010). This produces a microcircuit whereby GABAergic projections of the tVTA contact and inhibit a/pVTA dopamine neurons (Kaufling et al., 2010).

VTA AFFERENTS

The VTA is a hub of converging inputs allowing for the integration of diverse stimuli. Strong inputs to the VTA come from the prefrontal cortex, lateral septum, medial septum diagonal-band complex, accumbens shell, ventral pallidum, medial and lateral preoptic areas, paraventricular nucleus of the hypothalamus, medial and lateral hypothalamus, lateral habenula, laterodorsal tegmentum, pedunculopontine tegmental nucleus, dorsal raphe, periaqueductal gray, and mesencephalic and pontine reticular formation (Geisler and Zahm, 2005). With regard to stress and addiction, the confluence of the

mesocorticolimbic dopamine system and the extended amygdala have been best characterized. The VTA receives both excitatory and inhibitory input from numerous areas (Geisler et al., 2007, Bromberg-Martin et al., 2010, Sesack and Grace, 2010, Jennings et al., 2013).

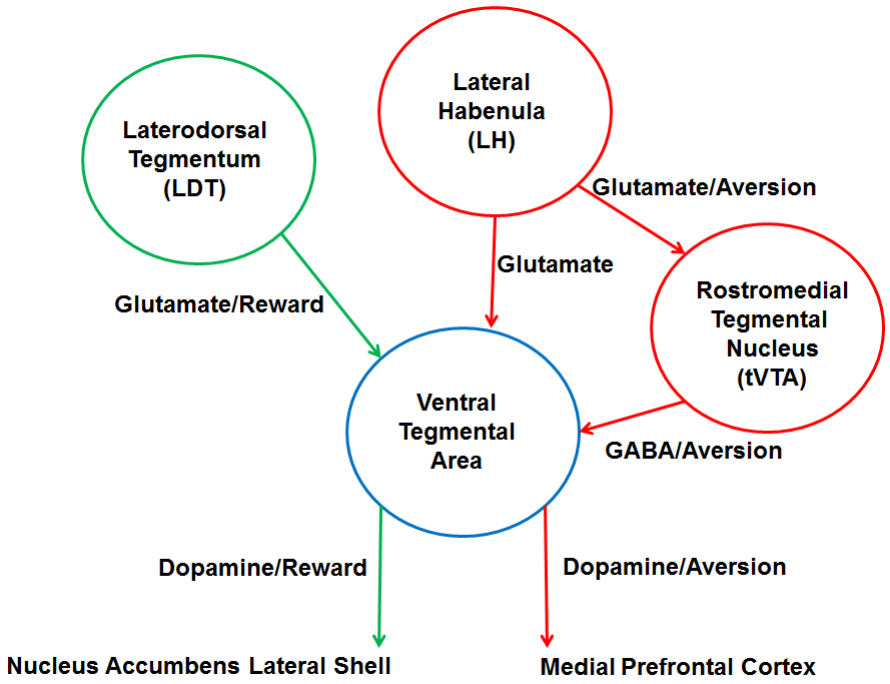
GLUTAMATERGIC VTA AFFERENTS

Glutamatergic inputs onto VTA dopaminergic neurons regulate their firing and the release of dopamine in VTA targets (Overton and Clark, 1997). The VTA receives excitatory glutamate inputs from numerous areas such as the ventromedial prefrontal cortex, ventral subiculum, subthalamic nucleus, parabrachial nucleus from the nucleus of the solitary tract (NTS), pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus, and the bed nucleus of the stria terminalis (Kalivas, 1993, Georges and Aston-Jones, 2001, 2002, Stuber et al., 2008).

One of the main glutamatergic afferent projections to the VTA is the prefrontal cortex (Christie et al., 1985, Sesack et al., 1989, Hurley et al., 1991, Sesack and Pickel, 1992, Lu et al., 1997). This glutamate input comes mainly from the infralimbic and prelimbic regions (Beckstead et al., 1979, Phillipson, 1979a, Sesack et al., 1989, Sesack and Pickel, 1992, Geisler and Zahm, 2005, Frankle et al., 2006). Furthermore, excitation and inhibition of the PFC results in increased and decreased activity of the VTA, respectively (Gariano and Groves, 1988, Svensson and Tung, 1989, Murase et al., 1993a, Tong et al., 1996). VTA PFC glutamate inputs selectively activate dopamine mesocortical neurons and

GABA-containing mesolimbic (accumbens) neurons (Sesack and Pickel, 1992, Carr and Sesack, 2000b). This is one mesocorticolimbic dopamine circuit that may become aberrant with drug-induced neuroplasticity. At least two different glutamate inputs into the VTA can signal reward or aversion (Figure 13). The laterodorsal tegmentum (LDT) and lateral habenula (LHb) have VTA afferents that preferentially project to different VTA dopamine neuron subpopulations. These dopamine populations then project to different target structures (nucleus accumbens lateral shell versus medial PFC) (Margolis et al., 2008, Lammel et al., 2011, Lammel et al., 2012) eliciting reward and aversion, respectively (Lammel et al., 2012).

Figure 13: Summary of circuitry proposed from Lammel et al., 2011. Rewarding versus aversive stimuli provide differential input upon midbrain dopamine neurons which in turn have different terminal field projections.



Reward involves excitation of VTA dopamine neurons that project to the nucleus accumbens shell (Lammel et al., 2011). Aversion involves excitation of the GABAergic tVTA neurons which then project to the a/pVTA signaling aversion by activating mesocortical dopamine projections through an unknown mechanism (Lammel et al., 2012). The tVTA also receives inputs from brain regions involved in aversive stimuli processing including the cingulate cortex (Devinsky et al., 1995, Vogt, 2005), septum (Sheehan et al., 2004), lateral habenula (Matsumoto and Hikosaka, 2009a, Sartorius et al., 2010, Winter et al., 2011), periaqueductal gray (Jhou, 2005, Berton et al., 2007, McNally et al., 2011), and extended amygdala (Davis et al., 2010).

GABAERGIC VTA AFFERENTS

The VTA receives substantial inhibitory GABAergic inputs from areas such as the pedunculopontine tegmental nucleus (PPTg), laterodorsal tegmentum (LDT), lateral hypothalamus, diagonal band, lateral septum, periaqueductal gray, dorsal raphe nuclei, parabrachial nucleus (PB) from the nucleus of the solitary tract (NTS), nucleus accumbens shell, ventral pallidum, lateral habenula, rostromedial tegmental nucleus, and the bed nucleus of the stria terminalis (Oades and Halliday, 1987, Floresco et al., 2003, Geisler and Zahm, 2005, Grace et al., 2007a, Matsumoto and Hikosaka, 2007, 2009b, Smith et al., 2009, Bourdy and Barrot, 2012, Kudo et al., 2012, Jennings et al., 2013). The VTA receives extensive inhibitory feedback from the extended amygdala and basal ganglia which originating from the nucleus accumbens shell and the ventral

pallidum, respectively (Zahm and Heimer, 1990, Heimer et al., 1991, Zahm et al., 1996, Usuda et al., 1998, Geisler and Zahm, 2005).

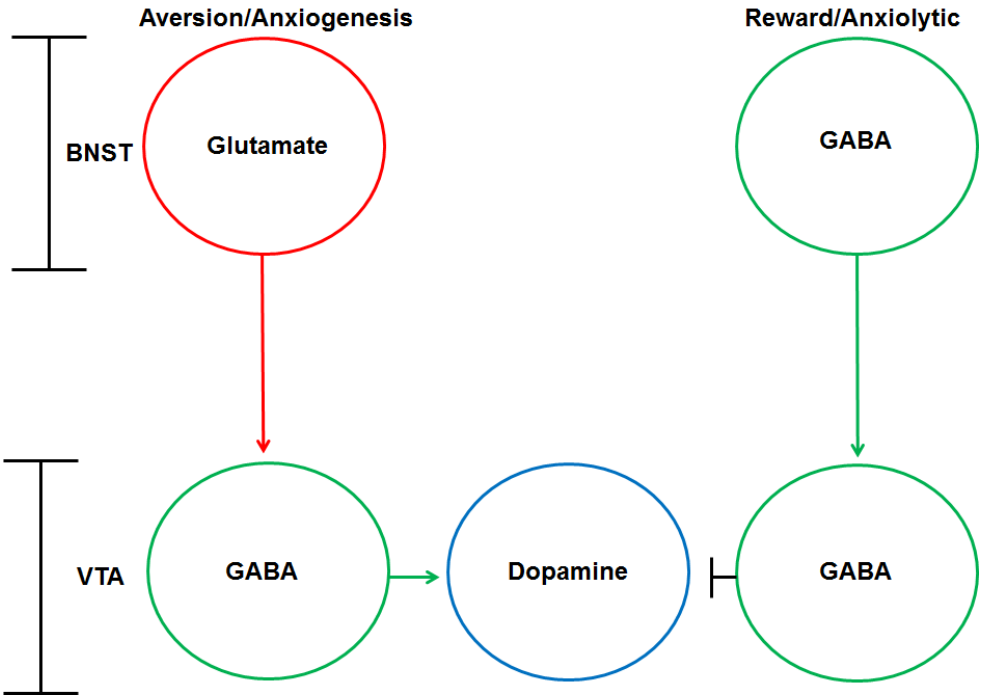
VTA GABA neurons provide local inhibition of dopamine neurons (Grace and Bunney, 1985, Johnson and North, 1992b, Tan et al., 2010) where local contacts between these neuronal phenotypes have been reported (Omelchenko and Sesack, 2009). In addition to local GABAergic interneurons, there are GABA neurons of the tail of the VTA (rostromedial tegmental nucleus) which inhibit DA neurons in the more rostral VTA (Johnson and North, 1992b, Olson and Nestler, 2007, Jhou et al., 2009, Kaufling et al., 2010). GABAergic local interneurons and projection neurons in the VTA not only regulate the excitatory state VTA dopamine and GABA neurons (Klitenick et al., 1992, Marinelli et al., 2006, Dobi et al., 2010) but also the excitatory state of VTA terminal fields including the PFC and NA (Pirot et al., 1992, Van Bockstaele and Pickel, 1995, Steffensen et al., 1998, Carr and Sesack, 2000a).

VTA AFFERENTS FROM THE BNST

One brain region upstream of the VTA that has received considerable attention is the bed nucleus of the stria-terminalis (BNST). The BNST sends both glutamatergic and GABAergic inputs into the VTA (Georges and Aston-Jones, 2002, Kudo et al., 2012, Jennings et al., 2013). The BNST also has aversive and reward specific inputs into the VTA, similar to the LDT and LH glutamate inputs, but through different mechanisms (Figure 14). Specifically, two differential BNST afferents into the VTA have been characterized. GABAergic BNST inputs onto VTA GABA neurons produces anxiolytic/rewarding behavioral responses, while

glutamatergic BNST inputs onto VTA GABA neurons produces anxiogenic/aversive behavioral responses (Jennings et al., 2013).

Figure 14: Summary of circuitry proposed from Jennings et al., 2013. Inhibition of VTA dopamine neurons by GABA input is aversive and anxiogenic while disinhibition is rewarding and anxiolytic.



The hypothesis that decreasing GABA neuron activity in the VTA is rewarding is supported by the finding that anxiolytic/rewarding behavioral responses can be induced by directly inhibiting intra-VTA GABA neurons (Jennings et al., 2013). Importantly, it has been reported that footshock stress selectively activates BNST glutamate projection neurons that increase intra-VTA GABA neuron activity (Jennings et al., 2013). These findings suggest that footshock stress increases GABA neuron firing in the VTA through excitatory drive from the BNST. Furthermore, footshock-induced increases in VTA GABA neuron activation

signals aversion and anxiogenesis. In summary, the findings from Lammel et al., (2012) and Jennings et al., (2013) suggest that increasing GABA input or intra-VTA GABA neuron activity produces aversive/anxiogenic behavioral responses. Alternatively, increasing glutamatergic input or disinhibiting VTA dopamine neurons both produce rewarding/anxiolytic behavioral responses (Lammel et al., 2012, Jennings et al., 2013).

VTA EFFERENTS

Neuroanatomical studies have characterized VTA projections using different criteria. One criteria used is medial/lateral topography of the mesolimbic projections to produce two different pathways (Fallon and Moore, 1978, Beckstead et al., 1979). These two pathways are: (1) the medial striatal projections to the medial nucleus accumbens shell, and (2) the lateral striatal projections to the lateral nucleus accumbens core and shell (Ikemoto, 2007). Another criteria used is dorsal/ventral topography to produce two distinct efferent pathways. These two pathways are: (1) the dorsal dopamine neurons that express lower levels of the dopamine active transporter (DAT) and high levels of calbindin calcium binding protein, and (2) the ventral dopamine neurons that project primarily to the striatum and have high levels of DAT and low levels of calbindin (Gerfen, 1992, Haber et al., 1995, Bjorklund and Dunnett, 2007). The latest criteria characterizing VTA efferents uses the forebrain targets, physical characteristics, and firing patterns of VTA dopamine neurons to produce distinct efferent pathways. These two pathways are: (1) the slow firing dopamine neurons that project to the lateral nucleus accumbens shell and dorsolateral

striatum, and (2) fast firing dopamine neurons that express lower levels of DAT and project to the PFC, nucleus accumbens core, and the medial nucleus accumbens shell (Lammel et al., 2008).

The majority of VTA dopamine neuron projections do not have overlapping target areas. This suggests that different VTA projections are separate and independent, but parallel (Fallon, 1981, Albanese and Minciacchi, 1983, Loughlin and Fallon, 1984, Lammel et al., 2008). For example, dopamine neurons of the medial posterior VTA project selectively to the nucleus accumbens core, medial nucleus accumbens shell, and to the medial prefrontal cortex. In contrast, dopamine neurons in the lateral posterior and anterior VTA project primarily to the nucleus accumbens lateral shell (Lammel et al., 2008). For these reasons, dopamine neurons that project to the medial prefrontal cortex and nucleus accumbens can be functionally distinct from one another, adding to the complexity of behavior that is preferentially regulated either by mesocortical or mesolimbic dopamine input.

Heterogeneous groups of VTA neurons which project to different terminal fields can be further divided into dopaminergic and non-dopaminergic (GABA) populations (Swanson, 1982). VTA GABA neurons are not only local inhibitory interneurons that regulate the excitatory state of VTA DA neurons (Klitenick et al., 1992, Marinelli et al., 2006) but also projection neurons that have terminal fields in the medial prefrontal cortex and nucleus accumbens (Pirot et al., 1992, Van Bockstaele and Pickel, 1995, Steffensen et al., 1998, Carr and Sesack, 2000a, Dobi et al., 2010). This makes GABA neurotransmission an essential

component to the mesocorticolimbic system and likely motivated behavior. In summary, the converging functional afferents, diverging efferents, and heterogeneous neuron populations of the VTA produces very complex neurocircuitry involved in motivated behavior. Although, the full complexity of VTA neurocircuitry is beyond the scope of this dissertation, there exists heterogenous circuits that control the differential response of the mesocorticolimbic system to different stimuli, including stress and reward.

STRESS REGULATION OF MESOCORTICOLIMBIC DOPAMINE SIGNALING

Distinct subpopulations of VTA dopamine neurons respond differently to aversive stimuli. Stressful stimuli can increase VTA dopamine neuron excitation and terminal field release of dopamine (Abercrombie et al., 1989, Mantz et al., 1989, Guarraci and Kapp, 1999, Bassareo et al., 2002, Joseph et al., 2003, Young, 2004, Anstrom and Woodward, 2005, Brischoux et al., 2009, Matsumoto and Hikosaka, 2009b, Bromberg-Martin et al., 2010, Cohen et al., 2012). Paradoxically, others have reported that the majority of VTA DA neurons are in fact inhibited by aversive stimuli (Ungless et al., 2004, Roitman et al., 2008, Badrinarayan et al., 2012, Oleson et al., 2012). These findings suggest that VTA dopamine neurons can both be excited and inhibited by stressful stimuli. In support, it has been reported that whether a VTA dopamine neuron is excited or inhibited by footshock depends on their ventral versus dorsal location in VTA sub nuclei (Brischoux et al., 2009).

Ventral VTA DA neurons have been reported to be preferentially excited by footshock stress while dorsal VTA DA neurons are inhibited (Brischoux et al.,

2009). This roughly translates to the dorsal parabrachial (PBP) and the more ventral parainterfascicular (PIF) and paranigral (PN) nuclei, respectively. The functional heterogeneity in VTA DA neurons in response to stress has not only been reported in the ventral dorsal topography but also in the rostral caudal topography (Ikemoto et al., 1997a, Ikemoto et al., 1998, Carlezon et al., 2000, Bolanos et al., 2003, Olson et al., 2005). These different regions also have specific connectivity within the mesocorticolimbic system (Ikemoto, 2007).

Dopamine neurons projecting to the medial prefrontal cortex (mPFC) are hypothesized to be preferentially activated by aversive stimuli, while dopamine neurons that project to the nucleus accumbens shell are hypothesized to signal reward and salience (Lammel et al., 2011, Lammel et al., 2012). The first evidence supporting heterogeneous mesocortical versus mesolimbic VTA DA neuron function was provided by looking at the effects of footshock stress on this system (Thierry et al., 1976). Dopamine levels were found to be significantly increased in the prefrontal cortex, but only slightly in the nucleus accumbens of drug naïve rats that had undergone short periods of intermittent footshock stress (Thierry et al., 1976). These findings have been replicated using footshock and other stressors across a range of different techniques (Fadda, 1978, Lavielle, 1978, Tassin et al., 1980, Herman et al., 1982, Bannon, 1983, Deutch et al., 1985, Roth et al., 1988, Deutch et al., 1990, Deutch and Roth, 1990, Deutch et al., 1991, Lammel et al., 2011). Mesocortical VTA DA neurons possess distinct characteristics (Bannon et al., 1983, Roth, 1984) such the lack of autoreceptors to regulate neurotransmission (Chiodo et al., 1984). However, upon initial

investigation the absence of functional autoreceptors does not appear to be a critical determinant in the preferential responsiveness of mesocortical DA neurons to footshock stress (Roth et al., 1988).

To summarize, there are conflicting results as to what stressful stimuli are doing to mesocorticolimbic dopamine neurotransmission in drug naïve animals, much less in cocaine experienced ones. These inconsistencies are likely the result of the complexity of the VTA neurocircuitry regulating both stress and reward. However, it is safe to say that we can no longer consider the VTA to be a homologous structure and its heterogeneity will need to be taken into consideration to fully characterize stress-induced reinstatement of drug seeking and other questions related to motivation and reward. Surely the complex circuitry of the VTA is converged upon by CRF release during stress in a way that regulates cocaine seeking.

VENTRAL TEGMENTAL AREA AND CORTICOTROPIN RELEASING FACTOR

CRF inputs to the VTA arise from multiple sources including afferents from the paraventricular nucleus of the hypothalamus (PVN), bed nucleus of the stria terminals (BNST), central nucleus of the amygdala (CeA), and (Beckstead et al., 1979, Phillipson, 1979a, Swanson et al., 1983, Wallace et al., 1989, Rodaros et al., 2007). CRF-containing axons and varicosities had been identified in the VTA (Swanson et al., 1983) and are co-expressed with classical neurotransmitters (Tagliaferro and Morales, 2008). There are two types of CRF-containing neurons that project to the VTA, these are CRF-asymmetric (assumed glutamatergic) and

CRF-symmetric (assumed GABAergic) (Tagliaferro and Morales, 2008). The majority of CRF terminals contacting dopamine neurons in the VTA are asymmetric (Tagliaferro and Morales, 2008). However, CRF containing terminals make contact onto the dendrites of both dopamine and GABA neurons (Tagliaferro and Morales, 2008). The exact origin of these two types of terminal inputs has not been determined.

CRF and both its receptors appear to be functionally expressed within the VTA (Ungless et al., 2003, Korotkova et al., 2006, Wanat et al., 2008). CRF-R1 mRNA has been found in both dopaminergic and GABAergic neurons of the VTA (Korotkova et al., 2006, Refojo et al., 2011). It should be noted that the VTA does express CRF-R1 but at significantly lower levels than other brain regions in the rat (Van Pett et al., 2000, Sauvage and Steckler, 2001). In contrast, *in situ* hybridization has failed to detect CRF-R2 mRNA in the VTA (Chalmers et al., 1995, Van Pett et al., 2000). This does not rule out a presynaptic mechanism for CRF-R2 in the VTA. Moreover, CRF-R2 mRNA has been detected in the VTA using single cell RT-PCR (Korotkova et al., 2006). Surprisingly, CRF-R2 mRNA levels were reported to greatly exceed those of CRF-R1 in VTA dopamine neurons (Korotkova et al., 2006). It should be noted that this was the only report to show expression of CRF-R2 mRNA in the VTA.

In contrast to *in situ* hybridization, immunohistochemical analysis of CRF receptor subtype specific expression has been met with significant skepticism because commercially available antibodies for the CRF receptors have failed sensitivity and specificity tests (Refojo et al., 2011). The inability to reliably detect

CRF receptor protein expression has greatly stymied the progress of the field. Pre- versus post-synaptic localization of both CRF receptor subtypes within the VTA has up to this point been ambiguous. Nevertheless, electrophysiological studies have implicated both CRF receptor subtype function in the regulation of VTA DA neuron activity (Ungless et al., 2003, Korotkova et al., 2006, Wanat et al., 2008, Beckstead et al., 2009). In summary, even without reliable protein detection, the field still supports the notion that both CRF-R1 and CRF-R2 are in fact expressed in the VTA (Van Pett et al., 2000, Ungless et al., 2003, Korotkova et al., 2006, Wang et al., 2007, Wanat et al., 2008, Beckstead et al., 2009, Blacktop et al., 2011, Wanat et al., 2013). CRF systems are in a good anatomical location to regulate dopaminergic neurotransmission of the mesocorticolimbic system and therefore motivated behavior.

CRF RECEPTORS AND STRESS-INDUCED REINSTATEMENT

CRF was originally hypothesized to be important to addiction-related actions in the VTA because the mesocorticolimbic dopamine system is implicated in both responsiveness to stress (Thierry et al., 1976) and cocaine self-administration (Roberts et al., 1977). A common theme to repeated cocaine administration is increased responsiveness throughout the brain to CRF (Sarnyai et al., 1992, Sarnyai et al., 1993, 1995, Erb et al., 1996, 1998, Shaham et al., 1998, Basso et al., 1999, Erb and Stewart, 1999, Koob, 1999b, Shaham et al., 2000, Shalev et al., 2000, Erb et al., 2001, Sarnyai et al., 2001, Erb et al., 2003, Erb et al., 2006a, Pollandt et al., 2006, Fu et al., 2007, Koob and Kreek, 2007,

Koob, 2008, Mantsch et al., 2008a, Specio et al., 2008, Shalev et al., 2010, Graf et al., 2011). One very important area in which this is occurring is the VTA (Kalivas et al., 1987, Goeders et al., 1990, Wang et al., 2007, Blacktop et al., 2011). Footshock stress which causes reinstatement of extinguished cocaine-seeking (Erb et al., 1996, Shaham et al., 1998, Mantsch et al., 2008a) also causes CRF release in the VTA in both drug-naïve and drug-experienced animals (Wang et al., 2005, Wang et al., 2007). Cocaine exposure alters the function of CRF in the VTA in such a way that it facilitates drug seeking (Wang et al., 2005, Wang et al., 2007, Blacktop et al., 2011).

GLUTAMATE AND VTA DOPAMINE NEURONS

The VTA receives both excitatory and inhibitory inputs (Watabe-Uchida et al., Geisler et al., 2007, Bromberg-Martin et al., 2010, Sesack and Grace, 2010). Stress and psychostimulants produce similar adaptations of excitatory (Ungless et al., 2001, Saal et al., 2003, Hahn et al., 2009) and inhibitory (Beckstead et al., 2009, Padgett et al., 2012) neurotransmission in the VTA. Several mechanisms have been postulated to explain how stress reinstates drug-seeking behavior. One hypothesis is that stressors activate the mesocorticolimbic DA system, in a similar way to both drugs and cues previously associated with use, resulting in craving and therefore relapse (Robinson and Berridge, 1993, Shaham and Stewart, 1995). Consistent with this, excitatory drive on this circuit through glutamate neurotransmission in the VTA is a leading hypothesis as to how stress-induced CRF release facilitates reinstatement. Glutamatergic afferent

projectionss are important to drug seeking through regulation of VTA dopamine neuron firing and release of downstream dopamine (Overton and Clark, 1997).

VTA DA neurons possess pacemaker like properties and fire in two distinct modes. These modes are tonic and phasic firing with phasic firing producing the highest DA levels (Grace and Bunney, 1984a, b, Cooper, 2002). Baseline dopamine neuron activity is regulated by pacemaker conductance bringing the membrane potential from a hyperpolarized state to a depolarized state, thereby decreasing the spike threshold and increasing burst firing (Grace and Bunney, 1983, 1984a, Grace and Onn, 1989). Phasic firing is a brief transient increase in dopamine cell firing that results in either episodic burst firing allowing for temporal summation (Gonon, 1988, Wightman and Zimmerman, 1990, Suaud-Chagny et al., 1992) or simultaneous firing of multiple cells projecting to the same target neurons allowing for spatial summation (Grace and Bunney, 1984a, b). In contrast, tonic firing is a slow pattern of dopamine neuron excitation resulting in dopamine concentration increases that can last from tens of seconds to days or longer (Grace, 1995, Goto and Grace, 2005).

Glutamatergic inputs stimulate midbrain dopamine neurons, promoting burst firing rather than single spike firing (Grace and Onn, 1989, Taber et al., 1995, Grillner and Mercuri, 2002, Floresco et al., 2003). Burst firing patterns of VTA DA neurons produce optimally efficient dopamine release at both terminals and somatodendritic sites (Wightman and Zimmerman, 1990). This results in the facilitation of supra-additive release or summation of dopamine, which saturates dopamine transporters limiting uptake. This optimization of VTA DA neuron firing

is ultimately responsible for the downstream behavioral responses to dopamine (Chergui et al., 1994, Floresco et al., 2003, Phillips et al., 2003) including the perception of both reward (Overton and Clark, 1997) and reward-predicting salient stimuli (Schultz, 1998). Endogenous burst activity of dopamine neurons is regulated by glutamate activation of the NMDA receptor (Johnson and North, 1992b, Overton and Clark, 1992, Chergui et al., 1993, Deister et al., 2009, Zweifel et al., 2009), which requires removal of its magnesium block by AMPA receptor activation (Calabresi et al., 1992). To the extent that increased levels of terminal field DA release are responsible for stress-induced cocaine seeking, glutamate inputs onto VTA dopamine neurons represent a possible mechanism.

CRF can augment glutamate signaling in the VTA. CRF-containing and glutamatergic VTA afferents can converge independently (Georges and Aston-Jones, 2001, Rodaros et al., 2007, Zahm et al., 2011, Jennings et al., 2013) or glutamate and CRF can be co-released (Rodaros et al., 2007, Tagliaferro and Morales, 2008). Intra-VTA CRF can increase dopamine and glutamate concentrations as measured by HPLC tissue or microdialysis sample analysis (Kalivas et al., 1987, Lavicky and Dunn, 1993, Wang et al., 2005). In slice preparations from drug-naïve rats, *ex vivo* electrophysiology studies have reported increased excitatory drive on VTA dopamine neurons by CRF through: (1) CRF-R2/CRF-BP/PKC-dependent increases in NMDA receptor conductance (Ungless et al., 2003), (2) CRF-R1/PKC-dependent increases in hyperpolarization-activated cation current I_H (Wanat et al., 2008), and (3) mGluR-

dependent increases in potassium-sensitive calcium channel conductance (Riegel and Williams, 2008).

Drug-induced neuroplasticity can augment glutamate-induced excitation of VTA dopamine neurons. A single exposure to cocaine, amphetamine, morphine, nicotine, ethanol, and notably, acute stress can enhance glutamate synaptic transmission upon midbrain dopamine neurons (Ungless et al., 2001, Saal et al., 2003). Drugs of abuse have consistently been reported to augment the function of ionotropic glutamate receptors on VTA dopamine neurons (Ungless et al., 2001, Saal et al., 2003, Bellone and Luscher, 2006, Argilli et al., 2008, Chen et al., 2008, Bowers et al., 2010, Luscher and Malenka, 2011, Mameli et al., 2011).

Cocaine- and stress-exposure induced neuroplasticity appears to target the AMPA receptor which in turn potentiates NMDA-mediated synaptic transmission by removing magnesium block (Ungless et al., 2010). Cocaine exposure has been reported to recruit a CRF-R1/PKA dependent mechanism that enhances NMDA and AMPA receptor signaling on VTA DA neurons (Hahn et al., 2009). These findings suggest that, following drug or stress exposure, the ability of CRF to augment glutamatergic signaling in the VTA is enhanced. However, these studies used *ex vivo* electrophysiology with brain slice preparations from animals that had received noncontingent cocaine administration followed by, at most, acute withdrawal without extinction.

STRESS-INDUCED RELAPSE CRF AND GLUTAMATE

In a set of experiments by Wise and colleagues, the role of glutamate and CRF in the VTA was characterized during footshock-induced reinstatement of

cocaine seeking. Footshock stress produced a significant increase in intra-VTA CRF release in both drug-naïve and drug-experienced animals (Wang et al., 2005). The importance of this CRF was highlighted by the finding that both footshock stress and reverse dialysis of CRF into the VTA was sufficient to reinstate extinguished cocaine-seeking behavior (Wang et al., 2005). Reinstatement was further characterized by increases in VTA glutamate and somatodendritic dopamine release, which were both blocked by a nonspecific CRF receptor antagonist (Wang et al., 2005). Furthermore, administration of kynurenic acid, a nonspecific ionotropic glutamate receptor antagonist (Stone, 1993), blocked footshock- and intra-CRF-induced reinstatement, along with the simultaneous increases in somatodendritic dopamine release but not the increases in extracellular glutamate (Wang et al., 2005). These data indicate that reinstatement involves excitatory drive on VTA dopamine neurons through CRF-dependent regulation of presynaptic glutamate release and subsequent ionotropic glutamate receptor activation on dopamine neurons. It was later reported that footshock and intra-VTA CRF-induced reinstatement and concomitant increases in glutamate and dopamine can be blocked by administration of CRF-R2 but not CRF-R1 specific antagonists (Wang et al., 2007). These findings were unexpected for reasons outlined below.

CRF has 10-fold higher affinity for the CRF-R1 than the CRF-R2 receptor (Perrin et al., 1995). CRF-R1 is much more widely distributed throughout the rodent brain than CRF-R2 (Van Pett et al., 2000). Moreover, the majority of experiments using ventricular administration of antagonists support the

conclusion that CRF-R1 and not CRF-R2 activation is necessary for footshock-induced reinstatement of not only cocaine- but also heroin-, alcohol-, and nicotine-seeking (Erb et al., 1998, Shaham et al., 1998, Le et al., 2000, Bruijnzeel et al., 2009). Although, there is a well-established role for CRF-R1 function in footshock-induced reinstatement of drug seeking, Wise and colleagues defined a clear role for the CRF-R2 receptor. Importantly, site-specific versus global ventricular administration of drugs targeting CRF receptors could help explain these disparate findings.

CRF receptor subtype-specific ligands (urocortins) that were sufficient to reinstate drug-seeking were also reported. Ligands sufficient to reinstate (CRF, Ucn1, and Ucn2) were not specific for a CRF-R receptor subtype but all bound to the CRF-BP (Wang et al., 2007). Paradoxically, when CRF-R2 specific ligands prevented binding to the CRF-BP, they also prevented the sufficiency of these ligands to reinstate cocaine seeking (Wang et al., 2007). CRF-BP is considered to be a functional CRF receptor antagonist due to its ability to sequester ligands (e.g., CRF) and prevent them from activating CRF receptors (Huising et al., 2008). Prevention of CRF binding to CRF-BP should increase the amount of free CRF available to activate endogenous CRF receptors. This would be hypothesized augment reinstatement and not block it.

Wang et al., (2007) suggested that in order for intra-VTA CRF to reinstate cocaine seeking it needs to bind to the CRF-BP and activate CRF-R2. Although these findings do not fit with the traditional role of CRF-BP they do support the hypothesis that a functional dimer with unique functions forms when CRF-BP

binds CRF (Potter et al., 1991, Behan et al., 1996b, Kemp et al., 1998, Sajdyk et al., 1999, Chan et al., 2000, Roseboom et al., 2007). Moreover, they are in congruence with a report by Ungless et al., (2003) of a CRF-R2/CRF-BP/PKC-dependent enhancement of NMDAR signaling on VTA dopamine neurons. However, both CRF-R1 and CRF-R2 have both been implicated in facilitating glutamate neurotransmission in the VTA through NMDA and AMPA receptors on dopamine neurons (Ungless et al., 2003, Pollandt et al., 2006, Wang et al., 2007, Wanat et al., 2008, Hahn et al., 2009). Therefore, there still remains conflicting evidence as to which CRF receptor subtype provides excitatory drive of VTA dopamine neurons through increased ionotropic glutamate receptor conductance.

In summary, there is strong evidence that CRF regulates VTA dopamine cellular activity in response to stress by facilitating glutamate signaling through ionotropic glutamate receptors on VTA dopamine neurons; an effect that appears to be enhanced following cocaine exposure (Ungless et al., 2003, Hahn et al., 2009). This enhancement of excitatory signaling through ionotropic glutamate receptors is one mechanism that could be involved in footshock stress-induced reinstatement of extinguished cocaine seeking. The specific role of both CRF and ionotropic glutamate receptor subtypes in the VTA has not been sufficiently characterized in stress-induced reinstatement of extinguished cocaine seeking, particularly following LgA self-administration. The LgA self-administration model of stress-induced relapse involves intake-dependent neuroplastic changes that regulate escalation of drug intake and the emergent ability of footshock stress to

cause relapse. Characterizing the interactions between drug-induced neuroplasticity and stress signaling in the VTA under conditions that reinstate extinguished cocaine seeking under these conditions will contribute to the field substantially.However, it is likely that the processes responsible for stress-induced relapse are more complex than simply increased excitatory drive on VTA DA neurons in response to a stressful stimulus.

GABA AND VTA DOPAMINE NEURONS

Footshock stress also results in increased activity of GABAergic inputs into the VTA as well as increased activity of VTA GABA neurons (Lammel et al., 2012, Tan et al., 2012). This increase in VTA GABA signaling may decrease the activity of neighboring dopamine neurons contributing to behaviors associated with aversion (Cohen et al., 2012, Kim et al., 2012, Tan et al., 2012, van Zessen et al., 2012). GABA inhibits neurons by activating two receptors, the ionotropic $GABA_A$ receptor and the metabotropic $GABA_B$ receptor. Fast inhibitory postsynaptic currents (IPSCs) are induced by $GABA_A$ receptor activation via intrinsic chloride channel influx (Rudolph et al., 2001), while slower inhibitory outward currents are induced by $GABA_B$ receptor activation (Otis and Mody, 1992). GABA exerts a direct $GABA_B$ receptor-mediated inhibitory influence by activation Gi/o protein-coupled receptors and hyperpolarization of neuronal membrane potentials through increases in inwardly rectifying potassium channel conductance (Lacey et al., 1988).

$GABA_A$ and $GABA_B$ receptors are both expressed in the VTA (Kalivas, 1993, Westerink et al., 1996, Westerink et al., 1998). In the VTA $GABA_A$ receptors are located predominantly, but not exclusively, on GABA neurons while $GABA_B$ receptors are predominantly (but not exclusively) located on dopamine neurons (Churchill et al., 1992, Klitenick et al., 1992, Xi and Stein, 1998, Magreta-Mitrovic, 1999, Laviolette and van der Kooy, 2001, Laviolette et al., 2004). Therefore, GABAergic interneuron inhibition of VTA DA neurons is largely regulated by ionotropic $GABA_A$ receptors (Sugita et al., 1992, Kalivas, 1993) while direct GABA inhibition of dopamine neurons is largely regulated by metabotropic $GABA_B$ receptors (Xi and Stein, 1998, Margeta-Mitrovic et al., 1999). In the VTA, GABA can also act presynaptically via $GABA_B$ receptor activation on both GABA and glutamate terminals (Bonci and Williams, 1997, Manzoni and Williams, 1999, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010) and postsynaptically upon dopamine and GABA neurons (Georges and Aston-Jones, 2002, Giorgetti et al., 2002, Beckstead et al., 2009, Cohen et al., 2012, Tan et al., 2012).

Altogether, phasic firing of DA neurons can be induced by glutamate input and NMDA receptor activation, and inhibited by GABA inputs and activation of both $GABA_A$ and $GABA_B$ receptors. NMDA receptor activation facilitates phasic firing of VTA dopamine neurons (Johnson and North, 1992b, Overton and Clark, 1992, Chergui et al., 1993, Deister et al., 2009, Zweifel et al., 2009) while both $GABA_A$ and $GABA_B$ receptor activation inhibits it (Lacey et al., 1988, Engberg et al., 1993, Seutin et al., 1994, Erhardt et al., 1998, Paladini et al., 1999a, Paladini

et al., 1999b, Paladini and Tepper, 1999, Wu et al., 1999, Erhardt et al., 2002). In addition to regulation of phasic firing $GABA_A$ and $GABA_B$ receptors provide tonic inhibition (Yim and Mogenson, 1980, Johnson and North, 1992b, a, Suaud-Chagny et al., 1992, Laviolette and van der Kooy, 2001, Erhardt et al., 2002, Giorgetti et al., 2002, Chen et al., 2005). Therefore, there is an important balance between excitatory and inhibitory effects on phasic and tonic firing of VTA DA neurons determined by activity of NMDA, $GABA_A$, and $GABA_B$ receptors.

GABA AND DRUGS OF ABUSE

Many drugs of abuse can directly inhibit VTA GABA neurons, thereby increasing VTA dopamine neuronal activity and facilitating the reinforcing effects of these drugs which includes: opioids, benzodiazepines, nicotine, cannabinoids, and cocaine (Johnson and North, 1992a, Mansvelder et al., 2002, Szabo et al., 2002, Steffensen et al., 2008, Bocklisch et al., 2013). This disinhibition can be mediated by increased GABA input on VTA GABA neurons and decreased responsiveness of VTA dopamine neurons to GABA (Johnson and North, 1992a, Beckstead et al., 2009, Arora et al., 2011, Bocklisch et al., 2013). Cocaine, on the other hand, provides excitatory drive on VTA dopamine neurons through both glutamatergic and, potentially, GABAergic mechanisms. These mechanisms include increased ionotropic glutamate receptor signaling on VTA DA neurons, increased GABA input on VTA GABA neurons, and decreased $GABA_B$-receptor mediated inhibition of dopamine neurons (Sun, 2005, Beckstead and Williams, 2007, Beckstead et al., 2009, Hahn et al., 2009, Arora et al., 2011, Bocklisch et al., 2013). $GABA_B$-receptor inhibition of VTA DA neurons occurs primarily

through G_i protein-gated inwardly rectifying potassium (GIRK) channels that actually results in potassium ion efflux (Johnson and North, 1992b, Cruz et al., 2004, Labouebe et al., 2007, Beckstead et al., 2009).

In the case of repeated cocaine, both increased ionotropic glutamate receptor conductance (Ungless et al., 2001, Hahn et al., 2009, Ungless et al., 2010) and decreased $GABA_A$- and $GABA_B$-induced inhibition of VTA dopamine neurons (Klitenick et al., 1992, Cameron and Williams, 1994, Kushner, 2001, Beckstead et al., 2009, Arora et al., 2011, Padgett et al., 2012, Graziane et al., 2013) have been reported. Notably, it has been reported that cocaine self-administration and increases in dopamine as a result of cocaine self-administration are both significantly inhibited by activation of the $GABA_B$ receptor following systemic administration of $GABA_B$ receptor agonists (Brebner et al., 2000, Fadda, 2003). Together, these mechanisms may facilitate a net shift in excitation of VTA dopamine neurons upon cocaine exposure. However, it is important to note that this shift is acute and does not extend into protracted abstinence, when reinstatement is typically assessed.

GABA AND CRF INTERACTIONS IN THE VTA

Although much research has focused on effects of CRF on excitatory synaptic transmission in the VTA, footshock-induced reinstatement of cocaine seeking may be regulated by intra-VTA CRF in such a way that reflects inhibition of VTA dopamine neurons. Intra-VTA CRF administration can decrease the motivation to work for food rewards; an effect that occurs through CRF-induced inhibition of reward-evoked dopamine release (Wanat et al., 2013). This suggests

that in the VTA CRF can exert inhibitory effects upon dopamine signaling in a way that can affect operant behavior. Intra-VTA CRF has been shown to exert inhibitory effects via enhancement of $GABA_B$-receptor regulated GIRK channel conductance on dopamine neurons (Beckstead et al., 2009). Specifically, activation of CRF-R1 receptors is necessary for CRF administration to augment this $GABA_B$/GIRK-induced inhibition (Beckstead et al., 2009). Notably, CRF-dependent enhancement of $GABA_B$/GIRK-induced inhibition of VTA dopamine neurons is acutely diminished by drug exposure (Beckstead et al., 2009).

Rewarding and aversive stressful stimuli can both reinstate extinguished cocaine seeking and by themselves have opposing effects on GABA regulation of VTA dopamine neuron activity. For example, cocaine increases VTA dopamine activity in part by decreasing GABAergic signaling in the VTA (Bocklisch et al., 2013) and induces conditioned place preference (Mueller and Stewart, 2000), while footshock stress decreases VTA dopamine activity by increasing GABAergic signaling in the VTA and induces conditioned place aversion (Tan et al., 2012). Both cocaine and footshock reinstate extinguished cocaine-seeking behavior (McFarland and Kalivas, 2001, McFarland et al., 2004). Moreover, prior history of exposure to drugs of abuse can change VTA GABAergic neurotransmission substantially (Johnson and North, 1992a, Bonci and Williams, 1997, Nugent et al., 2007, Madhavan et al., 2010). The role of GABA in footshock-induced reinstatement of extinguished LgA cocaine seeking has not been investigated despite its clear involvement with both CRF and dopamine neuron signaling in the VTA.

BACKGROUND AND SIGNIFICANCE SUMMARY

The high propensity for relapse in drug-abstinent addicts has made relapse prevention a key target for pre-clinical research aimed at improving approaches for the long-term management of drug addiction. Further, understanding of the neurobiological processes that contribute to relapse is needed for the development of new and/or more effective treatment for drug addiction. Primary questions being asked about addiction are: 1) what are the primary triggers for relapse, 2) what brain systems regulate these triggers of relapse, and 3) what maintains the vulnerability for these triggers to cause relapse even following periods of prolonged drug abstinence? One unpredictable and unavoidable cause of relapse in human addicts is the occurrence of a stressful life event. Importantly, stress-induced relapse can be modeled using the long-access self-administration/reinstatement model in rodents. Specific questions being asked in this dissertation are: 1) what are the primary mechanisms of stress-induced reinstatement, 2) what part of the brain regulates stress-induced reinstatement, and 3) what maintains the vulnerability for stress to trigger reinstatement even after extinction of drug seeking?

Very little is known about the neuromechanisms through which stress contributes to the relapse process. Previous work from our laboratory has demonstrated that intake-dependent neuroplasticity and its interactions with the stress-related neuropeptide, corticotropin-releasing factor, determines the ability of stress to facilitate reinstatement (Mantsch et al., 2008a, Graf et al., 2011). However, the exact brain region where CRF is interacting with this neuroplasticity

is unknown. Areas of convergence between motivational and reward neurocircuitry represent likely neurobiological substrates through which stress can facilitate relapse. A major area of convergence between motivational- and stress-related neurocircuitry is the ventral tegmental area (VTA). This area involves the convergence of resident dopamine neurons and inputs that release the stress-related neuropeptide corticotropin releasing factor (CRF). This dissertation will primarily characterize CRF-related neurobiological mechanisms within the VTA that contribute to stressor-induced reinstatement to cocaine use using the long-access (LgA) self-administration/reinstatement model of relapse in rats.

Chapter two characterizes whether CRF actions in the VTA represent a primary mechanism of stress-induced reinstatement, and, if so, which CRF receptor CRF is acting through. This is accomplished using site specific pharmacological manipulations within the ventral tegmental area and the reinstatement approach in rats. The hypotheses of chapter two are that intra-VTA CRF administration is sufficient to reinstate drug seeking in high- (long-access) but not moderate intake (short-access) animals and that, CRF-R1, but not CRF-R2, activation in the VTA is necessary and sufficient for this reinstatement.

Chapter three examines whether stressor-induced reinstatement of long-access (LgA) cocaine seeking involves an increase or a decrease in VTA dopamine neuron activation. This is done using dual immunohistochemistry to characterize stress-induced expression of an indicator of neuronal activation, c-Fos (Sagar et al., 1988), with tyrosine hydroxylase a known marker for dopamine

neurons in the VTA (Hokfelt, 1984). It is hypothesized that a significant increase in dopamine neuronal activation will only be observed under conditions in which footshock stress reinstates cocaine seeking. Therefore, it is hypothesized that there will be a significant increase in intra-VTA dopamine neurons that co-express c-Fos in response to footshock stress in long-access (which show stress-induced cocaine seeking) -but not short-access animals (which do not).

Lastly, chapter 4 determines whether both stress- and intra-VTA CRF-induced reinstatement is dependent on excitatory or inhibitory receptor activation in the VTA. More specifically, the necessity of VTA AMPA, NMDA, $GABA_A$, and $GABA_B$ receptor activation in reinstatement of extinguished cocaine seeking will be tested. This is also accomplished using site-specific pharmacological manipulations within the VTA using the long-access reinstatement rodent model of relapse. It is hypothesized that both stress- and intra-VTA CRF-induced reinstatement of extinguished long-access cocaine-seeking behavior is dependent on excitatory and not inhibitory receptor activation within the VTA. Therefore, it is more specifically hypothesized that both NMDA and AMPA antagonists will block while both $GABA_A$ and $GABA_B$ antagonists will augment both stress- and intra-VTA CRF-induced reinstatement following LgA self-administration.

CHAPTER 2

AUGMENTED COCAINE SEEKING IN RESPONSE TO STRESS OR CRF DELIVERED INTO THE VENTRAL TEGMENTAL AREA FOLLOWING LONG-ACCESS SELF-ADMINISTRATION IS MEDIATED BY CRF-R1 BUT NOT CRF-R2 RECEPTORS

ABSTRACT

Excessive cocaine use may increase susceptibility to stressor-induced relapse through alterations in brain corticotropin releasing factor (CRF) regulation of neurocircuitry involved in drug seeking. Reinstatement of cocaine seeking by a stressor (footshock) is CRF-dependent and is augmented in rats that self-administered cocaine under long-access (LgA; 6 hrs daily) conditions for 14 days when compared to rats provided shorter daily cocaine access (ShA rats; 2 hrs daily). Further, reinstatement in response to ventricular CRF administration is heightened in LgA rats. This study examined the role of altered ventral tegmental area (VTA) responsiveness to CRF in intake-dependent increases in CRF- and stress-induced cocaine seeking. Bilateral intra-VTA administration of CRF (250 or 500 ng/side) produced reinstatement in LgA but not ShA rats. In LgA rats, intra-VTA CRF-induced reinstatement was blocked by administration of the CRF-R1 receptor antagonists antalarmin (500 ng/side) or CP-376395 (500 ng/side) but not the CRF-R2 receptor antagonists astressin-2B (500 ng or 1 µg/side) or ASV-30 (500 ng/side) into the VTA. Likewise, intra-VTA antalarmin, but not astressin-2B, blocked footshock-induced reinstatement in LgA rats. By contrast, neither intra-VTA antalarmin nor CP-376395 altered food-reinforced lever pressing. Intra-VTA injection of the CRF-R1 receptor-selective agonist, cortagine (100 ng/side) but not the CRF-R2 receptor-selective agonist rat urocortin 2 (250 ng/side) produced reinstatement. Excessive cocaine use increases susceptibility to stressor-induced relapse in part by augmenting CRF-R1 receptor dependent regulation of addiction-related neurocircuitry in the VTA.

INTRODUCTION

Cocaine addiction is associated with a persistent susceptibility to drug relapse that emerges in an intake-dependent manner with repeated use. Understanding the neurobiological mechanisms that underlie drug relapse in cocaine addicts is critical to the development of effective treatment. Much evidence suggests that stress contributes to relapse. Stress promotes craving in abstinent human cocaine addicts (Sinha et al., 1999) and precipitates reinstatement in rodent relapse models (Erb et al., 1996, Ahmed and Koob, 1997). The preclinical study of addiction-related drug-induced neuroplasticity has involved the use of the long-access self-administration approach (Ahmed and Koob, 1998) in which rats provided repeated daily long access to cocaine for self-administration (6-10 hrs/daily; LgA rats) are compared to rats provided shorter daily drug access (1-2 hrs/daily; ShA rats). We have reported that, compared to ShA rats, LgA rats are more susceptible to reinstatement by a stressor, footshock, suggesting that repeated cocaine use can produce intake-dependent alterations in how stress regulates neurocircuitry subserving motivation and relapse susceptibility (Mantsch et al., 2008a). Our findings are consistent with reports that the magnitude of stress-induced craving is influenced by the amount of prior use in human addicts (Fox et al., 2005).

The neuropeptide, corticotropin releasing factor (CRF), is a key mediator of stress-induced cocaine seeking (Shalev et al., 2010). CRF receptor antagonists block stress-induced reinstatement (Erb et al., 1998, Shaham et al., 1998, Graf et al., 2011) while delivery of CRF directly into the brain reinstates

cocaine seeking (Erb et al., 2006b, Mantsch et al., 2008a). One key site at which CRF promotes reinstatement is the ventral tegmental area (VTA) (Wise and Morales, 2010) where CRF delivery precipitates reinstatement following self-administration (Wang et al., 2005, Wang et al., 2007). The VTA receives CRF-containing projections from a number of brain regions (Rodaros et al., 2007), and *in vivo* microdialysis studies have found that, during footshock-induced reinstatement, VTA extracellular CRF levels are elevated (Wang et al., 2005).

Both CRF-R1 and CRF-R2 receptor subtypes have been reported to be expressed in the VTA (Korotkova et al., 2006), but the receptor mechanism through which CRF regulates cocaine seeking remains unclear. Wang et al., (2005; 2007) have reported that reinstatement by intra-VTA CRF involves an activation of CRF-R2 receptors that relies on an interaction with CRF binding protein, consistent with the mechanism through which CRF acutely enhances NMDA receptor-mediated currents in the VTA (Ungless et al., 2003). However, others have found that stress-induced reinstatement and evoked increases in VTA and nucleus accumbens dopamine rely on CRF-R1 and not CRF-R2 receptors (Shaham et al., 1998, Lu et al., 2001, Lu et al., 2003a).

We have reported that reinstatement by centrally administered CRF is also augmented following LgA self-administration, suggesting that enhanced CRF responsiveness contributes to heightened stress-induced relapse (Mantsch et al., 2008a). It has been shown that repeated cocaine administration recruits CRF-R1 receptor regulation of excitatory signaling in the VTA (Hahn et al., 2009). In this study, we examine intake-dependent augmentation of intra-VTA CRF-

induced reinstatement and role of VTA CRF-R1 versus CRF-R2 receptors in reinstatement in response to intra-VTA CRF and stress.

MATERIALS AND METHODS

Adult male Sprague–Dawley rats (Harlan Laboratories, St. Louis, MO) were housed individually under a 12 h/12 h reversed light/dark cycle (lights on at 7:00 PM) in a temperature and humidity controlled AAALAC-accredited animal facility. All procedures were approved by the Marquette University IACUC and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Catheter and cannula implantation

For the reinstatement studies, rats were implanted with chronic indwelling jugular catheters under ketamine HCl (100 mg/kg, ip) and xylazine (2 mg/kg, ip) anesthesia as previously described (Mantsch et al., 2008a, Graf et al., 2011) and with bilateral 2.1-cm 23 gauge guide cannulae aimed at the VTA for intracranial injections. The tips of the guide cannulae were aimed 0.5 mm above the target injection site using the following coordinates determined from Paxinos and Watson (2000): 12˚ angle away from midline; A/P − 5.6 mm from bregma; M/L ± 2.2 mm from midline; and D/V − 6.7 mm from the skull surface. Placements for cannula targeting the VTA for rats from each of the experiments are depicted in Figure 15 (Paxinos, 2000).

Self-Administration Training

After recovery from surgery, rats were trained to self-administer cocaine (1.0 mg/kg/inf, iv; NIDA Drug Supply Program) by pressing a lever under a FR1 schedule during daily 2-h sessions, within which the active (i.e., front) lever was extended into the chamber and the corresponding stimulus light was illuminated. Pressing the lever resulted in an iv infusion of drug or saline solution (200 µl over 5 s) followed by a 25-s time-out period during which the stimulus light was extinguished but the lever remained extended. Responding on a second, inactive (i.e., back) lever was recorded but had no programmed consequences. Response requirements were gradually increased until rats displayed stable responding (within 10% of the 3-session mean) under an FR4 schedule at which time they entered into a 14-day period of self-administration testing.

Effects of LgA Self-Administration on intra-VTA CRF-induced reinstatement

To examine intake-dependent effects of cocaine self-administration on later reinstatement by intra-VTA CRF, rats were assigned to ShA or LgA groups after self-administration training according to their access conditions for cocaine self-administration for the next 14 days. ShA rats (n=6) continued to have access to cocaine for two hrs daily as described above. LgA rats (n=8) had access to the same cocaine dose for six hrs daily. Additionally a third group of rats with no prior history of cocaine self-administration had access to infusions of saline during 14 daily 2-h sessions and served as a control to examine non-specific effects of intra-VTA CRF on lever pressing (n=6). Following 14 days of self-administration, rats underwent extinction during ten consecutive 2-h sessions

within which the cocaine solution was replaced by saline. After extinction, rats received a bilateral sham intra-VTA injection prior to testing for reinstatement in response to bilateral intra-VTA delivery of CRF (250 or 500 ng/side; 210 or 420 µM; Sigma-Aldrich) or vehicle (0.9% NaCl) delivered in a volume of 0.25 µl/side over a 1-min period ten minutes prior to the reinstatement session. Responding on both the cocaine and inactive levers were recorded during the 2-h reinstatement sessions which were otherwise identical to extinction conditions. The order of testing with the two CRF doses and vehicle varied across rats in each group to avoid potential sequence effects. Rats underwent additional extinction sessions between reinstatement test sessions and were required to display less than 20 cocaine lever responses during an intervening extinction session in order to be tested again for reinstatement.

Effects of CRF Receptor Antagonists on Intra-VTA CRF-Induced Reinstatement

To examine the role of CRF-R1 and CRF-R2 receptors in reinstatement by intra-VTA administration of CRF, the ability of CRF (500 ng/side) to reinstate cocaine seeking following a 15-min bilateral intra-VTA pretreatment (0.25 µl/side over 1 min) with the CRF-R1 receptor selective antagonists antalarmin (500 ng/side; 4.8 mM; Sigma Aldrich; n=7) or CP-376395 (500 ng/side; 5.5 mM; Tocris Biosciences; n=6) or the CRF-R2 receptor-selective antagonists astressin-2B (500 ng and 1 µg/side; 495 and 990 µM; Sigma-Aldrich; n=6) or anti-sauvagine 30 (ASV-30; 500 ng/side; 548 µM; Sigma-Aldrich; n=6) was determined in separate groups of LgA rats. The astressin-2B and ASV-30 doses that were

used were based on those previously used for intracranial injections (Henry et al., 2006, Forster et al., 2008). After 14 days of LgA self-administration and extinction, rats were tested twice for CRF-induced reinstatement in counterbalanced sequence: once following intra-VTA pretreatment with CRF receptor antagonist and once following pretreatment with vehicle. In the case of the CRF-R2 receptor antagonist astressin-2B, rats were also treated with a second, higher antagonist dose (1 μg/side).

Effects of CRF Receptor Antagonists on Stress-Induced Reinstatement

To examine the role of CRF-R1 and CRF-R2 receptor activation in the VTA in stress-induced reinstatement, separate groups of rats were tested for the ability of electric footshock, delivered though the stainless steel grid floors of the self-administration chambers, to reinstate cocaine seeking following bilateral intra-VTA delivery of the CRF-R1 receptor-selective antagonist antalarmin (500 ng/side; n=7) or the CRF-R2 receptor-selective antagonist astressin-2B (500 ng/side; n=8). During the 15-min footshock period, the houselight was illuminated and the levers were retracted and stimulus lights extinguished. Shocks (0.5 mA, 0.5” duration) were delivered an average of every 40 sec (range 10-70 sec). We have reported that these parameters produce robust reinstatement after long-access, but not short-access, self-administration (Mantsch et al., 2008a). As was the case with intra-VTA CRF, rats were tested twice for footshock-induced reinstatement in counterbalanced fashion: once following intra-VTA pretreatment with CRF receptor antagonist and once following pretreatment with vehicle.

Food Self-Administration

In order to confirm that the effects of intra-VTA antalarmin and CP-376395 on reinstatement were not attributable to non-specific motor impairments, rats were tested for effects on sucrose pellet-reinforced lever pressing (data not shown). These rats were maintained at 90% of their free-feeding body weights and trained to self-administer 45 mg sucrose-sweetened food pellets (BioServ) by pressing a response lever under a FR4 schedule of reinforcement during 30-min sessions. Once stable response patterns were observed (responding within 10% of the mean over 3 sessions), separate groups of rats were tested for the effects of intra-VTA delivery of antalarmin (500 ng/side; n=10) or CP-376395 (500 ng/side; n=6), as described above, on responding. Each rat was tested twice with intra-VTA treatment in counterbalanced sequence: once with the CRF-R1 receptor antagonist and once with vehicle.

Reinstatement by Intra-VTA Administration of CRF Receptor Agonist Drugs

To further examine the role of CRF-R1 and CRF-R2 receptors in reinstatement, a separate group of LgA rats (n=5) was tested for reinstatement following bilateral intra-VTA administration of the CRF-R1 receptor-selective agonist, cortagine (100 ng/side; 90 μM; Phoenix Pharmaceuticals) (Tezval et al., 2004), the CRF-R2 receptor-selective agonist rat Urocortin 2 (rUcn2; 250 ng/side; 212.5 μM; Phoenix Pharmaceuticals) (Reyes et al., 2001), or vehicle (5 mM acetic acid in sterile saline). Following self-administration and extinction, each rat was tested with each intra-VTA treatment in counterbalanced sequence.

After the first test, rats were only tested again for reinstatement if they emitted less than 20 responses during an intervening extinction session.

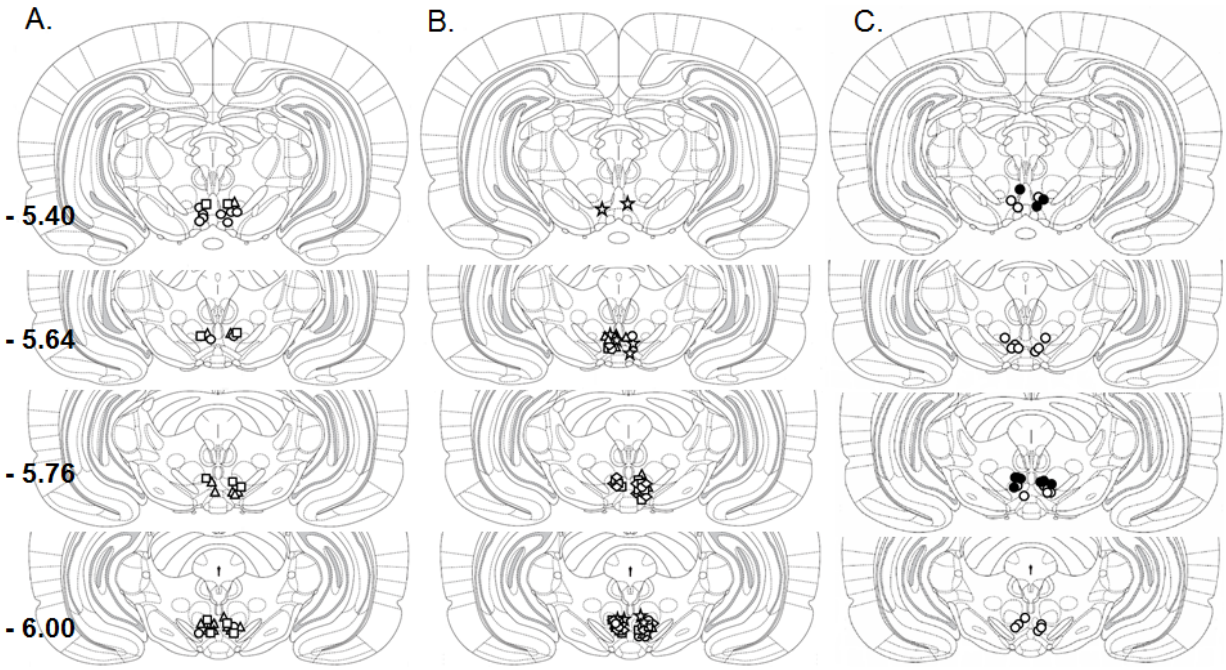
Histological Confirmation of Injection Sites

The accuracy of cannula implantation was confirmed postmortem in each rat after cardiac perfusion with 60-ml NaCl followed by 60-ml 2.5% buffered neutral formalin under sodium barbital anesthesia (55 mg/kg). Brains were removed and stored in 2.5% buffered formalin for at least one day. 200-μm sections were cut using a vibrotome, slide-mounted, and stained with cresyl violet for placement confirmation using a light microscope. Rats with injection sites outside of the VTA were excluded from data analysis.

Statistical Analyses

Statistical analyses were conducted using Predictive Analytics SoftWare statistics software (SPSS, Inc.). Statistical significance was determined using ANOVA or Student's t-tests followed, when appropriate, by further analyses of main effects using ANOVA and/or post-hoc testing using Bonferroni-corrected t-tests.

Figure 15: Injection sites within the VTA for: A) LgA rats (triangles), ShA rats (squares) and Sal rats (circles); B) LgA rats tested for CRF-induced reinstatement following CP-376395 (triangles), ASV-30 (circles), antalarmin (diamonds), and astressin-2B (squares) or LgA rats tested for EFS-induced reinstatement (stars); and C) rats tested for effects on food-reinforced responding (open circles) and LgA rats tested for reinstatement by CRF receptor agonists (closed circles).



RESULTS

Effects of Self-Administration Access Condition on Reinstatement by Intra-VTA CRF Self-Administration

As expected, escalated self-administration was observed in rats provided daily long access (6 hrs, LgA, n=8) but not short access (2 hrs, ShA, n=6) to cocaine for self-administration and was not observed in rats provided access to saline (n=6, Sal). For analysis, the mean daily hourly

self-administration (infusions/hr) was compared across groups over the 14-day time period (Figure 16A). Two-way ANOVA examining effects of self-administration condition (ShA, LgA, and Sal) and day (1-14, repeated measure) on the hourly number of self-administered infusions showed a significant overall main effect of self-administration condition ($F_{2,16}$=23.561; P<0.001) but not day and a significant condition x day interaction ($F_{26,208}$=2.708; P<0.01). The daily number of self-administered infusions increased across the 14 days of self-administration in LgA but not ShA or Sal rats (one-way ANOVA: $F_{13,78}$=2.520; P<0.01). Post-hoc testing showed that self-administration was increased in LgA rats on days 5 and 9-14 compared to short-access rats (Bonferroni-corrected t-test; P<0.05 for each comparison). The mean net cocaine intake over the 14 sessions was marked higher in LgA rats compared to ShA rats (t_{11}=5.889; P<0.001) and is shown in the insert for Figure 16A.

Extinction and Intra-VTA CRF-Induced Reinstatement

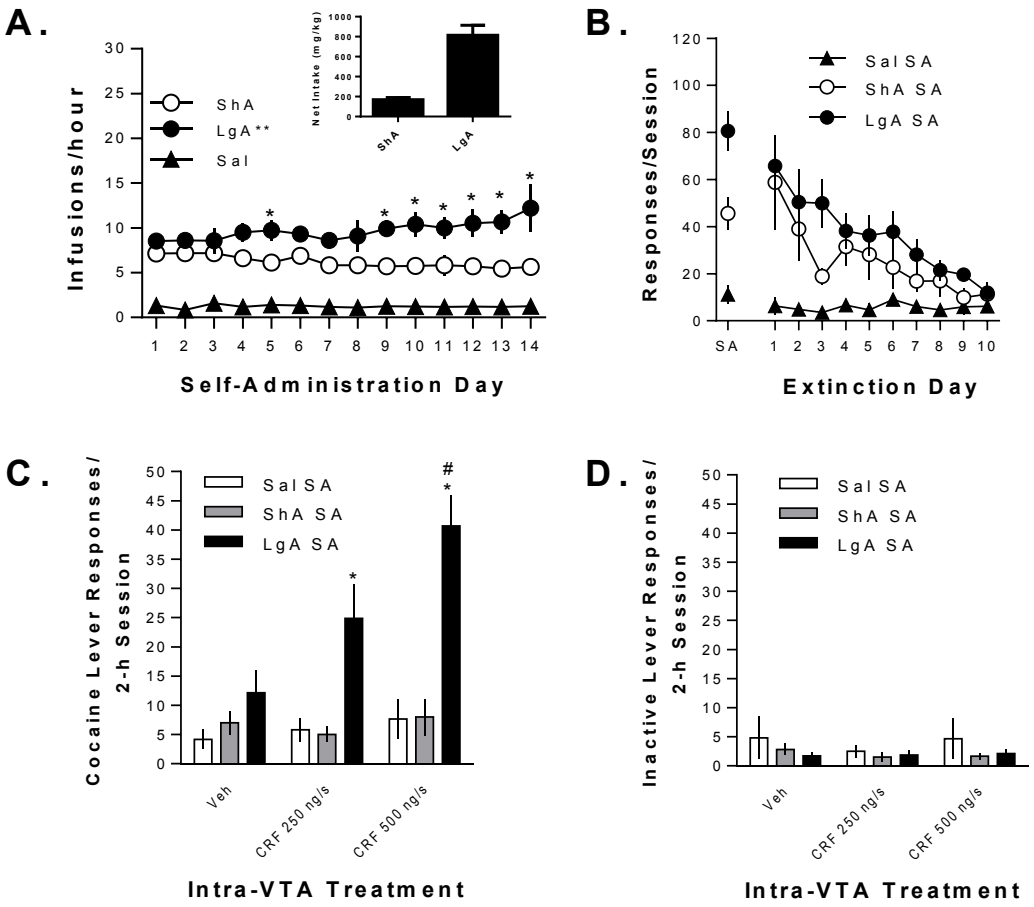
Responding did not differ between ShA and LgA rats during the ten extinction sessions prior to reinstatement testing (Fig 16B). However, differences in reinstatement in response to intra-VTA CRF (250 or 500 ng/side) were observed (Figure 16C). Reinstatement by intra-VTA CRF was only observed in rats with a history of LgA self-administration. Two-way self-administration group (ShA, LgA, Sal) x reinstatement condition (Veh, 250 ng/side CRF, 500 ng/side CRF; repeated measure) ANOVA showed significant main effects of self-administration group ($F_{2,17}$=16.774; P<0.001) and reinstatement condition

($F_{2,34}$=7.934; P=0.001), and a significant group x reinstatement condition interaction ($F_{4,34}$=5.969; P=0.001).

CRF-induced increases in responding were only found in rats with a prior history of LgA self-administration (one-way repeated measures ANOVA; $F_{2,14}$=10.639; P<0.01). Post-hoc testing showed that responding following intra-VTA administration of the 500 ng/side CRF dose was significantly increased compared to vehicle (P<0.05). Additionally, responding on the previously active lever was significantly increased in LgA rats compared to ShA and Saline rats following intra-VTA delivery of either 250 ng/side CRF (one-way ANOVA, $F_{2,19}$=4.179, P<0.05; significant increase in LgA vs. either ShA or Sal, P<0.05, Bonferroni-corrected t-test) or 500 ng/side CRF (one-way ANOVA, $F_{2,19}$=23.103, P<0.001; significant increase in LgA vs. either ShA or Sal, P<0.05). By contrast, we failed to find reinstatement effects or self-administration group effects on responding on the previously inactive lever or a reinstatement x group interaction (Figure 16D).

Figure 16: Self-administration, extinction, and reinstatement of cocaine seeking by intra-VTA injections of CRF in rats that self-administered cocaine under short-access (ShA; 14 x 2 h/day) and long-access (LgA; 14 x 6h/day) conditions and in saline self-administration control rats. Data in Figure 16A represent the daily mean hourly numbers of self-administered infusions (±S.E.) in ShA, LgA, and Sal rats across the 14-day test period. Escalation was observed in LgA, but not ShA or Sal rats (**P<0.05 overall effect) and self-administration was increased in LgA rats compared to ShA (and Sal) rats on days 5 and 9-14 of self-administration (*P<0.05). The cumulative total cocaine intake (mg/kg ± S.E.) in ShA and LgA rats is shown in the insert for Figure 16A. Intake was markedly and significantly increased in LgA rats (**P<0.001 vs. ShA rats). Responding (± S.E.) during the final self-administration session (represented as the 2-h mean in LgA rats) and the ten consecutive 2h extinctions sessions is shown in Figure 16B. Significant differences in extinction responding between ShA and LgA rats were not found. Significant intra-VTA CRF-induced reinstatement was observed in LgA but not

ShA rats, and CRF-induced responding was significantly higher in LgA rats compared to ShA rats and Sal controls at both doses tested (*P<0.05; 16C) and increased compared to vehicle at the 500 (but not 250) ng/side CRF dose ($^{\#}$P<0.05). Effects on responding on the previously inactive lever during reinstatement testing were not found (16D).

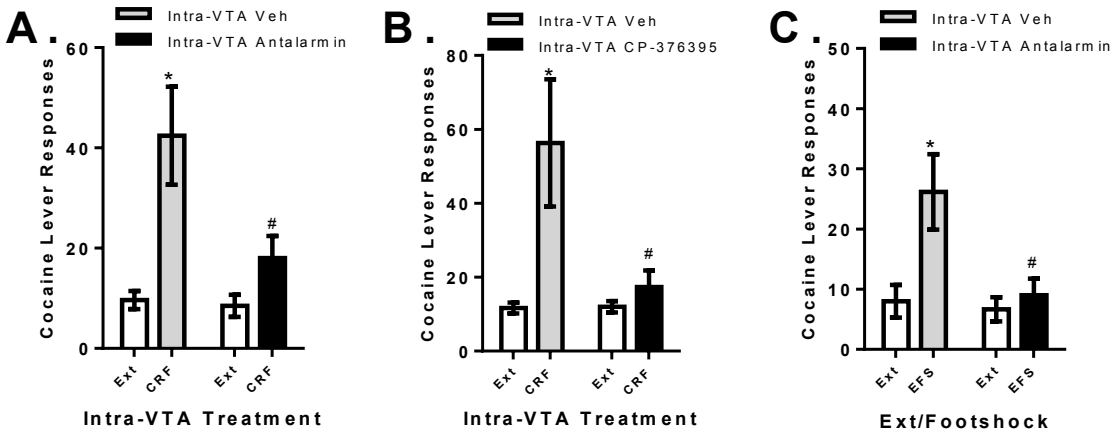


Effects of Intra-VTA CRF-R1 Receptor Antagonists on CRF-Induced Reinstatement

Bilateral intra-VTA pretreatment with the CRF-R1 receptor-selective antagonist antalarmin or CP-376395 blocked reinstatement by intra-VTA CRF (500 ng/side) in LgA rats (Figure 17). The effect of antalarmin on CRF-induced

reinstatement (n=7) is shown in Figure 17A and the effect of CP-376395 on CRF-induced reinstatement (n=6) is shown in Figure 17B. In both cases, 2-way repeated measures (CRF vs. extinction; antagonist vs. vehicle) ANOVA showed significant main effects of CRF delivery ($F_{1,6}$=12.603, P<0.05 for antalarmin; $F_{1,5}$=6.920, P<0.05 for CP-376395), but not antagonist treatment, and significant interactions between CRF-R1 antagonist pretreatment and CRF-induced reinstatement ($F_{1,6}$=5.696, P=0.05 for antalarmin; $F_{1,5}$=9.120, P<0.05 for CP-376395). Intra-VTA CRF produced significant reinstatement following pretreatment with vehicle (P<0.01 for each experiment), but not antalarmin or CP-376395, and responding following intra-VTA CRF administration was significantly lower following antalarmin or CP-376395 pretreatment compared to vehicle pretreatment (P<0.01).

Figure 17: Effects of intra-VTA injections of CRF-R1 receptor antagonists on reinstatement by intra-VTA CRF delivery and footshock stress in LgA rats. Data represent the effects of bilateral injections of antalarmin (500 ng/side; 17A; n=7) or CP-376395 (500 ng/side; 17B; n=6) or vehicle on reinstatement by bilateral intra-VTA delivery of CRF (500 ng/side) and the effects of bilateral injections of antalarmin (500 ng/side) on reinstatement (responses/2-h session ± S.E.) by electric footshock (EFS; 17C; n=6). In all cases, significant reinstatement was observed in rats pretreated with vehicle (*P<0.05 vs. Ext) but not CRF-R1 receptor antagonists and responding during reinstatement was significantly lower following CRF-R1 receptor antagonists compared to vehicle ($^{\#}$P<0.05 vs. Veh).



Effects of Intra-VTA CRF-R1 Receptor Antagonism on Stress-Induced Reinstatement

Antagonism of CRF-R1 receptors in the VTA also blocked footshock-induced reinstatement in LgA rats (n=6; Figure 17C). Two-way (footshock vs. extinction; antalarmin vs. vehicle) repeated measures ANOVA showed a significant overall effect of footshock ($F_{1,5}$=28.817, P<0.01), but not antalarmin pretreatment, and a significant antalarmin x footshock interaction ($F_{1,5}$=6.294, P=0.05). Footshock stress produced significant reinstatement following pretreatment with vehicle (P<0.01), but not antalarmin, and responding following EFS was significantly lower following antalarmin pretreatment compared to vehicle pretreatment (P<0.01).

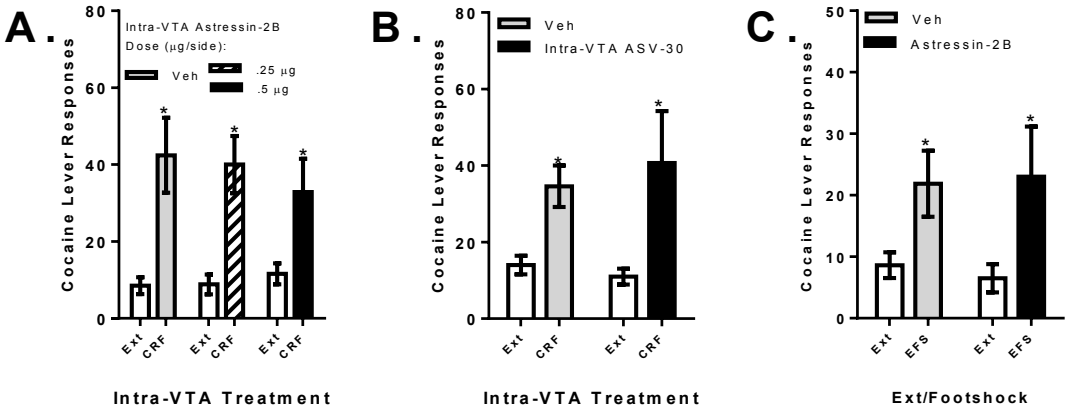
Effects of Intra-VTA CRF-R1 Receptor Antagonists on Food-Reinforced Lever Pressing

In order to confirm that the effects of intra-VTA antalarmin and CP-376395 on reinstatement were not attributable to non-specific motor impairments, effects on sucrose pellet-reinforced lever pressing were examined. Neither intra-VTA antalarmin (185.83 ± 28.86 resp/30-min session vs. 211.80 ± 13.41 resp/session for vehicle) nor intra-VTA CP-376395 (192.67 ± 23.73 resp/30-min session vs. 203.10 ± 13.17 resp/session for vehicle) significantly decreased food-reinforced responding at doses that blocked CRF- or footshock-induced reinstatement. When interpreting these findings, it is important to note that in contrast to our self-administration rats, these rats had no history of cocaine intake and were food-restricted, possibly altering their sensitivity to CRF-R1 receptor antagonism.

Effects of Intra-VTA CRF-R2 Receptor Antagonists on CRF- and Stress-Induced Reinstatement

The effects of bilateral intra-VTA pretreatment with CRF-R2 receptor-selective antagonists astressin-2B or ASV-30 on reinstatement by intra-VTA CRF (500 ng/side) are shown in Figures 18A and 18B. The effects of the astressin-2B on CRF-induced reinstatement (n=6) are shown in Figure 18A.

Figure 18: Effects of intra-VTA injections of CRF-R2 receptor antagonists on reinstatement by intra-VTA CRF delivery and footshock stress in LgA rats. Data represent the effects of bilateral injections of astressin-2B (500 ng and 1 µg/side; 18A; n=6) or ASV-30 (500 ng/side; 18B; n=6) or vehicle on reinstatement by bilateral intra-VTA delivery of CRF (500 ng/side) and the effects of bilateral injections of astressin-2B (500 ng/side) on reinstatement (responses/2-h session ± S.E.) by electric footshock (EFS; 18C; n=8). In all cases, significant reinstatement was observed in rats pretreated with vehicle or CRF-R2 receptor antagonists (*P<0.05 vs. Ext), while CRF-R2 receptor antagonist pretreatments failed to attenuate reinstatement by intra-VTA CRF or shock.



We initially tested rats for the effect of astressin-2B at the 250 ng/side dose. However, since this dose of astressin-2B did not attenuate reinstatement, we also tested rats with a 2-fold higher dose of astressin-2B (500 ng/side; 18A). Two-way repeated measures ANOVA examining the effects of both astressin-2B doses on reinstatement showed a significant overall effect of CRF-induced reinstatement ($F_{1,5}$=52.077, P=0.001), but not astressin-2B pretreatment, and no significant astressin-2B x CRF reinstatement interaction. Significant intra-VTA CRF-induced reinstatement was observed in rats pretreated with vehicle or either astressin-2B dose (P<0.01 for each comparison) and reinstatement was not significantly different following astressin-2B delivery when compared to vehicle

administration. Likewise, intra-VTA pretreatment with ASV-30 failed to alter reinstatement by intra-VTA CRF (n=6; Figure 18B). Two-way repeated measures ANOVA showed a significant overall effect of CRF-induced reinstatement ($F_{1,5}$=8.462, P<0.05), but not ASV-30 pretreatment, and no significant ASV-30 x CRF reinstatement interaction. Significant intra-VTA CRF-induced reinstatement was observed in rats pretreated with vehicle or ASV-30 (P<0.01) and reinstatement was not different following ASV-30 delivery when compared to vehicle administration.

Effects of Intra-VTA CRF-R2 Receptor Antagonism on Stress-Induced Reinstatement

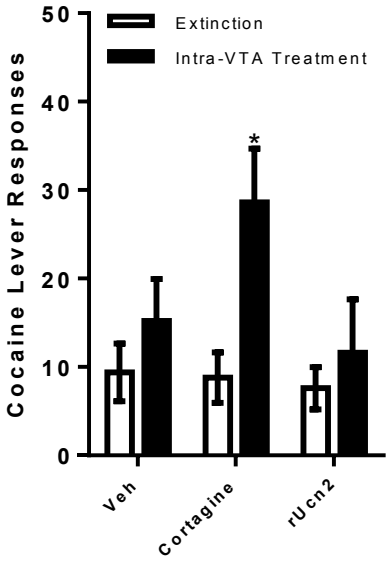
Intra-VTA pretreatment with the CRF-R2 receptor-selective antagonist astressin-2B also failed to alter footshock-induced reinstatement (n=8; Fig 18C). As was the case with CRF-induced reinstatement, ANOVA showed a significant overall effect of footshock reinstatement ($F_{1,7}$=20.587, P<0.01), but not astressin-2B pretreatment, and no significant interaction. Significant footshock-induced reinstatement was observed in rats pretreated with vehicle or astressin-2B (P<0.01) and reinstatement was not different following astressin-2B delivery when compared to vehicle.

Reinstatement by Intra-VTA Administration of the CRF Receptor Agonists

To further examine the role of VTA CRF-R1 and CRF-R2 receptors in reinstatement, the ability of bilateral intra-VTA injection of the selective CRF-R1

receptor agonist, cortagine (100 ng/side) or the selective CRF-R2 receptor agonist, rat Urocortin 2 (rUCN2; 250 ng/side), to reinstate cocaine seeking was examined in a separate group of LgA rats (n=5). Reinstatement was observed following intra-VTA delivery of cortagine but not rUCN2 (Figure 19).

Figure 19: Reinstatement by intra-VTA administration of the CRF-R1 receptor-selective agonist, cortagine, and the CRF-R2 receptor-selective agonist, rUcn2, in LgA rats (n=5). Cortagine (100 ng/side), but not rUcn2 (250 ng/side) or vehicle reinstated extinguished cocaine seeking (*P<0.05 vs. vehicle and the preceding extinction session). Data represent responding on the cocaine lever (responses/2-h session ± S.E.) during extinction (ext) or following bilateral intra-VTA cortagine or vehicle (Veh).



A 2-way repeated measure ANOVA with reinstatement condition (extinction vs. intra-VTA treatment) and drug treatment (cortagine vs. vehicle) as factors showed a significant overall main effect of reinstatement ($F_{1,4}$=17.316, P<0.05), but not cortagine treatment, and a significant reinstatement x cortagine treatment interaction ($F_{1,4}$=7.626, P=0.05). By contrast, an identical analysis examining rUCN2-induced reinstatement failed to show effects of rUcn2

treatment or reinstatement testing or a rUcn2 x reinstatement testing interaction. Intra-VTA cortagine treatment increased responding compared to the preceding extinction session ($P<0.05$) and compared to vehicle-treated rats ($P<0.05$).

DISCUSSION

We have reported that self-administration under LgA/high-intake conditions augments later reinstatement by footshock stress and ventricular CRF (Mantsch et al., 2008a) and that footshock-induced reinstatement following LgA self-administration is CRF-dependent (Graf et al., 2011). These findings are consistent with prior reports that stress-induced reinstatement of drug seeking following self-administration of cocaine (Erb et al., 1998), heroin (Shaham et al., 1997), alcohol (Le et al., 2000) or nicotine (Bruijnzeel et al., 2009) involves CRF, as does stress-induced reinstatement of palatable food-seeking behavior (Ghitza et al., 2006).

Our current findings demonstrate that heightened CRF responsiveness in the VTA likely contributes to intake dependent increases in vulnerability to stress-induced relapse that emerge with repeated drug use. Previous studies have reported that VTA CRF levels are increased during stress-induced reinstatement and that stress-induced cocaine seeking involves CRF actions in the VTA (Wang et al., 2005, Wang et al., 2007). Here we report that, similar to stress-induced reinstatement, reinstatement by intra-VTA CRF was augmented and, in fact was only observed, in rats with a history of self-administration under long-access/high-intake conditions. Further, we find that cocaine seeking induced by

CRF delivery into the VTA or by footshock is mediated by VTA CRF-R1 and not CRF-R2 receptors, a finding that contrasts with previous reports implicating CRF-R2 receptors in the VTA in stress-induced reinstatement (Wang et al., 2005, Wang et al., 2007).

A circuit involving the regulation of VTA dopaminergic neurons that project to the dorsal medial prefrontal cortex has been implicated in stress-induced relapse (McFarland et al., 2004). According to this model, inputs from several regions, including the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala (CeA), and the nucleus accumbens (NA) shell positively regulate mesocortical dopamine neurons in the VTA, thereby promoting cocaine seeking through activation of corticostriatal glutamatergic inputs into the NA core. It has been reported that the VTA receives CRF-containing afferents from two of these regions, the CeA and BNST (Rodaros et al., 2007) and dual immunolabeling studies indicate that these CRF-containing projections are primarily glutamatergic (Tagliaferro and Morales, 2008). CRF containing afferents form synapses on dopaminergic and GABAergic cells in the VTA (Tagliaferro and Morales, 2008) and the activity of both cell types is altered upon local CRF application (Korotkova et al., 2006). In the case of dopaminergic cells, CRF-positive synapses on are primarily asymmetric (Tagliaferro and Morales, 2008), suggesting that CRF-releasing glutamatergic projections into the VTA, likely originating in the BNST and CeA, positively regulate the activity of dopaminergic cells. Accordingly, delivery of CRF into the VTA stimulates local and terminal field dopamine release (Kalivas et al., 1987, Wang et al., 2005).

Although CRF-R1 receptors are abundant in the VTA, their expression on dopaminergic cells has been reported to be limited (Korotkova et al., 2006). However, preliminary findings from the Seasholtz laboratory reports more CRF-R1 expression in dopamine than GABA neurons. This suggests that actions of CRF on dopaminergic cells may contribute to its effects on cocaine seeking. GABAergic and dopaminergic cells in the VTA likely express CRF-R1 receptors (Korotkova et al., 2006), however, the picture is less clear for CRF-R2 receptors. Despite pharmacological evidence for CRF-R2 receptor expression in the VTA (Wang et al., 2007), examination of the VTA using in situ hybridization suggests that there is little or no cell body expression (Van Pett et al., 2000). However, CRF-R2 receptor expression has been reported as determined by reverse transcription-PCR (Korotkova et al., 2006). The purported ability of CRF-R2 receptors to locally regulate glutamate release suggests a potential presynaptic localization of CRF-R2 receptors on glutamate terminals (Wang et al., 2007). A clear understanding of the localization of CRF-R2 receptors in the VTA awaits the availability of better antibodies for immunohistochemical characterization. The precise mechanism of CRF regulation of dopaminergic cells in the VTA is also unclear and has been reported to involve both CRF-R1 receptor-mediated activation of protein kinase C (Wanat et al., 2008) and possibly protein kinase A (PKA) (Riegel and Williams, 2008) signaling and/or CRF-R2 facilitation of NMDA receptor function (Ungless et al., 2003). The CRF-R2 receptor effects on signaling also appear to require CRF interaction with CRF binding protein

(Ungless et al., 2003, Wang et al., 2007) and may also involve presynaptic enhancement of glutamate release (Wang et al., 2005, Wang et al., 2007).

While our data implicate CRF actions in the VTA in stress-induced reinstatement, others have reported that CRF can act elsewhere within this circuit, including at sites upstream from the VTA, to regulate cocaine seeking, most notably in the BNST, where CRF receptor antagonism blocks stress-induced cocaine seeking (Erb and Stewart, 1999). As the CRF actions in the BNST involve projections from the CeA (Erb et al., 2001) and both regions send CRF containing efferents to the VTA (Rodaros et al., 2007), it is likely that CRF exerts actions at multiple sites in the circuit that contribute to stress-induced cocaine seeking.

Similar to what we previously reported with stress-induced reinstatement (Mantsch et al., 2008a), reinstatement by intra-VTA CRF appeared to represent an emergent phenomenon in that it was only observed following LgA self-administration. This finding is consistent with a report by Wang et al. (2005), who found that while footshock-induced increases in VTA CRF were observed in the VTA, the ability of CRF to regulate dopamine and glutamate levels in the VTA required a history of cocaine self-administration. The apparent augmentation of CRF responsiveness is also consistent with reports that CRF responsiveness in a number of brain regions is heightened following repeated cocaine administration (Erb et al., 2005, Liu et al., 2005, Pollandt et al., 2006, Orozco-Cabal et al., 2008, Francesconi et al., 2009, Guan et al., 2009). Although the effects of LgA cocaine self-administration on CRF-R1 receptor-mediated actions

in the VTA have not been examined, some insight into potential mechanisms underlying heightened CRF regulation of cocaine seeking can be provided by studies involving repeated experimenter-delivered cocaine. In the VTA, CRF-R1 receptor activation has been reported to produce both excitatory effects on dopamine neurons via potentiation of NMDA and AMPA receptor-mediated neurotransmission (Hahn et al., 2009), and inhibitory effects via enhancement D2 dopamine- and $GABA_B$-receptor mediated regulation of G-protein activated inwardly rectifying potassium channels (Beckstead et al., 2009). With repeated cocaine exposure, the excitatory effects of CRF-R1 activation are augmented (Hahn et al., 2009), while the inhibitory effects diminish (Beckstead et al., 2009), likely resulting in a net shift towards positive CRF-R1 receptor regulation of dopaminergic cells in the VTA. Further, CRF binding in the VTA, as measured using autoradiography, is increased with repeated cocaine delivery (Goeders et al., 1990).

Our findings that VTA CRF-R1 receptors mediate CRF- and stress-induced cocaine seeking are inconsistent with previous reports suggesting involvement of CRF-R2 receptors in the VTA, apparently through a mechanism that also involves CRF binding protein (Wang et al., 2005, Wang et al., 2007). However, others have reported that stress-induced reinstatement is inhibited by systemic or ventricular delivery CRF-R1, but not CRF-R2, receptor antagonists (Shaham et al., 1998, Lu et al., 2001) and that CRF-R1 but not -R2 receptor antagonism can reduce evoked increases in VTA and NAc dopamine (Lu et al., 2003a, Lodge and Grace, 2005). The reason for the discrepancy between our

findings and those of Wang et al. is unclear but may involve different modes of CRF and receptor antagonist delivery (microinjection vs. reverse dialysis), different CRF receptor antagonist and agonist doses, different rat strains, and differential experimental histories. Most importantly, it may be that the regulation of cocaine seeking by CRF-R1 receptors in VTA requires a prior history of very high levels of cocaine intake (daily cocaine intake reported by Wang et al. was approximately 33 mg/kg compared to more than 70 mg/kg in our LgA rats), consistent with reports that the selective CRF-R1 antagonists only reduce cocaine self-administration following escalation in LgA rats (Specio et al., 2008).

The ability of stressful life events to precipitate drug use through actions involving CRF-R1 receptors in the VTA may represent an emergent consequence of excessive cocaine use. Identification of the precise mechanisms through which VTA CRF-R1 receptor activation produces cocaine seeking and the neuroadaptations that contribute to heightened susceptibility to CRF-dependent reinstatement should provide important insight into how stressor responsiveness changes in cocaine addicts in a way that promotes further use.

CHAPTER 3

EFFECTS OF COCAINE SELF-ADMINISTRATION ON BASAL AND STRESS-INDUCED ACTIVATION OF DOPAMINE CELLS IN THE VTA IN RATS: RELATIONSHIP TO STRESS-INDUCED COCAINE SEEKING

ABSTRACT

The ventral tegmental area (VTA) is a key site for stress-induced regulation of illicit drug use. Here we investigate the relationship between stress-induced cocaine seeking, measured using the self-administration (SA)/reinstatement approach in rats, and activation of VTA dopamine (DA) cells. Previous work has demonstrated that footshock-induced reinstatement is more pronounced and DA alterations are more evident in rats that have a history of cocaine SA under daily long-access (LgA) conditions. We examined basal and stress-induced Fos expression in DA (tyrosine hydroxylase/TH-positive) cells in the VTA using dual immunohistochemistry following testing for footshock-induced reinstatement or under basal conditions in rats with a history of LgA (14 x 6 hrs/day), short-access (ShA; 14 x 2 hrs/day) and saline (Sal) SA. Despite overall differences in TH-positive cell Fos expression across groups (ShA, but not LgA rats had more Fos-expressing TH-positive cells vs. saline controls), significant stress-induced increases in DA cellular Fos reactivity were not observed in any SA access group. However, when examined across groups, the percentage of TH-positive cells expressing Fos following stress-induced reinstatement was positively correlated with reinstatement magnitude. Compared to non-stress and saline controls and non-reinstating rats, the number and percentage of VTA Fos-expressing TH-positive cells were significantly increased in rats that displayed stress-induced reinstatement. These data suggest that stress-induced cocaine use is associated with the activation of DA cells in the VTA and likely with elevated DA levels in target regions.

INTRODUCTION

It is well established that stress contributes to relapse to drug use in cocaine users (Marlatt, 1980). Reports that stressful life events promote relapse are paralleled by findings that, in a laboratory setting, audiotaped scripts describing past stressful events can elicit craving in cocaine-dependent individuals (Sinha et al., 1999). Notably, prior history of higher-frequency cocaine use is associated with augmented stress-induced craving (Fox et al., 2005), with measures of stress-induced craving positively correlated with higher relapse rates (Sinha et al., 2006). Thus, the ability of stress to regulate cocaine use may intensify with excessive use as a result of intake-dependent drug-induced neuroadaptations.

In rats, stress-induced relapse can be examined by determining the ability of electric footshock to reinstate extinguished cocaine seeking following intravenous self-administration (SA) (Shaham et al., 2003). As is the case with stress-induced craving in humans, the ability of footshock to reinstate cocaine seeking is dependent on use history. We have reported that reliable footshock-induced reinstatement is observed in rats with a history of daily long-access (LgA) SA (14 x 6 hrs daily), a condition that results in high levels of drug intake and escalating patterns of use, but not in rats that undergo daily testing under short-access (ShA) SA conditions (14 x 2 hrs daily) (Mantsch et al., 2008a). Understanding the mechanisms that underlie stress-induced reinstatement and

its intake-dependent augmentation in rats could guide the development of treatment aimed at relapse prevention.

VTA dopamine (DA) cells have been implicated in stress-induced cocaine seeking. Extracellular DA in the VTA, likely reflecting somatodendritic release, is elevated during footshock-induced reinstatement following SA in rats (Wang et al., 2005), while transient inhibition of the VTA prevents stress-induced cocaine seeking (McFarland et al., 2004). In particular, DA projections to the medial prefrontal cortex (mPFC) appear to mediate stress-induced cocaine seeking, as administration of DA D1 receptor antagonists into the mPFC prevent reinstatement (Capriles et al., 2003, Sanchez et al., 2003, McFarland et al., 2004). Although these findings suggest that stressors activate DA cells in the VTA to promote drug use, studies examining the effects of stressors on VTA DA neuronal activity and mesocorticolimbic DA neurotransmission have produced mixed results, with reports of both increases and decreases in cellular activity (Ungless et al., 2004, Brischoux et al., 2009) and terminal field DA release (Deutch et al., 1985, Abercrombie et al., 1989, Kalivas and Duffy, 1995, Tidey and Miczek, 1996, Di Chiara et al., 1999, Roitman et al., 2008, Oleson et al., 2012).

One approach for studying neurocircuitry that contributes to cocaine seeking involves examination of the expression of the immediate early gene c-Fos. For example, cue-induced reinstatement of cocaine seeking is associated with increased Fos immunoreactivity in the VTA (Kufahl et al., 2009) and in regions from which it receives projections (Briand et al., 2010, Mahler and Aston-

Jones, 2012). Stressors also increase VTA Fos expression (Deutch et al., 1991), and augment the subsequent induction of VTA Fos expression by other stimuli, including abused drugs (Nikulina et al., 2004, Miczek et al., 2011). However, the exact relationship between stress-evoked increases in Fos expression in VTA DA cells and stress-induced cocaine seeking is unclear.

We have found that augmented stress-induced reinstatement in rats with a history of LgA cocaine SA involves increased VTA responsiveness to corticotropin releasing factor (CRF) (Blacktop et al., 2011), suggesting that heightened stressor-induced reactivity of the mesocorticolimbic DA system may determine relapse susceptibility. In this study we investigate this possibility by examining stress-induced activation of VTA DA cells, defined by the number/percentage of Fos expressing tyrosine hydroxylase- (TH) positive cells, in rats with a history of ShA or LgA SA or control rats with a history of access to saline for SA. We hypothesize that footshock-induced increases in the number of Fos-expressing TH-positive cells would be more pronounced after LgA SA and therefore would correspond to the magnitude of stress-induced reinstatement.

MATERIALS AND METHODS

Subjects

Adult male Sprague–Dawley rats (Harlan Laboratories, St. Louis) were housed individually under a 12-h/12-h reversed light/dark cycle (lights on at 7:00 PM) in a temperature and humidity controlled AAALAC-accredited animal facility.

All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Catheter Implantation and Self-Administration

Rats were implanted with jugular catheters under ketamine HCl (100 mg/kg, ip) and xylazine (2 mg/kg, ip) anesthesia (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). After recovery, rats were assigned to a cocaine SA or saline control (Sal) group. Rats in the SA groups were trained to self-administer cocaine (1.0 mg/kg/inf, iv; NIDA) by pressing a lever under a FR1 schedule during daily 2-h sessions, within which the active lever was extended into the chamber and the corresponding stimulus light was illuminated. Rats in the Sal group were tested under identical conditions except that saline was available. Lever pressing resulted in an iv infusion of drug or saline solution (200 µl over 5 s) followed by a 25-s time-out period during which the stimulus light was extinguished but the lever remained extended. Response requirements were increased until rats displayed stable responding (within 10% of the 3-session mean) under an FR4 schedule was observed, at which time rats were assigned to groups according to their SA conditions for the next 14 days: ShA rats (n=20) continued to have access to cocaine for two hours daily; LgA rats (n=20) were provided access to the same cocaine dose for six hours daily. Sal rats (n=20) continued to have access to saline for SA.

Stress-Induced Reinstatement

Following SA testing, ShA and LgA rats underwent extinction training for ten consecutive 2-h sessions during which the cocaine solution was replaced by saline. Sal rats continued to have saline access over this 10-day period. After extinction, ShA, LgA and Sal rats were further divided into subgroups according to their test conditions prior to sacrifice. Half of the rats in each group (n=10/subgroup) were placed in the chambers and underwent testing for footshock-induced reinstatement. Over a 15-min period prior to the reinstatement session, intermittent shocks (0.5 mA, 0.5" duration, average every 40 sec; range 10-70 sec) were delivered through the grid floors of the chambers. During the shock period, the houselight was illuminated, the levers were retracted, and stimulus lights were extinguished. After the shock period, the response levers were extended into the chamber, the houselight was extinguished and the stimulus light above the previously active lever was illuminated. Reinstatement was defined as responding on the lever previously reinforced by cocaine during the 2-h session, which was otherwise identical to extinction sessions. We have reported that these shock parameters produce robust reinstatement after LgA, but not ShA, SA and do not increase responding in Sal rats (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011)). Non-shocked control rats (n=10/ subgroup) were placed into the chambers for fifteen minutes with no shock exposure prior to undergoing an additional extinction session.

Tissue Processing

Immediately following the 2-h reinstatement/control session, rats were perfused transcardially with 300 ml of 0.5 M potassium phosphate buffered saline (KPBS) followed immediately by 300 ml of 4% paraformaldehyde in potassium phosphate buffer pH 6.8. Brains were removed and post-fixed in 4% paraformaldehyde overnight at 4°C, then stored in 30% sucrose until they sank, which occurred within 48 hours. Brains were then cut into a series of 40-µm sections with a sliding microtome and placed immediately into cryoprotectant at -20°C to prevent freezing during storage.

Immunohistochemistry

Fos is an indicator of neuronal activation (Sagar et al., 1988) and TH is a marker for DA neurons (Hokfelt, 1984). Therefore, co-localization of nuclear Fos and cytoplasmic TH is indicative of a recently activated DA neuron. In order to detect co-labeling, contrasting compartmentalized labeling of Fos and TH is necessary. The immunohistochemical approach described was optimized for this purpose in preliminary experiments using acute ip treatment of 5 mg/kg D-amphetamine, a treatment shown to significantly induce Fos in VTA neurons (Rotllant et al., 2010).

Sequential identification of co-labeled Fos and TH positive neurons via color immunoperoxidase staining was conducted using a protocol modified from Hoffman et al., (2008). Four consecutive sections containing the VTA (~ -5.52 to -

5.76 mm A/P from bregma) were collected from each rat in each treatment condition. Free floating sections were washed in 0.5 M KPBS and incubated in 1% hydrogen peroxide in KPBS for 15 min to block endogenous peroxidase activity. Sections were washed again and incubated in primary polyclonal rabbit anti-Fos antibody (sc-52, Santa Cruz Biotechnology; 1:10,000) in KPBS containing 0.4% triton X-100 first for one hr at room temperature then 24 hr at 4°C. The next day sections were washed again then incubated for one hour in biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories; 1:600) in KPBS containing 0.4% triton X-100. Sections were washed once again then incubated for one hr in A/B solution (Vectastain Elite ABC kit; Vector Laboratories). After another series of washes, sections were incubated in 0.175 M sodium acetate (pH 7.0). Activity was visualized with nickel diaminobenzidine (DAB substrate kit; Vector Laboratories) and the reaction was terminated after 20 min by washing first in 0.175 M sodium acetate then KPBS (Hoffman et al., 2008).

After Fos processing, sections were treated with blocking solutions to block nonspecific avidin and biotin binding sites (Avidin/Biotin Blocking Kit; Vector Laboratories). Sections were incubated in avidin blocking solution for 15 min, washed, incubated in biotin blocking solution for 15 min, and then washed. This step is critical for specific biotinylated goat anti-mouse antibody binding to the mouse anti-TH primary antibody. Following the avidin biotin blocking step, sections were incubated in primary monoclonal mouse anti-TH antibody (MAB-318, Chemicon; 1:300,000) in KPBS containing 0.4% triton X-100 first for 1 hr at

room temperature, then 24 hr at 4°C. Sections were washed then incubated for one hour in biotinylated goat anti-mouse IgG secondary antibody (Vector Laboratories; 1:600) in KPBS containing 0.4% triton X-100. Sections were washed again then incubated in for one hour in A/B solution (Vectastain Elite ABC kit; Vector Laboratories). After another series of washes, sections were incubated in 0.1 M Tris buffer (pH 7.5). Activity was visualized with DAB (DAB substrate kit; Vector Laboratories) as described for Fos. Following termination of the last reaction after 4 mins with 0.1 M Tris buffer (PH 7.5), sections were mounted onto superfrost plus glass slides (VWR International) and allowed to air dry overnight, dehydrated, xylene cleared and coverslipped.

Counting of labeled cells

Bilateral photomicrographs of four consecutive 40-µm sections VTA sections (~ -5.52 to -5.76 A/P coordinates; (Paxinos, 2004)) containing the parabrachial (PBP), parainterfascicular (PIF), and paranigral nuclei (PN) of the VTA were acquired at 20X using a Zeiss Axioscop microscope (Axioscop-2; Zeiss, Thornwood, NY) and Axiovision software (Zeiss) by an experimenter blind to treatment condition. Selection of this area was based on earlier findings that CRF-R1 receptor antagonist administration into this region prevents footshock-induced reinstatement while CRF delivery into this region reinstates (Paxinos, 2004, Blacktop et al., 2011). The DA neurons in this VTA subregion are most responsive (Fos) to rewarding stimuli and have the highest percent of DA neurons (Zhao-Shea et al., 2011).

Fos immunoreactive (ir) neurons were identified by a dark purple/black oval-shaped immunoprecipitate in the nucleus. TH-ir neurons were identified by light brown cytoplasmic staining. This allowed for the identification of TH-ir cells with or without Fos-ir. TH-ir/Fos-ir neurons were identified as cells with dark purple/black nuclear Ni-DAB staining surrounded by light brown cytoplasmic DAB staining. The numbers of TH-ir, Fos-ir, and TH/Fos-ir neurons in the VTA were counted in each series of sections by an experimenter blind to the treatment condition. In addition to the number, the percentage of TH-ir cells that co-expressed Fos was calculated.

Data Analysis

In order to ensure similar withdrawal periods across conditions, all rats were perfused no more than two weeks after the last SA session. As a result, cocaine seeking was not fully extinguished in some rats at the time of testing. SA rats displaying more than 25 responses during the final extinction session prior to sacrifice (i.e., non-extinguished rats) were removed from the analyses. A total of ten rats (five ShA and five LgA) were excluded based on this criterion (60-10 = n of 50). Statistical analyses were conducted using Predictive Analytics SoftWare statistics software (SPSS). Differences in SA among Sal, ShA and LgA rats were assessed using 2-way SA condition x test day (repeated measure) ANOVA. Reinstatement in each group was defined as a significant increase in responding relative to the prior extinction session using paired Student's t-tests. Differences in the numbers of Fos-ir, TH-ir, and TH-ir/Fos-ir cells and percentage

of TH-ir cells expressing Fos across SA groups and in response to footshock were determined using 2-way SA condition x stress condition ANOVA followed by post-hoc testing using Bonferroni-corrected t-tests. The relationship between reinstatement and Fos reactivity was assessed using linear regression analysis. Since, in contrast to earlier finding (Mantsch et al 2008), about 30% of ShA rats showed footshock-induced reinstatement while about 40% of LgA rats did not, we examined differences in Fos reactivity between rats that reinstated, rats that did not reinstate, and rats not exposed to shock, as well as Sal controls, using one-way ANOVA followed by post-hoc testing using Bonferroni-corrected t-tests. For this analysis, reinstatement was defined as an increase in responding by at least ten responses relative to the prior extinction session. For all analyses, significance was defined as $P<0.05$.

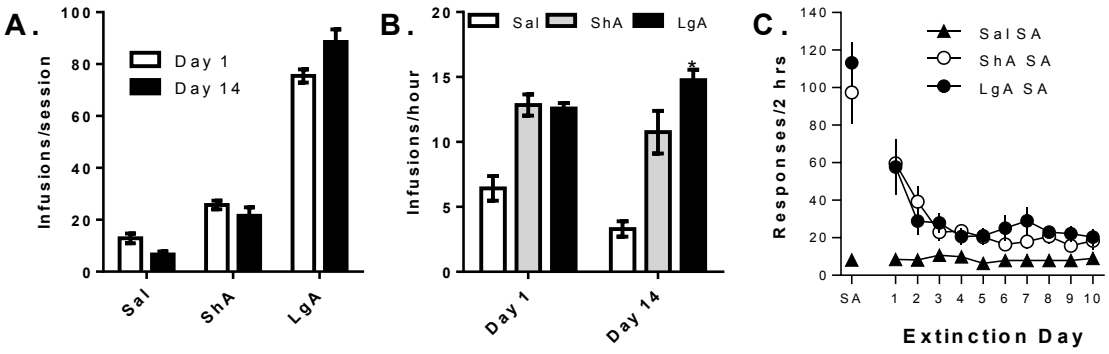
RESULTS

Cocaine SA and Extinction

SA on days one and 14 of testing in rats provided 14 days of ShA (2 hrs) or LgA (6 hrs) to cocaine or Sal access is shown in Figure 20A. To permit comparison across groups, hourly SA was assessed and is shown in Figure 20B. Two-way SA group x day ANOVA comparing day one and 14 infusions in ShA, LgA and Sal rats, showed a significant SA group x test day interaction ($F_{2,47}=3.67$; $P<0.05$). Post-hoc testing showed that the number of hourly infusions was significantly increased in LgA, but not ShA or Sal, rats on day 14 vs. day one ($P<0.05$) and that on day 14, but not day one, SA was greater in LgA

vs. ShA rats (P<0.05). Thus, as has been reported (Ahmed and Koob, 1998, Mantsch et al., 2004), escalated SA was observed in LgA, but not ShA rats. ShA and LgA rats did not significantly differ in extinction responding (Figure 20C).

Figure 20: Self-administration and extinction responding in ShA, LgA, and Sal control rats. Data represent A) the mean total (infusions/session) and B) hourly (infusions/hr) self-administration (SA) on days 1 and 14 of daily SA and C) extinction (responses/2 hrs) of cocaine seeking in rats tested under short-access (ShA; 14 x 2 hrs/day) or long-access (LgA; 14 x 6 hrs/day) conditions or under saline control (Sal) conditions. Escalation of SA was determined based on differences in hourly SA. Two-way ANOVA followed by post-hoc testing showed that escalation was observed in LgA (P<0.05 day 14 vs. day) but not ShA and Sal rats and that SA on day 14 was increased in LgA rats compared to ShA rats (P<0.05). Differences between ShA and LgA rats in extinction responding were not observed.

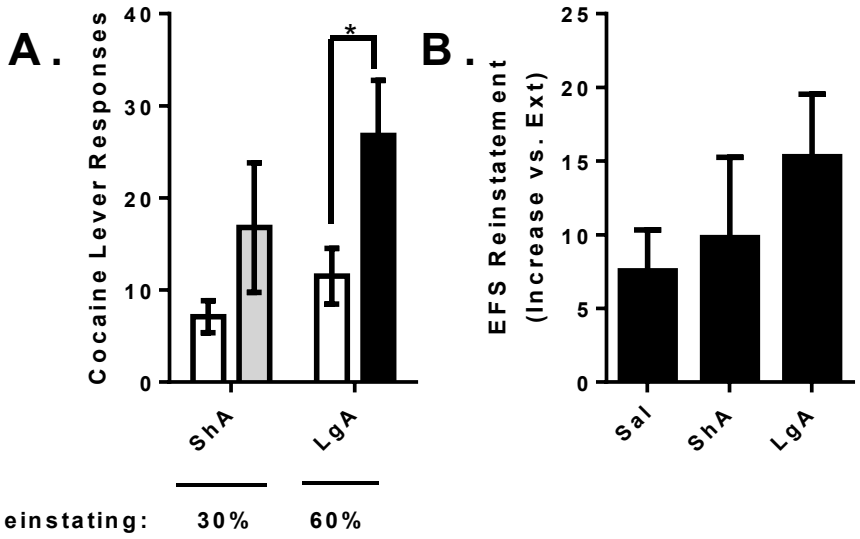


Footshock-Induced Reinstatement of Cocaine Seeking

Footshock-induced reinstatement in ShA, LgA and Sal rats is shown in Figure 21. Responding on the active lever on the final day of extinction and following footshock is shown in Fig. 21A. Significant reinstatement was observed in LgA (increased responding vs. extinction; paired 2-tailed t_8=3.126; P<0.05) but not ShA rats. However, in contrast to previous findings that footshock-induced

reinstatement was observed in virtually all LgA rats and no ShA rats (Mantsch et al., 2008a), in this study, reinstatement was observed in 30% of ShA rats and only 60% of LgA animals. The mean change in lever pressing in each group relative to the previous extinction session is shown in Figure 21B. One-way ANOVA failed to show an overall effect of SA group on footshock-induced reinstatement.

Figure 21: Footshock-induced reinstatement of cocaine seeking in ShA and LgA rats. Data represent A) responding on the active lever on the final day of extinction (Fig. 21A; white bar) and following footshock, prior to Fos measurement, in ShA (grey bar) and LgA (black bar) rats and B) Footshock-induced increases in active lever presses in ShA and LgA animals as compared to saline controls. Although significant reinstatement was observed in LgA, but not ShA, rats (*P<0.05 vs. extinction), significant differences in the magnitude of reinstatement were not observed across SA groups and individual variability in reinstatement was observed in each group.



Effects of SA Condition on Fos Expression in VTA DA Cells

Table 1 shows the numbers of TH- and Fos-ir cells in the VTA following footshock or under control conditions in ShA, LgA and Sal rats. Two-way ANOVA failed to show significant main effects of SA condition or footshock on the numbers of TH- or Fos-ir VTA cells or significant group x footshock interactions. However, although no differences were observed, there was much within-group variability. For this reason, when examining Fos reactivity, both the total number of Fos-/TH-ir and percentage of TH-ir cells showing Fos-ir were assessed.

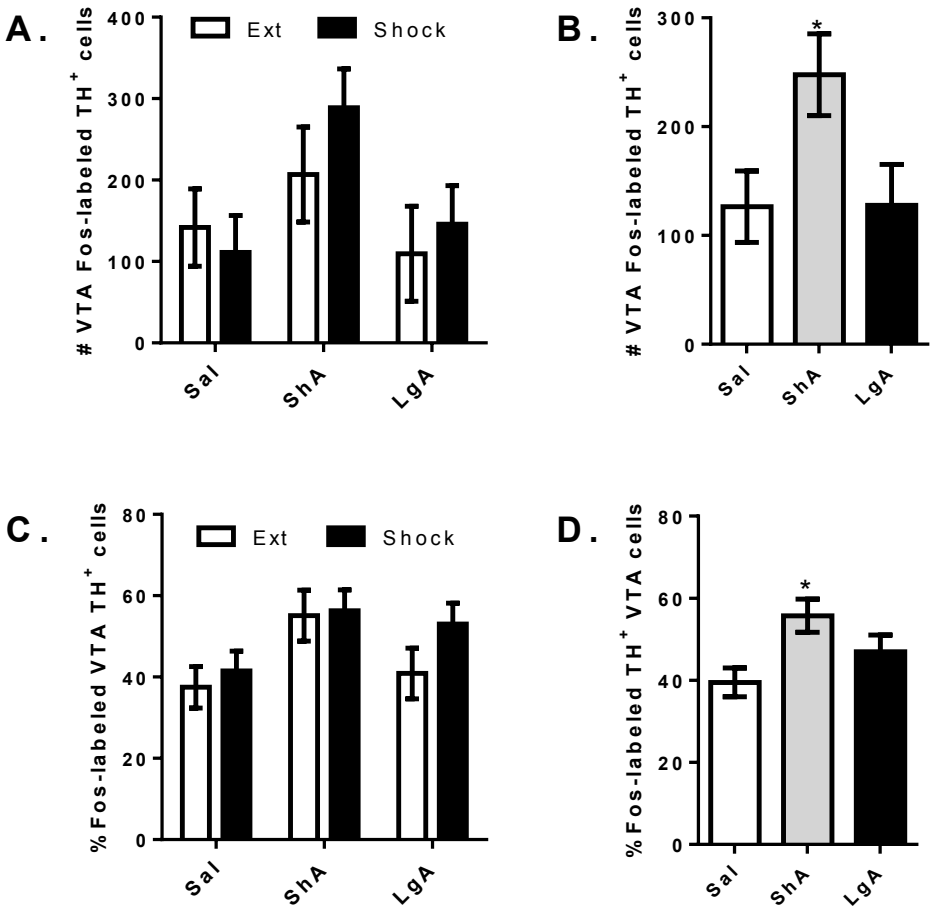
Table 1: Total numbers of TH-positive (TH^+) and Fos-positive (Fos^+) cells in the VTA (±S.E.) following footshock (Shock) or under control (No Shock) conditions in rats with a history of Saline, ShA or LgA SA.

SA CONDITION:	**Saline**		**ShA**		**LgA**	
	Ctrl	***EFS***	***Ctrl***	***EFS***	***Ctrl***	***EFS***
TOTAL TH^+ VTA CELLS	367.33 ±64.74	280.90 ±61.42	316.00 ±79.29	465.44 ±64.74	234.00 ±79.29	255.89 ±64.74
TOTAL FOS^+ VTA CELLS	310.78 ±130.59	354.3 ±104.45	504.00 ±159.94	573.56 ±130.59	282.00 ±159.94	451.78 ±130.59

The number and percentage of TH-ir cells co-expressing Fos under footshock reinstatement or control conditions following Sal, ShA or LgA SA are shown in Figures 22A (number of cells) and 22C (percentage of cells). Two-way ANOVA revealed a significant overall effect of SA condition on both the number ($F_{2,43}$=3.612;P<0.05) and percentage ($F_{2,43}$=4.591;P<0.05) of TH-ir cells that

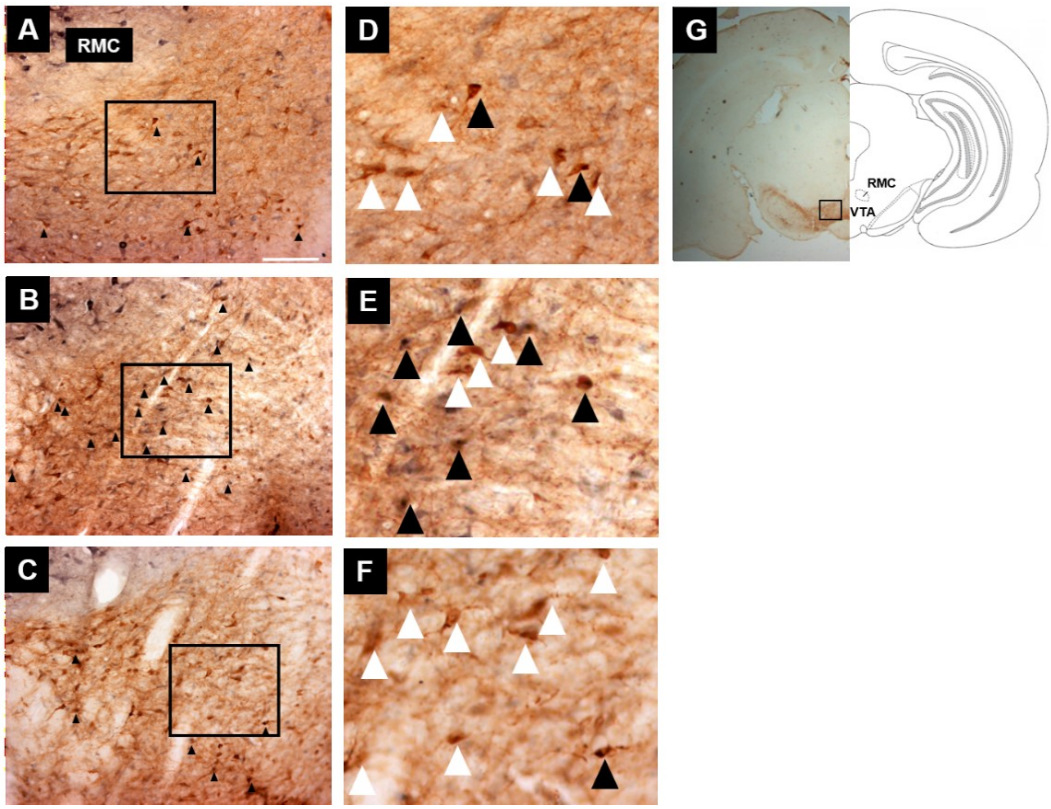
were also Fos-ir. However significant overall effects of footshock or SA condition x footshock interactions were not observed. Figures 22B (number of Fos-ir DA cells) and 22D (percentage of Fos-ir DA cells) represent the data collapsed across shock conditions. Post-hoc analyses showed that, overall, both the number and percentage of Fos-ir VTA DA cells were increased in ShA, but not LgA rats, compared to Sal controls ($P<0.05$), suggesting that 1) DA cells are activated in rats with a history of ShA SA, regardless of whether or not rats are exposed to shock and 2) the activation of DA cells is blunted or absent in LgA rats.

Figure 22: Fos-expressing DA cells in the VTA following footshock in ShA, LgA, and Sal rats. Data represent the number (A and B) and percentage (C and D) of TH-positive cells co-expressing Fos in the VTA of Sal, ShA, and LgA rats. Panels A and C depict Fos expression in DA cells in rats tested under extinction conditions (Ext) and in rats that underwent testing for footshock-induced reinstatement of cocaine seeking (Shock). Two-way ANOVA showed significant main effects of SA condition ($P<0.05$) but not footshock on both the numbers and percentages of Fos-expressing DA cells and failed to show a significant interaction between SA condition and footshock. Overall differences across treatment conditions are shown in panels B and D. Post-hoc testing showed that overall increases in both the number (B) and percentage (D) of Fos-expressing DA cells were increased in ShA, but not LgA, rats compared to Sal controls ($P<0.05$).



Representative photomicrographs showing immunoperoxidase staining for nuclear Fos and cytoplasmic TH in Sal (23A/D.), ShA (23B/E), and LgA (23C/F) rats are presented in Figure 23. Figures 23D, 23E, and 23F are higher magnification photomicrographs of the area within the black perimeter in 23A, 23B, and 23C, respectively. Figure 23G illustrates the area of interest in the VTA used for analysis, which includes the PBP, PIF, and PN of the VTA.

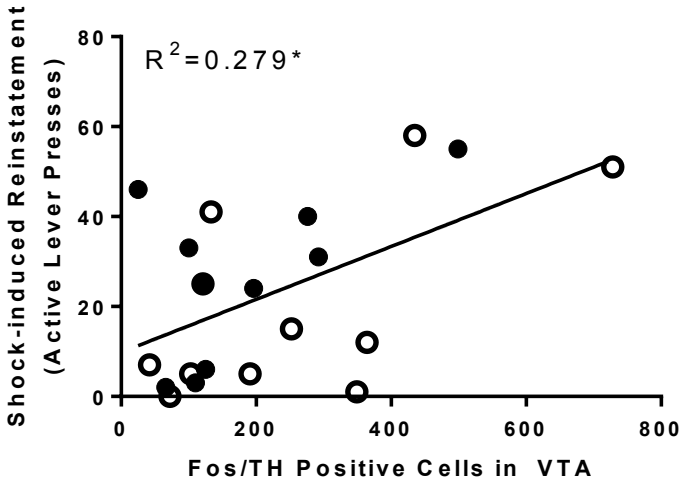
Figure 23: Photomicrographs showing Fos-, TH-, and combined Fos-/TH-immunoreactive cells in the VTA from representative sections from ShA, LgA, and Sal rats. The photomicrographs display the midbrain magnified at 20X from sections labeled using anti-Fos and anti-TH antibodies from a Sal control rat (A) or rats that underwent ShA (B) or LgA (C) SA testing. D, E, and F are higher magnification photomicrographs of the area within the black perimeter in A, B, and C, respectively. TH-positive only cells are indicated by white arrows, and TH-positive cells with Fos-positive nuclei are indicated by black arrows. Panel G shows the target area (box) including the parabrachial (PBP), parainterfascicular (PIF), and paranigral nuclei (PN) of the VTA at -5.76 mm posterior to bregma using the RMC (magnocellular part of the red nucleus) as an anatomic reference (Paxinos and Watson, 2004). The reticule in panel A represents 100 μm.



Relationship between Fos Expression in VTA DA Cells and Shock-Induced Reinstatement

Since there was variability in footshock-induced reinstatement in both ShA and LgA rats, we conducted linear regression to examine the relationship between the magnitude of cocaine seeking following footshock and the number of VTA Fos-ir DA cells (Figure 24). A significant positive correlation was observed between the number of TH-ir/c-fos-ir cells and EFS-induced reinstatement defined as either the total number of responses during testing (R^2=0.279; $F_{1,19}$= 6.976; P<0.05; Figure 24) or the increase relative to the prior extinction session (R^2=0.245; $F_{1,19}$= 5.854; P<0.05; not shown). The relationship between VTA DA cell Fos expression and footshock-induced cocaine seeking was stronger in ShA rats (R^2=0.449; P<0.05), but was also present, although not statistically significant, in LgA rats (R^2=0.314; P=0.09). By contrast, no significant correlations between TH-ir/Fos-ir cell numbers in the VTA and cocaine seeking in the absence of footshock were observed.

Figure 24: Scatter plot with regression line depicting the relationship between stress-induced cocaine seeking and the activation of DA cells in the VTA. When rats with a history of ShA and LgA SA were combined, a significant positive correlation was observed between the total number of Fos-labeled TH-positive cells in the VTA and active lever responding during testing for footshock-induced reinstatement (R^2=0.279; P<0.05). Closed circles represent LgA rats. Open circle represent ShA rats.



To further the examine the relationship between the activation of DA cells in the VTA and stress-induced cocaine seeking, we compared Fos-ir in TH-positive cells among rats that 1) demonstrated footshock-induced reinstatement (increase ≥ 10 responses vs. extinction), 2) failed to demonstrate reinstatement, and 3) were not exposed to footshock - in each case regardless of if they had undergone ShA or LgA SA. Immunoreactivity in these rats was also compared to shocked and non-shocked Sal rats. Differences in footshock-induced cocaine seeking between "reinstated" and "non-reinstated" rats are shown in Figure 25. Due to an imbalanced design, differences in the numbers and percentages of Fos-ir and/or TH-ir cells in the VTA were analyzed using one-way ANOVA comparing the following groups: 1) rats with a history of SA (ShA or LgA) but not exposed to footshock; 2) rats with a history of SA (ShA or LgA) that displayed footshock-induced reinstatement; 3) rats with a history of SA (ShA or LgA) that

did not display footshock-induced reinstatement; 4) Sal control rats the received footshock; and 5) Sal control rats that did not receive footshock.

Figure 25: Footshock-induced cocaine seeking in rats classified according to footshock-induced reinstatement. Rats were classified as reinstated and non-reinstated according to whether or not they displayed footshock-induced reinstatement (see Materials and Methods). Active lever presses (± S.E.) under extinction conditions and during testing for footshock-induced reinstatement in responders and non-responders is shown in panel A (*P<0.001 vs. extinction). The magnitude of reinstatement defined as the mean increase in responding relative to the prior reinstatement session (± S.E.) is shown in panel B.

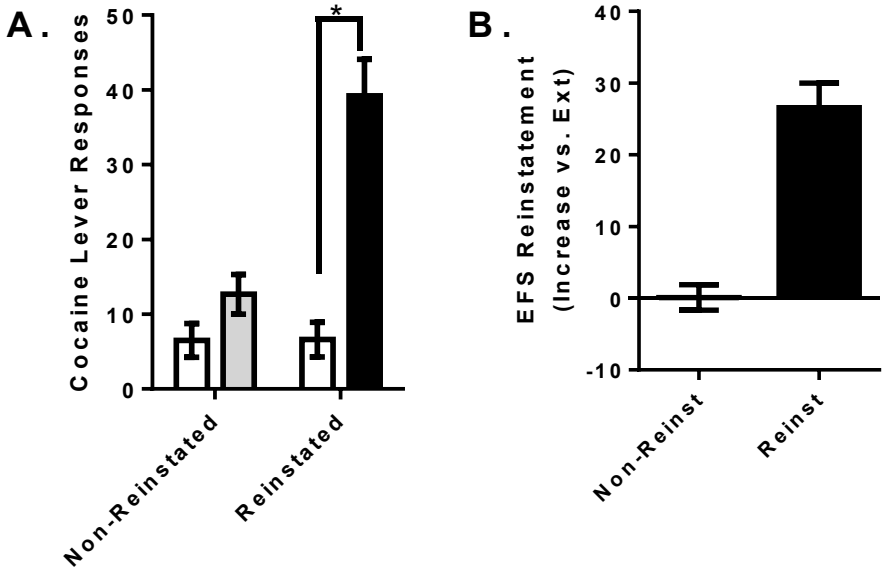


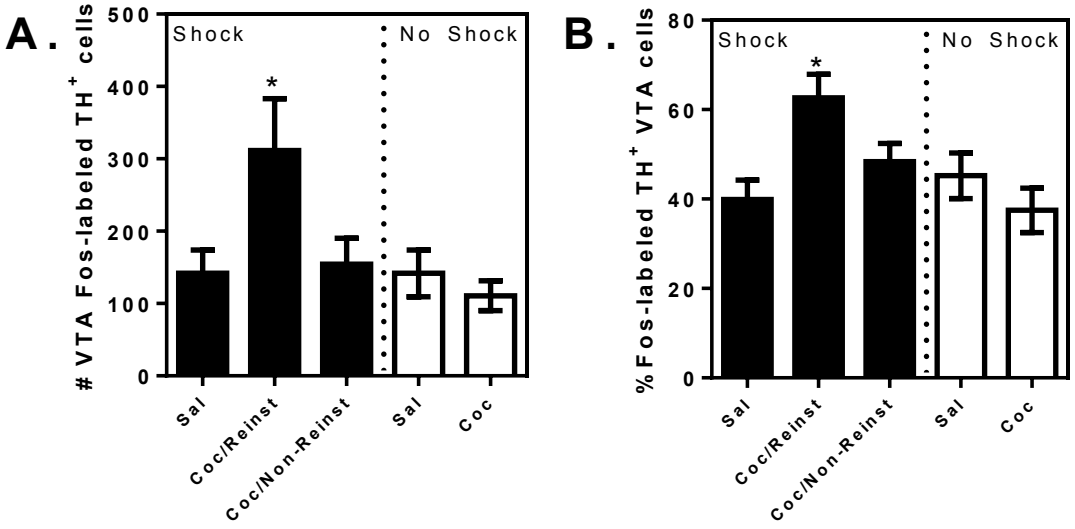
Table 2 shows the numbers of TH-ir and Fos-ir cells in the VTA in each of the groups. One-way ANOVA failed to show differences across conditions in TH-positive or Fos-positive cell numbers. However, since there was much within-group variability, when examining Fos reactivity, both the total number of Fos-/TH-ir and percentage of TH-ir cells showing Fos-ir were assessed.

Table 2: Total numbers of TH-positive (TH^+) and Fos-positive (Fos^+) cells in the VTA (±S.E.) following footshock (Shock) in saline control rats (Sal SA), cocaine self-administering rats that displayed footshock-induced reinstatement (Coc SA/Resp), and self-administering rats that did not reinstate (Coc SA/Non-Resp) and in cocaine self-administering rats (Coc SA) and saline controls (Sal SA) that did not receive footshock (No Shock).

SA CONDITION:	**SHOCK**			**NO SHOCK**	
	Sal SA	***Coc SA/ Resp***	***Coc SA/ Non-Resp***	***Sal SA***	***Coc SA***
TOTAL TH^+ VTA CELLS	280.90 ±43.63	476.56 ±97.74	307.90 ±62.14	367.33 ±63.44	275.00 ±59.03
TOTAL FOS^+ VTA CELLS	354.3 ±110.32	620.56 ±138.06	414.70 ±140.49	310.78 ±87.26	393.00 ±117.47

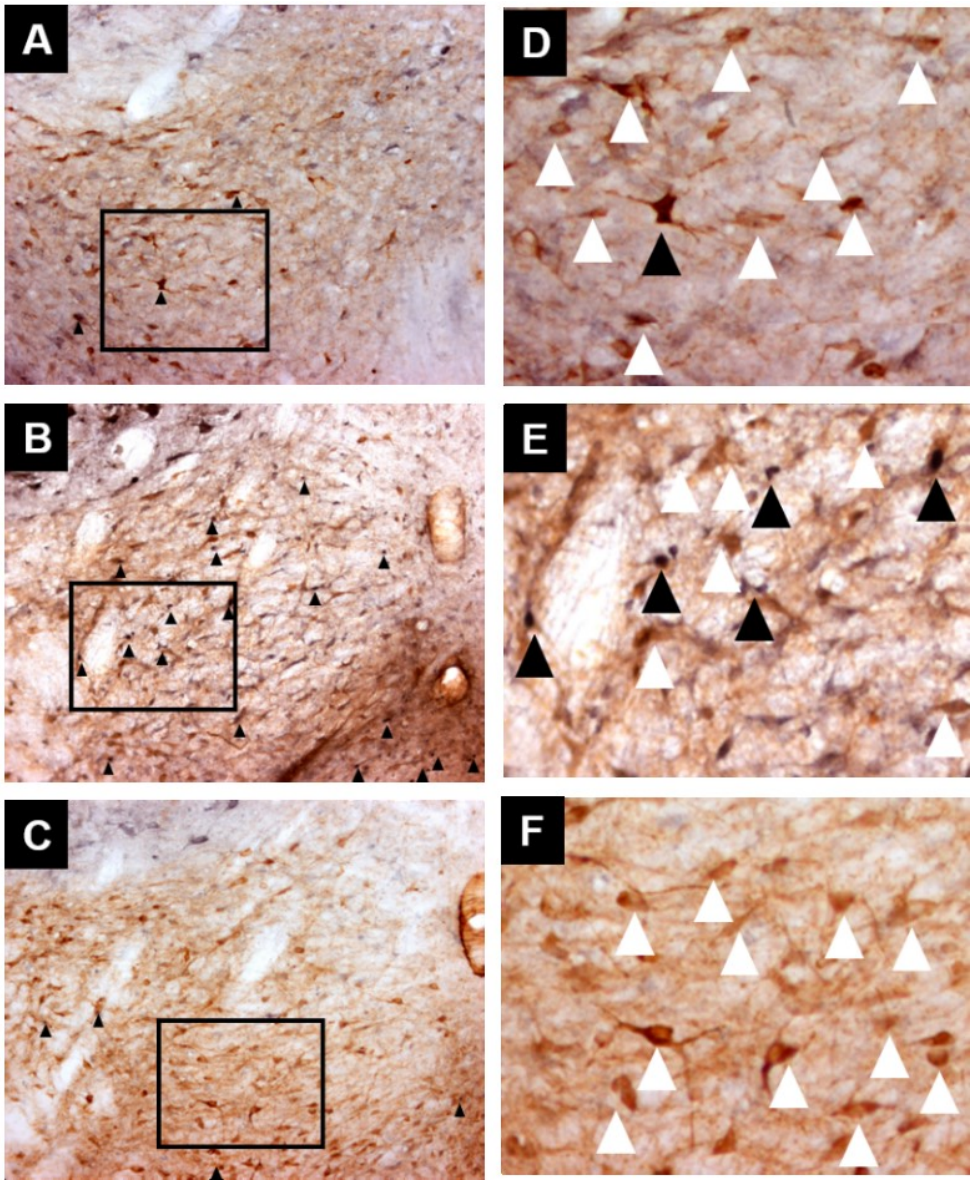
The number and percentage of VTA DA cells expressing Fos under each condition are depicted in Figure 26. One-way ANOVA showed significant group effects on both the number of Fos-expressing TH-positive cells ($F_{2,28}$=5.346; $P<0.05$; 26A) and the percentage of TH-positive cells expressing Fos ($F_{2,28}$=6.690; $P<0.01$; 26B). Post-hoc testing showed that both the number and percentage of VTA cells that displayed dual TH-/Fos-ir were significantly increased in rats in which reinstatement was observed, but not in non-reinstating rats or rats not exposed to footshock, compared to Sal controls ($P<0.05$). Thus, DA cells in the VTA were only activated when footshock produced cocaine seeking.

Figure 26: Fos-expressing DA cells in the VTA following footshock in rats classified based on the expression of footshock-induced cocaine seeking. Data represent the number (panel A) and percentage (panel B) of TH-positive cells co-expressing Fos in the VTA following footshock (Shock) in saline control rats (Sal), cocaine self-administering rats that displayed footshock-induced reinstatement (Coc/Reinst), and self-administering rats that did not reinstate (Coc/Non-Reinst) and cocaine self-administering rats (Coc) and saline controls (Sal) that did not receive footshock (No Shock). Shock only increased the number of Fos-expressing DA cells in the VTA in rats that expressed footshock-induced cocaine seeking (Coc/Resp rats; $P<0.05$ vs. Sal rats).



Representative photomicrographs showing staining for VTA nuclear Fos and cytoplasmic TH in cocaine experienced animals that did not receive footshock (Fig. 27A/D), cocaine experienced that received footshock and reinstated (Fig. 27B/E), and experienced animals that received footshock and did not reinstate (Fig. 27C/F) are shown in Figure 27. Figures 27D, 27E, and 27F are higher magnification photomicrographs of the area within the black perimeter in 27A, 27B, and 27C, respectively.

Figure 27: Photomicrographs showing footshock-induced increases Fos-, TH-, and dual Fos-/TH- immunoreactive cells in the VTA in reinstating but not non-reinstating rats. The photomicrographs display the VTA magnified at 20X from sections labeled using anti-Fos and anti-TH antibodies from a representative rat that self-administered but did not receive footshock (A), a rat that displayed footshock-induced reinstatement (B) and a rat that did not engage in cocaine seeking following footshock exposure (C). D, E, and F are higher magnification photomicrographs of the area within the black perimeter in A, B, and C, respectively. TH-positive only cells are indicated by white arrows, and TH-positive cells with Fos-positive nuclei are indicated by black arrows.



DISCUSSION

Evidence suggests that activation of the VTA is involved in stress-induced relapse to cocaine use. However, the exact relationship between stress-induced cocaine seeking and the activity of DA cells in the VTA is not well understood. Here we demonstrate that stress-induced reinstatement following cocaine SA and extinction in rats is associated with the recruitment of VTA DA cells VTA, as defined by Fos expression in TH-positive cells. In rats with a history of cocaine SA, regardless of whether it was ShA or LgA, footshock stress only increased the number of Fos-expressing DA cells in the VTA when it reinstated cocaine seeking. Furthermore, the magnitude of footshock-induced reinstatement was positively correlated with both the number and percentage of Fos-expressing DA cells. The association between stress-induced cocaine seeking and activation of DA cells is consistent with reports that extracellular VTA DA levels are increased during footshock-induced cocaine seeking following SA (Wang et al., 2005). Furthermore, it has been reported that 1) pharmacological manipulations of the VTA disrupt stress-induced reinstatement of cocaine seeking (McFarland et al., 2004, Blacktop et al., 2011); and 2) DA D1 receptor antagonist administration into the mPFC prevents stress-induced reinstatement (Capriles et al., 2003, Sanchez et al., 2003, McFarland et al., 2004), suggesting that there is a causal relationship between stress-induced activation of DA cells in the VTA, particularly those that project to the mPFC, and stress-induced cocaine seeking.

Since we previously reported that footshock reinstates cocaine seeking in LgA but not ShA rats (Mantsch et al., 2008a), the initial objective of this study was to compare stress-induced activation of DA cells between rats with a history of ShA and LgA cocaine SA and saline controls. Not only were there no differences in the numbers or percentages of Fos-expressing TH-positive cells in the VTA following footshock across ShA, LgA, and Sal rats, but footshock-induced increases in DA cell activation were not observed in any SA group. The lack of differences was likely attributable in part to a greater variability in footshock effects than was previously reported. In contrast our prior findings, 30% of the ShA rats tested and only 60% of LgA rats tested displayed footshock-induced reinstatement and the overall magnitude of reinstatement did not differ between ShA and LgA rats. These differences were not the result of altered intake, as regression analyses found that cocaine intake over the 14-day SA period failed to predict Fos reactivity (data not shown). It is not clear why we failed to observe the clear differences in drug seeking between ShA and LgA rats that we previously found. Nonetheless, these findings indicate that while prior intake is one predictor of stress-induced cocaine seeking, other factors (e.g., pre-existing individual differences) that dictate the ability of stress to engage VTA DA cells are also important determinants.

While stress-induced activation of DA cells in the VTA did not differ with SA history, overall Fos expression in DA cells did. In ShA rats, the number of Fos-expressing TH-positive cells was higher than in Sal controls. Since these rats were re-introduced into an environment in which they previously self-

administered cocaine prior to Fos measurement, it is likely that the exposure to the SA context accounted for the overall increase in DA cell activation. Consistent with this interpretation, it has been reported that Fos expression in the VTA (Kufahl et al., 2009) and in regions that project to the VTA (Mahler and Aston-Jones, 2012) is increased by exposure to cocaine-conditioned stimuli that reinstate drug seeking. Alternatively, simply having a history of prior drug exposure may produce long-term basal increases in Fos expression in TH-positive cells.

In contrast to ShA rats, the overall number of Fos-positive DA cells in the VTA in LgA rats was no different than Sal controls. One interpretation of this finding is that, with repeated LgA SA, overall deficits in DA cell function emerge and mask the processes that elevate DA activity in ShA rats. This interpretation is in line with reports that basal extracellular NA DA levels are decreased following extended or continuous access SA in rats (Weiss et al., 1992b). Thus, while we were unable to detect cocaine access-/intake-dependent changes in stressor-reactivity in the DA system, the overall activity of DA cells appears to be progressively reduced as cocaine intake increases, consistent with the theory that hedonic deficits attributable to dysfunction of dopaminergic systems emerge with excessive use and contribute to the onset of addiction (Koob, 2009).

Based on the lack of differences in stress-induced activation of DA cells across SA conditions and the unexpected variability in the magnitude of footshock-induced reinstatement, we compared the number of Fos-expressing TH-positive cells in the VTA in rats that displayed reinstatement with those that

did not, regardless of whether they were ShA or LgA rats. Footshock only activated VTA DA cells in rats that displayed cocaine seeking. The finding that footshock failed to produce a response in rats with no prior SA history (Sal control rats) or in rats in which footshock failed to evoke cocaine seeking, is somewhat surprising. Studies examining the effects of stressors and aversive stimuli on mesocorticolimbic DA neurotransmission have yielded mixed results, with reports of both increases and decreases in VTA DA cell activity (Ungless et al., 2004, Brischoux et al., 2009) and terminal field DA release (Deutch et al., 1985, Abercrombie et al., 1989, Kalivas and Duffy, 1995, Tidey and Miczek, 1996, Di Chiara et al., 1999, Roitman et al., 2008, Oleson et al., 2012). The observation that increases in DA cell Fos expression were only observed in a subgroup of SA rats, suggests that either 1) ability of stress to regulate DA cells in the VTA is influenced by prior history of drug use and/or 2) DA cell activation requires footshock exposure in a context associated with drug SA. In either case, the regulation of DA cells by stress is clearly determined by additional factors that were not controlled for in this study, as reactivity was not solely predicted by SA history.

The possibility that cocaine SA recruited the ability of footshock to regulate DA cells, is supported by a report that shock-induced increases in extracellular VTA DA are only observed in animals that have acquired SA (Wang et al., 2005) and a report that footshock-induced increases in VTA Fos expression are observed following morphine-induced conditioned place preference, but not in morphine-naïve rats (Ahmadi et al., 2008). It should, however, be noted that

stress-induced activation of DA cells using Fos as a marker has been previously reported in rats with no history of cocaine SA, albeit only in a subgroup of DA cells that project to the mPFC (Deutch et al., 1991).

The prospect that footshock-induced activation of DA cells is context dependent and therefore relies on other inputs into the VTA is also intriguing. The VTA receives projections from a number of structures, many of which respond to both drug-associated stimuli and stress and some of which do not (Briand et al., 2010, Mahler and Aston-Jones, 2012). We hypothesize that, in some cases, stress-activated VTA afferent projections that are otherwise insufficient to activate DA cells, may evoke DA responses when inputs relaying context-related information are concurrently active. In support of this hypothesis, it has been reported that, in situations where stress itself does not reliably induce cocaine seeking, it can effectively evoke cocaine seeking in the presence of other stimuli, including drug-associated cues (Buffalari and See, 2009).

While our findings suggest that activation of DA cells in the VTA is associated with stress-induced cocaine seeking, the terminal regions to which these cells project was not determined. The VTA provides DA projections to a number of forebrain regions, including the NA and the mPFC. Although stress has been reported to elevate DA in both structures (Deutch et al., 1985, Abercrombie et al., 1989, Kalivas and Duffy, 1995, Tidey and Miczek, 1996, Di Chiara et al., 1999), stress-induced stimulation of these projections does not appear to be uniform (Deutch et al., 1991, Brischoux et al., 2009, Refojo et al., 2011, Chaudhury et al., 2013). It has been reported that footshock selectively

increases Fos expression in DA cells that project to the mPFC (Deutch et al., 1991). This finding is interesting in light of reports that mPFC DA D1 receptor activation is required for stress-induced cocaine seeking (Capriles et al., 2003, Sanchez et al., 2003, McFarland et al., 2004). Future studies will determine if stress-induced cocaine seeking is selectively associated with activation of mesocortical DA projections.

The mechanisms through which stress activates DA cells in the VTA likely involves a number of stress-responsive processes, including actions of the neuropeptide CRF. CRF is released into the VTA during footshock-induced reinstatement (Wang et al., 2005) and has been reported to promote excitatory regulation of DA cells (Ungless et al., 2003, Wanat et al., 2008, Hahn et al., 2009). We and others have reported that intra-VTA CRF delivery is sufficient to evoke cocaine seeking (Wang et al., 2005, Blacktop et al., 2011) and that antagonism of VTA CRF receptors prevents footshock-induced reinstatement (Wang et al., 2005, Blacktop et al., 2011). Like footshock-induced cocaine seeking, the ability of intra-VTA CRF to induce reinstatement is augmented in rats with a history LgA cocaine SA (Blacktop et al., 2011). Further, the ability of CRF to increase VTA DA levels requires a history of cocaine SA (Wang et al., 2005). The likely involvement of CRF in the stress-induced activation of DA cells in the VTA does not rule out potential contributions by other stress-responsive systems. Indeed, it has recently been reported that kappa opioid receptor antagonist administration into the VTA can prevent stress-induced cocaine seeking (Graziane et al., 2013).

In conclusion, we report that the activation of DA cells in the VTA is associated with stress-induced cocaine seeking and likely contributes to stress-induced relapse to cocaine use. Understanding the mechanisms through which DA cells are engaged and the downstream processes that lead to drug use may facilitate the development of new and more effective approaches for the management of addiction.

CHAPTER 4

ROLE OF GABA AND GLUTAMATE RECEPTORS IN AUGMENTED COCAINE SEEKING IN RESPONSE TO STRESS OR CRF DELIVERED INTO THE VENTRAL TEGMENTAL AREA FOLLOWING LONG-ACCESS SELF-ADMINISTRATION

ABSTRACT

In the rodent model of relapse, excessive cocaine use increases susceptibility to stressor-induced reinstatement of drug-seeking behavior. Reinstatement of extinguished cocaine seeking by footshock stress is dependent on extended LgA cocaine self-administration, corticotropin releasing factor (CRF), and augmented CRF receptor 1 (CRF-R1) regulation of addiction-related neurocircuitry in the ventral tegmental area (VTA). Moreover, stress-induced reinstatement is associated with increased dopamine neuron activation in the VTA, likely resulting in elevated DA levels in downstream target regions. CRF may alter DA cell activity either directly or indirectly through regulation of GABA or glutamate mechanisms to induce cocaine seeking. This study examined the role of intra-VTA GABA and glutamate receptors in the reinstatement of extinguished cocaine seeking by footshock or intra-VTA CRF delivery. Bilateral intra-VTA administration of the $GABA_A$ receptor antagonist bicuculline (1.0 and 10.0 ng/side), $GABA_B$ receptor antagonist 2-hydroxysaclofen (2 µg/side), NMDA receptor antagonist AP-5 (1.0, 3.0, and 10 µg/side), AMPA receptor antagonist NBQX (1.0, 3.0, and 10.0 µg/side), and the nonspecific ionotropic glutamate receptor antagonist kynurenic acid (24.0 µg/side) were tested for their ability to block footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine-seeking behavior. 2-hydroxsaclofen and kynurenic acid but not AP-5 nor NBQX blocked reinstatement. These findings reveal that $GABA_B$ receptor signaling and nonspecific ionotropic glutamate signaling are both necessary for CRF actions in the VTA to reinstate extinguished LgA cocaine-seeking behavior.

INTRODUCTION

Drug addiction research is aimed to better understand the neuromechanisms of relapse to aid in the development of medications that prevent drug craving, drug seeking, and relapse. However, there are currently no FDA approved medications for the treatment of cocaine addiction (Vocci and Elkashef, 2005). Stress is a major factor in causing relapse in cocaine dependent individuals. Clinical evidence in humans and experimental animal models report that stressful events and activation of stress neurocircuitry regulates cocaine use, cocaine craving, and relapse to cocaine use (Marlatt, 1980, Wallace, 1989, Goeders and Guerin, 1994, Erb et al., 1996, Sinha et al., 1999, Grimm et al., 2001, Karlsgodt et al., 2003, Sinha et al., 2006).

Corticotropin releasing factor (CRF) is a 41-amino acid neuropeptide that plays an important role in both the stress response and stress-induced reinstatement of extinguished cocaine-seeking behavior (Shaham et al., 1997, Erb et al., 1998, Shalev et al., 2010). CRF is released during footshock stress producing its effects through the coordinated action of two G-protein coupled receptors, CRF-R1 and CRF-R2, in brain regions associated with the effects of drugs of abuse, notably the ventral tegmental area (VTA) (Van Pett et al., 2000, Dautzenberg and Hauger, 2002, Wang et al., 2005, Wang et al., 2007, Blacktop et al., 2011). We have previously reported that reinstatement of extinguished cocaine seeking by footshock stress and intra-VTA CRF administration is dependent on excessive LgA cocaine use, and that CRF-R1 activation in the VTA is necessary and sufficient for reinstatement (Blacktop et al., 2011).

Regulation of cocaine seeking by CRF-R1 receptors appears to require a prior history of very high levels of cocaine intake as seen in the long-access rodent model of relapse (Ahmed and Koob, 1998, Specio et al., 2008, Blacktop et al., 2011). However, very little is known about the mechanism of action of CRF-R1 in the VTA during reinstatement.

Excitatory drive on VTA dopamine neurons through glutamate neurotransmission is a leading hypothesis as to how intra-VTA CRF promotes reinstatement. Glutamatergic afferents to VTA dopamine neurons can produce somatodendritic dopamine release, indicative of phasic firing (Rice et al., 1997, Adell and Artigas, 2004), which has been repeatedly implicated in drug-seeking behavior (Vorel et al., 2001, Wang et al., 2005, You et al., 2007). Following drug exposure intra-VTA CRF can augment ionotropic glutamate receptor signaling on VTA dopamine neurons (Ungless et al., 2001, Saal et al., 2003, Ungless et al., 2003, Wanat et al., 2008, Hahn et al., 2009). In support of an excitatory role, we have previously reported that the magnitude of footshock stress-induced reinstatement is positively correlated with increased VTA dopamine responsiveness at the time of the reinstatement session (Chapter 3). However, emerging evidence suggests that inhibitory GABA signaling in the VTA may also be involved in stress-induced relapse.

Footshock stress increases VTA GABA neuron activity (Lammel et al., 2012, Tan et al., 2012) which can transiently suppress neighboring DA neurons (Jhou et al., 2009, Tan et al., 2012). VTA GABA neuron activation is sufficient to not only reduce reward consumption (van Zessen et al., 2012) but also produce

conditioned place aversion (Tan et al., 2012). Intra-VTA CRF can augment inhibition of VTA dopamine neurons through the coordinated action of CRF-R1 and $GABA_B$ receptors (Beckstead et al., 2009) and decrease the motivation to work for food rewards through inhibition of reward-evoked dopamine release (Wanat et al., 2013). In other brain regions binge pattern self-administration can augment CRF-R1 dependent GABAergic signaling (Nie et al., 2009). Furthermore, $GABA_A$ receptor regulation of VTA dopamine neuron activity has been recently reported to be essential in stress-induced reinstatement (Graziane et al., 2013). In summary, intra-VTA CRF appears to induce inhibitory and excitatory effects upon dopamine signaling to regulate reward seeking.

The goal of this set of experiments is to characterize the role of intra-VTA glutamate and GABA receptor activation in both footshock- and intra-VTA CRF-induced reinstatement. Particularly, the role of ionotropic glutamate receptor subtypes to build upon the work of Wise and colleagues. It is hypothesized that reinstatement will be dependent on ionotropic glutamate receptor activation. Specifically, we want to confirm the role of intra-VTA NMDA versus AMPA receptor activation in reinstatement. Additionally, further characterization of what little is known about GABA receptor signaling in footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine seeking.

MATERIALS AND METHODS

Adult male Sprague–Dawley rats (Harlan Laboratories, St. Louis, MO) were housed individually under a 12 h/12 h reversed light/dark cycle (lights on at

7:00 PM) in a temperature and humidity controlled AAALAC-accredited animal facility. All procedures were approved by the Marquette University IACUC and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Catheter and cannula implantation

For the reinstatement studies, rats were implanted with chronic indwelling jugular catheters under ketamine HCl (100 mg/kg, ip) and xylazine (2 mg/kg, ip) anesthesia and with bilateral 2.1-cm 23 gauge guide cannulae aimed at the VTA for intracranial injections as previously described (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). The tips of the guide cannulae were aimed 0.5 mm above the target injection site using the following coordinates determined from Paxinos and Watson (1998): 12° angle away from midline; A/P − 5.6 mm from bregma; M/L ± 2.2 mm from midline; and D/V − 6.7 mm from the skull surface. Placements for cannula targeting the VTA for rats from each of the experiments are depicted in Figure 28.

Self-Administration Training

After recovery from surgery, rats were trained to self-administer cocaine (1.0 mg/kg/inf, iv; NIDA Drug Supply Program) by pressing a lever under a FR1 schedule during daily 2-h sessions, within which the active (i.e., front) lever was extended into the chamber and the corresponding stimulus light was illuminated. Pressing the lever resulted in an iv infusion of drug or saline solution (200 µl over

5 s) followed by a 25-s time-out period during which the stimulus light was extinguished but the lever remained extended. Responding on a second, inactive (i.e., back) lever was recorded but had no programmed consequences. Response requirements were gradually increased until rats displayed stable responding (within 10% of the 3-session mean) under an FR4 schedule at which time they entered into a 14-day period of self-administration testing.

Long-Access, Extinction, and Reinstatement

To examine GABA and glutamate receptor signaling in intake-dependent intra-VTA CRF- and footschock-induced reinstatement of extinguished LgA cocaine-seeking-behavior, rats were assigned to LgA groups following acquisition which had access to cocaine for six hrs daily for the next 14 days. Following 14 days of LgA self-administration, rats underwent extinction during ten consecutive 2-h sessions within which the cocaine solution was replaced by saline. After 14 days of LgA self-administration and extinction, rats were tested twice for footshock- and twice for intra-VTA CRF-induced reinstatement in counterbalanced sequence: once following intra-VTA pretreatment with an antagonist and once following pretreatment with vehicle (aCSF; Tocris Biosciences). All microinfusions delivered a volume of 0.25 µl/side over a 1-min period ten minutes prior to the reinstatement session.

Stress-induced reinstatement, was tested for the ability of electric footshock, delivered though the stainless steel grid floors of the self-administration chambers, to reinstate cocaine seeking. During the 15-min

footshock period, the houselight was illuminated and the levers were retracted and stimulus lights extinguished. Shocks (0.5 mA, 0.5” duration) were delivered an average of every 40 sec (range 10-70 sec). We have reported that these parameters produce robust reinstatement after long-access, but not short-access, self-administration (Mantsch et al., 2008). As was the case with footshock, rats were tested twice for intra-VTA CRF-induced reinstatement in counterbalanced fashion: once following intra-VTA pretreatment with an antagonist and once following pretreatment with vehicle. Responding on both the active and inactive levers were recorded during the 2-h reinstatement sessions which were otherwise identical to extinction conditions. The order of testing with drug and vehicle pretreatment to both footshock and intra-VTA CRF-induced reinstatement varied across rats in each group to avoid potential sequence effects. Rats underwent additional extinction sessions between reinstatement test sessions and were required to display less than 20 cocaine lever responses during an intervening extinction session in order to be tested again for reinstatement.

Effects of GABA Receptor Antagonists on both Footshock and Intra-VTA CRF-induced Reinstatement in LgA Animals

The ability of both footshock and intra-VTA CRF (500 ng/side; 420 μM; Sigma Aldrich) delivery to reinstate extinguished LgA cocaine seeking was tested following a 10-min bilateral intra-VTA pretreatment (0.25 μl/side over 1 min) of the $GABA_A$ receptor selective antagonist, bicuculline, and the $GABA_B$ receptor selective antagonist, 2-hydroxysaclofen. The more water soluble and stable form of a selective $GABA_A$ receptor antagonist (-)-bicuculline methiodide (1.0 and 10.0

ng/side; 7.85 and 78.5 μM; Tocris Bioscience; n=19) was utilized as a pretreatment to both footshock and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine seeking. Bicuculline doses were chosen from primary literature demonstrating behavioral effects through intra-VTA $GABA_A$ receptor blockade (Sandner et al., 1996, David et al., 1997, Trojniar and Klejbor, 1999, Grubb et al., 2002). Higher doses of bicuculline were omitted due to seizure activity. $GABA_A$ receptor pharmacological blockade can induce DA neuron burst firing (Paladini et al., 1999b, Paladini and Tepper, 1999).Intra-cerebral injection of GABAergic antagonists such as bicuculline have been reported to elicit convulsions at high doses (Florio and Longo, 1972). Convulsions were not observed at a 1.0-1.5 ng dose (David et al., 1997).

The more water soluble and stable form of a prototypical $GABA_B$ receptor antagonist 2-hydroxysaclofen (2 μg/side; 30 mM; Tocris Bioscience; n=14) prior to both footshock and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine-seeking was additionally characterized. The 2-Hydroxysaclofen dose was chosen from primary literature demonstrating behavioral effects through intra-VTA $GABA_B$ receptor blockade (Xi and Stein, 1999, Ackerman et al., 2003, Miner et al., 2010).

Effects of Ionotropic Glutamate Receptor Antagonists on both Footshock and Intra-VTA CRF-induced Reinstatement in LgA Animals

The ability of both footshock and intra-VTA CRF (500 ng/side; Sigma Aldrich) delivery to reinstate cocaine seeking was tested following a 10-min bilateral intra-VTA pretreatment (0.25 μl/side over 1 min) with an NMDA, AMPA,

and nonspecific ionotropic glutamate receptor antagonist. The more active and water soluble form of a selective and competitive NMDA receptor antagonist D-AP5 was administered at three different doses (1.0, 3.0, and 10 µg/side; 20.3, 60.9, and 203 mM; Tocris Bioscience; n=23). Doses were initially chosen from primary literature demonstrating behavioral effects through intra-VTA NMDA receptor blockade (Ranaldi et al., 2011) and scaled up proportionately due to failure to attenuate reinstatement (Park et al., 2002, Famous et al., 2007). The more water soluble form of a potent, selective, and competitive AMPA receptor antagonist NBQX disodium salt (1.0, 3.0, and 10.0 µg/side; 10.5, 31.5, 105 mM; Tocris Bioscience; n=26) was determined in separate groups of LgA rats.

A pilot study in another separate group of LgA rats with the more water soluble form of the nonspecific ionotropic glutamate receptor antagonist kynurenic acid sodium salt (24.0 µg/side; 454 mM; Tocris Bioscience; n=3) was administered to measure the ability of both footshock and intra-VTA CRF to reinstate. At this dose kynurenic acid antagonizes both the NMDA (Kessler et al., 1989, Stone, 1993) and the AMPA/Kainate family of ionotropic glutamate receptors (Stone, 1993).

Food Self-Administration

In order to confirm that the effects of intra-VTA 2-hydroxysaclofen and kynurenic acid on reinstatement were not attributable to non-specific motor impairments, rats were tested for effects on sucrose pellet-reinforced lever pressing. These rats were maintained at 90% of their free-feeding body weights and trained to self-administer 45 mg sucrose-sweetened food pellets (BioServ)

by pressing a response lever under a FR4 schedule of reinforcement during 30-min sessions. Once stable response patterns were observed (responding within 10% of the mean over 3 sessions), separate groups of rats were tested for the effects of intra-VTA delivery of 2-hydroxysaclofen (2 μg/side; n=7) or kynurenic acid (multiple doses; n=7), as described above, on responding. Each rat was tested two or three times with intra-VTA treatment in counterbalanced sequence with the 2-hydroxysaclofen, kynurenic acid, or vehicle. 2-hydroxysaclofen and kynurenic acid were administered in separate groups of rats.

Histological Confirmation of Injection Sites

The accuracy of cannula implantation was confirmed postmortem in each rat after cardiac perfusion with 60-ml NaCl followed by 60-ml 2.5% buffered neutral formalin under sodium barbital anesthesia (55 mg/kg). Brains were removed and stored in 2.5% buffered formalin for at least one day. 200-μm sections were cut using a vibrotome, slide-mounted, and stained with cresyl violet for placement confirmation using a light microscope. Rats with injection sites outside of the VTA were excluded from data analysis.

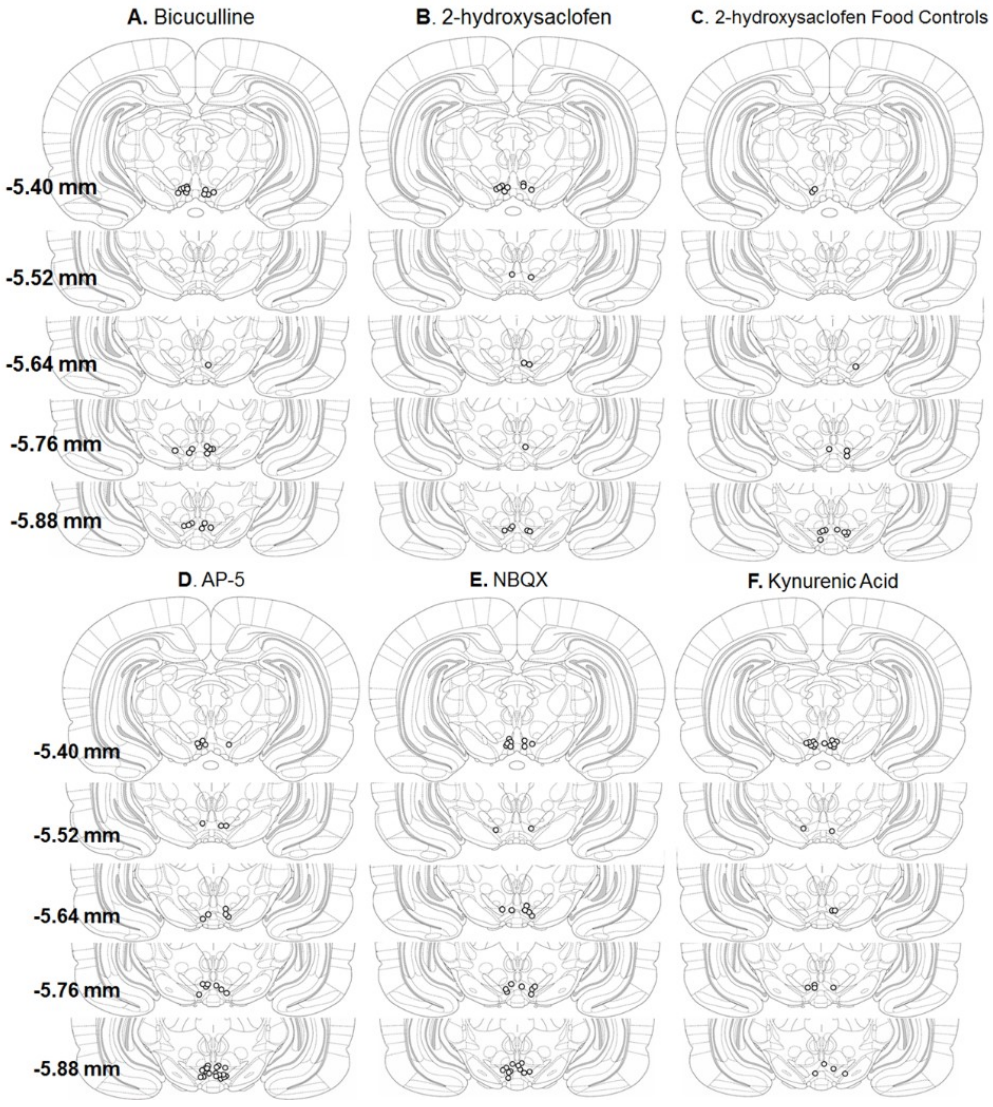
Statistical Analyses

Statistical analyses were conducted using Predictive Analytics SoftWare statistics software (SPSS, Inc.). Statistical significance was determined using paired students t-test, 1-way ANOVA, and 2-way ANOVA followed, when appropriate, by further analyses of main effects and interactions using Bonferroni-corrected t-test post-hoc testing.

Histology

The histological confirmation of all injection sites in animals included in data analysis is shown in the Figure 28. All placements are in the VTA and depicted by cohort.

Figure 28: Schematic depiction of injection sites within the VTA for different treatment conditions in LgA rats including: A) bicuculline B) 2-hydoxysaclofen C) 2-hydroxysaclofen food controls, D) AP-5, E) NBQX, and F) kynurenic acid. Open circles represent the injection site upon coronal brain section images from the atlas of Paxinos and Watson (2005).



RESULTS

Cocaine Self-Administration and Extinction

Cocaine self-administration (SA) on days one and 14 of testing in rats provided 14 days of LgA (6 hrs) along with the first and last day of extinction training is shown is shown in Table 1 for animals pretreated with bicuculline, 2-hydroxysaclofen, AP-5, NBQX, and kynurenic acid. Cocaine SA and extinction did not vary across groups. The mean and standard error data are shown in Table 3.

Table 3: Total number of infusions on days 1 and 14 of LgA cocaine self-administration and the total number of responses on the active lever on extinction days 1 and 10 (±S.E.) in animals that were tested with intra-VTA pretreatments of bicuculline, 2-hydroxysaclofen, AP-5, NBQX, and kynurenic acid to both footshock- and intra-VTA CRF-induced reinstatement. Self-administration infusions and extinction responses did not significantly vary amongst animals treated with different antagonists.

Reinstatement Group	SA Day 1 (Inf ± S.E.)	SA Day 14 (Inf ± S.E.)	EXT Day 1 (Resp ± S.E.)	Final EXT Day (Resp ± S.E.)
Bicuculline	81.25 (±4.14)	92.67 (±7.75)	47.0 (±8.49)	11.33 (±1.35)
Saclofen	72.63 (±3.68)	83.38 (±3.78)	66.0 (±10.70)	7.88 (±1.60)
AP-5	84.65 (±5.22)	88.30 (±4.01)	49.15 (±8.28)	6.8 (±1.58)
NBQX	84.24 (±5.88)	87.88 (±4.16)	77.18 (±13.55)	10.53 (±1.52)
Kynurenic Acid	80.42 (±4.77)	84.17 (± 5.28)	69.75 (±9.27)	10.42 (±0.97)

Effects of Intra-VTA GABA Receptor Antagonists on Footshock and CRF-Induced Reinstatement

As the effects of GABA antagonists administered into the VTA on stress and CRF-induced reinstatement have not been previously tested, we initially investigated the effects of intra-VTA administration of the $GABA_A$ receptor antagonist, bicuculline, and the $GABA_B$ antagonist, 2-hydroxylsaclofen on reinstatement in response to footshock or intra-VTA CRF delivery (500 ng/side; Sigma-Aldrich).

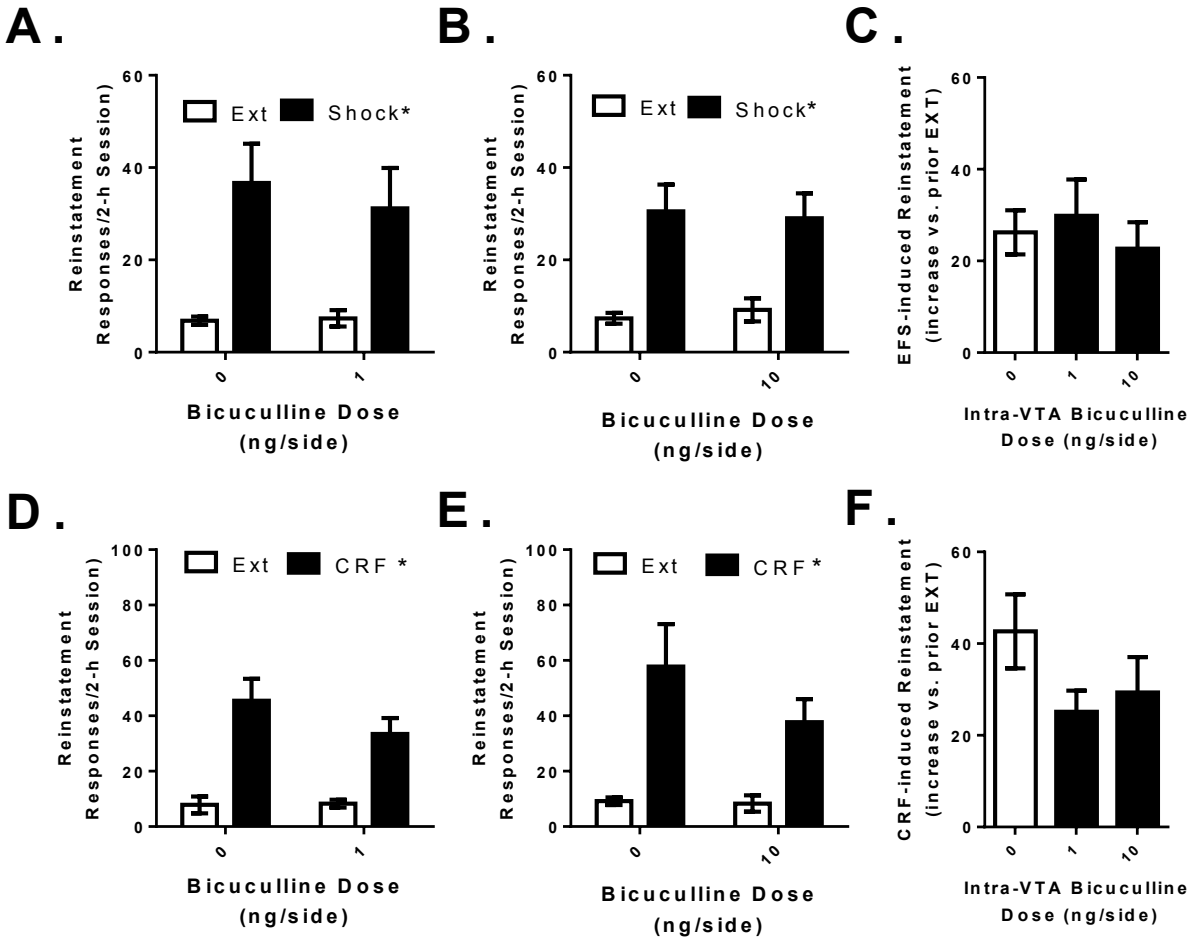
Effects of Intra-VTA $GABA_A$ Receptor Antagonists on Footshock and CRF-Induced Reinstatement

We initially tested rats for the effects of intra-VTA bicuculline at a dose of 1 ng/side (David et al., 1997, Trojniar and Klejbor, 1999). As this dose failed to produce effects on reinstatement, we subsequently tested rats for the effects of a 10-fold higher bicuculline dose (10 ng/side) (Sandner et al., 1996, Grubb et al., 2002, Ackerman et al., 2003). Notably, higher doses than 10 ng/side tend to generate aberrant electrical activity and promote seizures (unpublished observation). Therefore, we did not test higher concentrations of bicuculline. The effects of intra-VTA bicuculline on reinstatement are shown in Figure 29. Neither bicuculline dose significantly attenuated reinstatement when administered into the VTA prior to testing with footshock or intra-VTA CRF. Since within subject testing for the effects of bicuculline on reinstatement was conducted in different groups of rats for each dose, we performed separate 2-way repeated measures ANOVA examining the effects of each dose (1 ng, 29A and 29D and 10 ng, 29B

and 29E) on reinstatement in response to each stimulus (footshock, 29A and 29B and CRF, 29D and 29E).

Two-way repeated measures reinstatement x drug pretreatment ANOVAs showed either overall reinstatement main effects of or near-significant trends for reinstatement testing in response to either footshock (1 ng/side bicuculline: $F_{1,5}$=9.967; P<0.05; 10 ng/side bicuculline: $F_{1,5}$=54.921; P=0.001) or intra-VTA CRF (1 ng/side bicuculline: $F_{1,6}$=35.698; P<0.01; 10 ng/side bicuculline: $F_{1,5}$=12.317; P<0.05). However, in no cases were significant main effects of bicuculline or interactions between bicuculline pretreatment and reinstatement testing condition observed. To permit analysis of reinstatement across dose conditions, we also consolidated veh-pretreated rats into one group and conducted one-way ANOVA assessing changes in cocaine lever responding relative to the prior extinction session following either footshock or intra-VTA CRF (29C and 29F). Despite some variability across groups, significant overall effects of bicuculline pretreatment were not observed in either case. Notably, to address the small sample size in some of the groups and within-group variability, additional rats are currently undergoing testing.

Figure 29: Effects of intra-VTA injections of the $GABA_A$ receptor antagonist bicuculline on reinstatement by footshock stress and intra-VTA CRF delivery in LgA rats. Data represent the effects of bilateral injections of 1ng/side bicuculline on reinstatement induced by footshock (29A; n=6) or intra-VTA CRF (29D; n=7) or 10ng/side bicuculline on reinstatement by footshock (29B; n=5) or intra-VTA CRF (500 ng/side; 29E; n=5). Overall reinstatement main effects of or near-significant trends for reinstatement testing in response to either footshock (1 ng/side bicuculline: $F_{1,5}$=9.967; P<0.05; 10 ng/side bicuculline: $F_{1,5}$=54.921; P=0.001) or intra-VTA CRF (1 ng/side bicuculline: $F_{1,6}$=35.698; P<0.01; 10 ng/side bicuculline: $F_{1,5}$=12.317; P<0.05) were observed. Significant overall effects of bicuculline pretreatment or interactions between bicuculline pretreatment and reinstatement were not observed (29C and 29F).



Effects of Intra-VTA $GABA_B$ Receptor Antagonists on Footshock and CRF-Induced Reinstatement

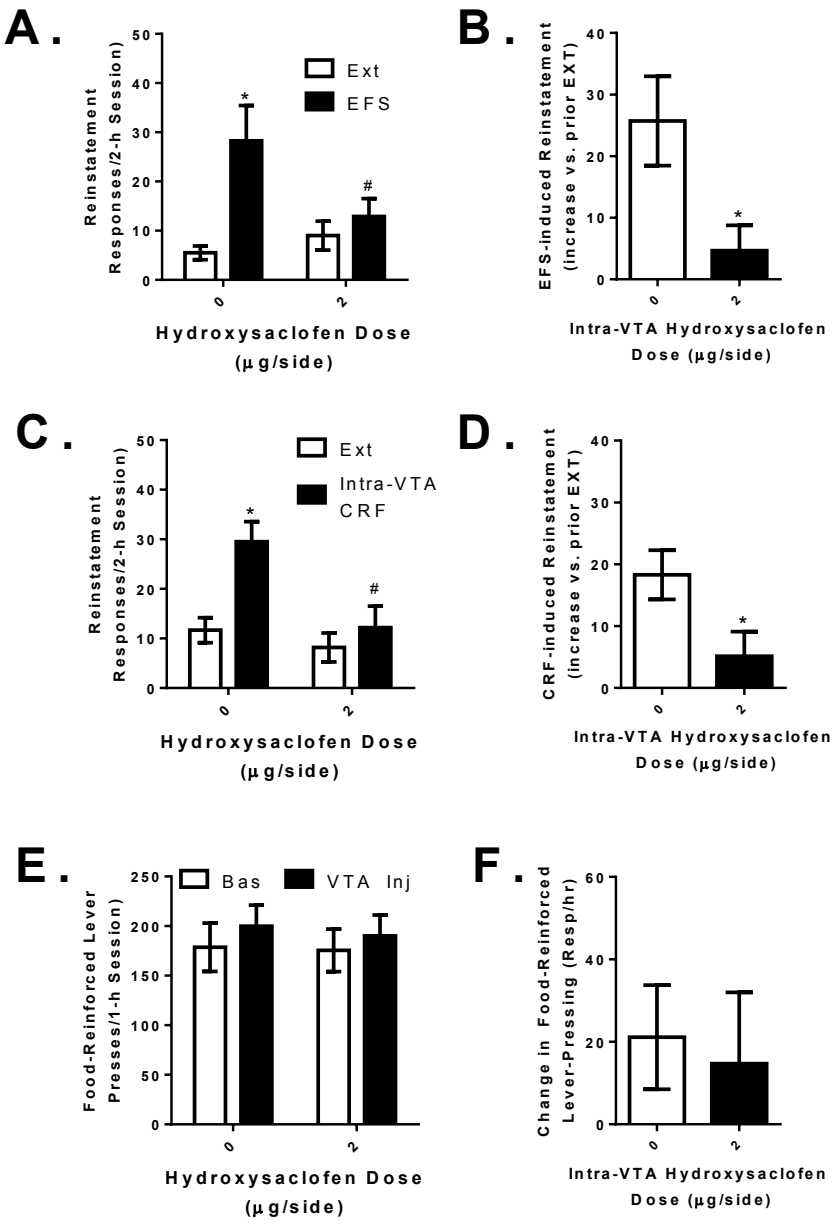
The effects of intra-VTA 2-hydroxysaclofen (2-HS) on reinstatement were tested at a single dose (2 µg/side) (Xi and Stein, 1999, Ackerman et al., 2003, Miner et al.) and are shown in Figure 30. The effects of 2-HS were assessed using a) 2-way repeated measures 2-HS treatment x reinstatement test condition ANOVA (30A and 30C) examining total cocaine lever responding and b) a paired t-test examining differences in reinstatement relative to the previous extinction

session following intra-VTA 2-HS or vehicle. 2-HS effects on footshock-induced reinstatement are shown in 30A and 30B.Two-way ANOVA examining effects of intra-VTA 2-HS footshock cocaine seeking showed a significant main effect of footshock ($F_{1,7}$=14.322;P<0.01) but not intra-VTA 2-HS pretreatment and a significant footshock x 2-HS interaction ($F_{1,7}$=6.666;P<0.05). Post-hoc testing (Bonferroni-corrected t-test) showed that footshock-induced reinstatement in rats pretreated with intra-VTA vehicle (P<0.05 vs. extinction) but not 2-HS and that cocaine seeking was greater following vehicle than after 2-HS (P<0.05). To further examine difference in shock-induced reinstatement conditions, we also used a paired t-test to compare differences in the magnitude of reinstatement (change in responding vs. prior extinction session). Intra-VTA 2-HS significantly decreased footshock-induced reinstatement (t_7=2.582; P<0.05).

2-HS effects on reinstatement in response to intra-VTA CRF delivery are shown in panels 30C and 30D. Two-way ANOVA examining effects of 2-HS on CRF-induced cocaine seeking showed significant main effects of CRF ($F_{1,6}$=12.655;P<0.05) and intra-VTA 2-HS pretreatment ($F_{1,6}$=8.897;P<0.05) and a significant CRF x 2-HS interaction ($F_{1,6}$=10.206;P<0.05; 30C). Post-hoc testing showed that intra-VTA CRF induced reinstatement in rats pretreated with intra-VTA vehicle (P<0.05 vs. extinction) but not 2-HS and that cocaine seeking was greater following vehicle than after 2-HS (P<0.05). A paired t-test comparing differences in the magnitude of reinstatement showed that intra-VTA 2-HS significantly decreased CRF-induced reinstatement (t_6=3.195;P<0.05; 30D).

To ensure that the attenuation of reinstatement by intra-VTA 2-HS was not attributable to impairment of motor activity that would prevent rats from displaying lever pressing behavior, an additional group of rats was trained to lever press under a FR4 schedule of sucrose-sweetened pellet (45 mg) reinforcement followed by testing for the effects of intra-VTA vehicle or 2-HS administration (30E and 30F). 2-HS failed to alter lever-pressing in these rats. Two-way repeated measures ANOVA showed no main effect of or interaction involving intra-VTA 2-HS. Likewise A paired student's t-test failed to show a significant reduction in food reinforced responding following intra-VTA 2-HS.

Figure 30: Effects of intra-VTA injections of $GABA_B$ receptor antagonists on reinstatement by footshock stress and intra-VTA CRF delivery in LgA rats. Data represent the effects of bilateral injections of 2-hydroxysaclofen (2-HS; 2 µg/side) as a pretreatment to footshock stress (30A/B; n=7) or intra-VTA CRF delivery (500 ng/side; 30C/D; n=7). In all cases, significant reinstatement was observed in rats pretreated with vehicle (*$P<0.05$ vs. Ext) but not 2-HS in both footshock and intra-VTA CRF-induced reinstatement, with cocaine seeking being greater following vehicle than after 2-HS ($P<0.05$). 2-HS failed to significantly alter or reduce food reinforced responding following intra-VTA 2-HS administration (30E/F; n= 7).



Effects of Intra-VTA Glutamate Receptor Antagonists on Footshock and CRF-Induced Reinstatement

Since other reports suggest a role for glutamatergic neurotransmission in the VTA in reinstatement in response to footshock or CRF (Wang et al., 2005, Wang et al., 2007) and CRF actions in the VTA (Ungless et al., 2003), we also

examined the effects of intra-VTA delivery of antagonists at NMDA (AP-5) and AMPA (NBQX) receptors on reinstatement.

Effects of Intra-VTA NMDA Receptor Antagonists on Footshock and CRF-Induced Reinstatement

The effects of intra-VTA AP-5 administration on footshock-induced reinstatement are shown in Figure 31. Since a number of rats were not tested under both vehicle and AP-5 conditions, mixed 2-way ANOVA with shock (vs. ext) as a repeated measure and intra-VTA AP-5 (vs. veh) as a between subjects measure was conducted at each dose. Initially AP-5 was tested at a dose of 1 µg/side (Cornish et al., 2001, Covington et al., 2008, Ranaldi et al., 2011). As this dose failed to attenuate reinstatement, we subsequently tested rats for effects of higher AP-5 doses (3 and 10 µg/side). In all cases, intra-VTA AP-5 failed to block footshock-induced reinstatement. For each dose tested, a significant main effect of footshock was observed (1 µg/side dose: $F_{1,7}$=11.086; 3 µg/side dose: $F_{1,7}$=9.776; 10 µg/side dose: $F_{1,4}$=18.434; $P<0.05$ for each), but no main effects of AP-5 pretreatment and no AP-5 x footshock interactions.

Additionally, one-way ANOVA was conducted to examine the magnitude of footshock reinstatement across AP-5 dose conditions. No effect of treatment was observed (31D). To confirm that our inability to block reinstatement was not due to the AP-5 dose, we tested two rats at 30 µg/side, a very large intracranial dose (Dunn et al., 2005). Footshock-induced reinstatement was observed, even at this dose (mean increase vs. extinction of 45.5 responses; data not shown). Intra-VTA AP-5 did not significantly alter inactive lever pressing at any dose

tested with footshock (Table 4), nor did it induce cocaine seeking, when tested alone, when tested in two rats (not shown).

Figure 31: Intra-VTA AP-5 failed to block footshock-induced reinstatement at all doses tested which included 1 µg/side (31A; n=4), 3 µg/side (31B; n=5), and 10 µg/side (31C; n=3). For each dose tested, a significant main effect of footshock was observed (1 µg/side dose: $F_{1,7}$=11.086; 3 µg/side dose: $F_{1,7}$=9.776; 10 µg/side dose: $F_{1,4}$=18.434; P<0.05 for each) but no main effects of AP-5 pretreatment and no AP-5 x footshock interactions. No effect of treatment was observed on the magnitude of footshock reinstatement across AP-5 dose conditions (31D).

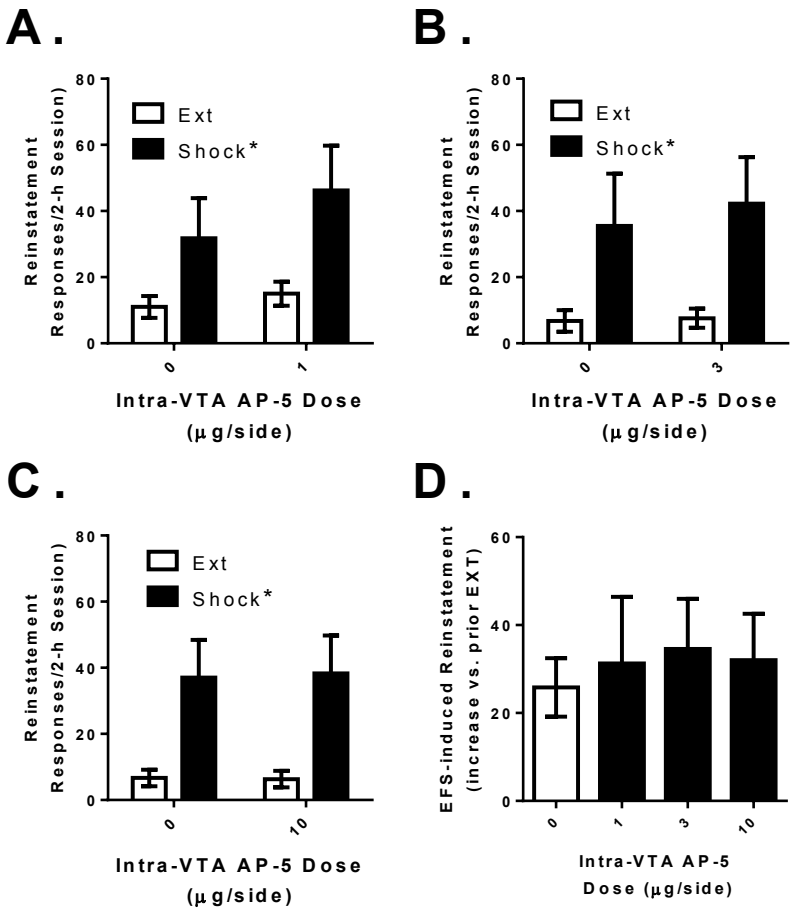


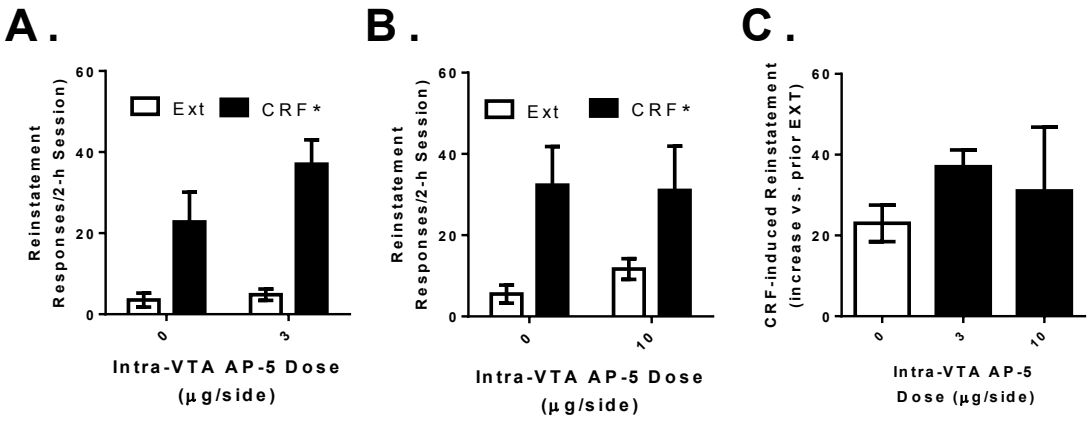
Table 4: Total number of inactive lever presses (±S.E.) in LgA rats receiving intra-VTA pretreatments of 1, 3, and 10 µg/side doses of either AP-5 or NBQX to footshock- and intra-VTA CRF-induced reinstatement.

Intra-VTA Drug	Reinstatement	"Inactive" Lever Responses (± S.E.)			
		Veh	1 µg/side	3 µg/side	10 µg/side
AP5	Shock	7.09 (±2.80)	5.67 (±1.77)	3.25 (±2.92)	10.00 (±4.00)
	CRF	6.13 (±2.57)	---	2.00 (±0.77)	2.67 (±2.18)
NBQX	Shock	4.60 (±2.28)	14.00 (±2.31)	16.33 (±9.24)	8.00 (±3.81)
	CRF	6.42 (±2.24)	---	7.20 (±1.96)	9.75 (±3.29)

The effects of intra-VTA AP-5 administration on reinstatement in response to intra-VTA CRF are shown in Figure 32. For these experiments, only the 3 and 10 µg/side AP-5 doses were tested. Neither dose blocked reinstatement in response to intra-VTA CRF. Effects were assessed using 2-way ANOVA. In both cases, main effects of CRF were observed (3 µg/side dose: $F_{1,8}$=30.958; 10 µg/side dose: $F_{1,5}$=10.016; $P<0.05$ for each; 32A/B), but main effects of AP-5 pretreatment or AP-5 x CRF interactions were not.

One-way ANOVA also failed to show that the magnitude of intra-VTA CRF-induced reinstatement varied across AP-5 dose conditions (32C). As was the case with shock-induced reinstatement, 30 µg/side AP-5 (a very high dose) also failed to block CRF-induced reinstatement (mean increase vs. extinction = 41.5 responses). Inactive lever pressing during the sessions was not altered by AP-5 (Table 4).

Figure 32: Intra-VTA AP-5 failed to block CRF-induced reinstatement at all doses tested which included 3 µg/side (32A; n=6), and 10 µg/side (32B; n=5). For each dose tested, main effects of CRF were observed (3 µg/side dose: $F_{1,8}$=30.958; 10 µg/side dose: $F_{1,5}$=10.016; P<0.05 for each), but main effects of AP-5 pretreatment or AP-5 x CRF interactions were not.



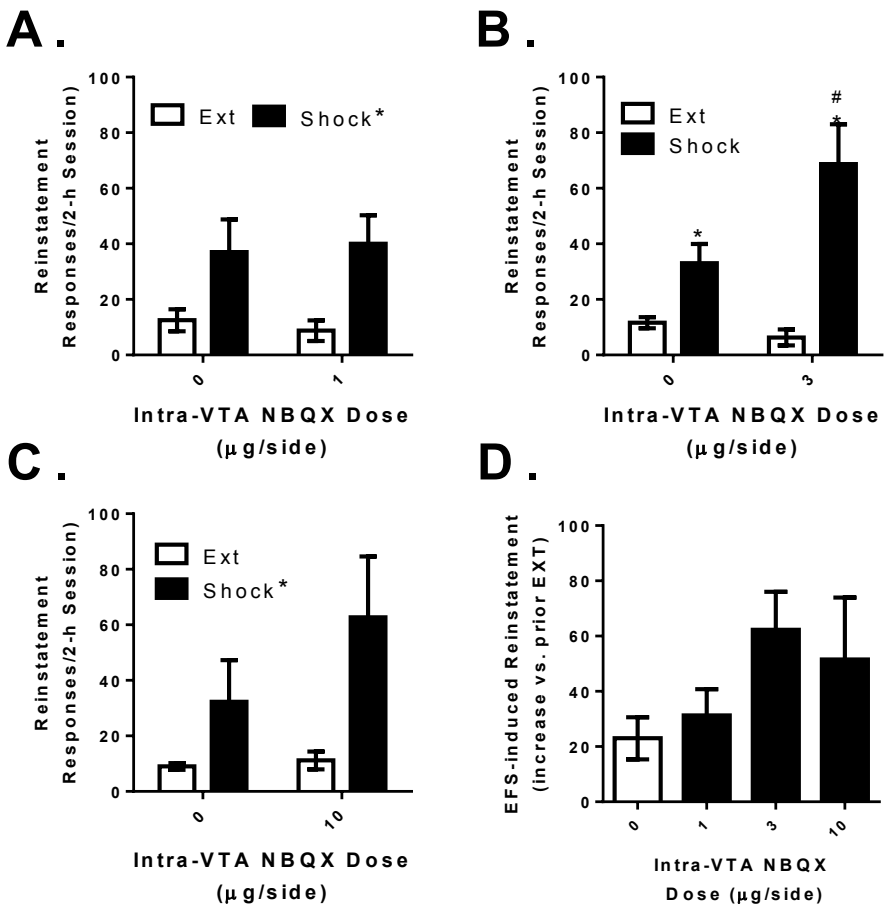
Effects of Intra-VTA AMPA Receptor Antagonists on Footshock and CRF-Induced Reinstatement

The effects of intra-VTA NBQX administration on shock-induced reinstatement are shown in Figure 33. As with AP-5 pretreated rats, mixed 2-way ANOVA with shock (vs. ext) as a repeated measure and intra-VTA NBQX (vs. veh) as a between subjects measure was conducted at each dose. Initially NBQX was tested at a dose of 1 µg/side (Nolan et al., 2010, Millan and McNally, 2011). However, since this dose failed to attenuate reinstatement, we subsequently tested rats for effects of higher NBQX doses (3 and 10 µg/side). Intra-VTA NBQX produces dose-dependent effects on reinstatement with no effects at the 1 µg/side dose and an apparent augmentation at the higher doses. At the 1 µg/side dose, a significant main effect of footshock was observed (1

μg/side dose: $F_{1,6}$=20.120; P<0.05), but no main effects of NBQX pretreatment and no NBQX x footshock interaction (33A). At the 3 μg/side dose, a significant main effect of footshock ($F_{1,5}$=40.457; P=0.001) and a significant interaction between footshock and intra-VTA NBQX pretreatment ($F_{1,5}$=9.627; P<0.05) were observed. Post-hoc testing showed that not only was significant reinstatement observed in both groups (P<0.05 vs. extinction) but that shock-induced reinstatement of cocaine seeking following intra-VTA NBQX was increased compared to vehicle controls (P=0.05; 33B). At the highest NBQX dose tested (10 μg/side), a similar pattern was present. However, while a significant main effect of shock was observed ($F_{1,10}$=7.717; P<0.05), a significant shock x NBQX pretreatment interaction was not (33C). Additionally, one-way ANOVA was conducted to examine the magnitude of footshock reinstatement across AP-5 dose conditions. However, despite dose-dependent increases in reinstatement magnitude following intra-VTA NBQX delivery, a significant overall effect was not observed (33D). Although also not significant, dose-dependent increases in responding on the previously inactive lever were also observed (Table 4).

The effects of intra-VTA NBQX administration on reinstatement in response to intra-VTA CRF are shown in Figure 34. As was the case with AP-5, for these experiments, only the 3 and 10 μg/side NBQX doses were tested. Neither NBQX dose blocked reinstatement in response to intra-VTA CRF.

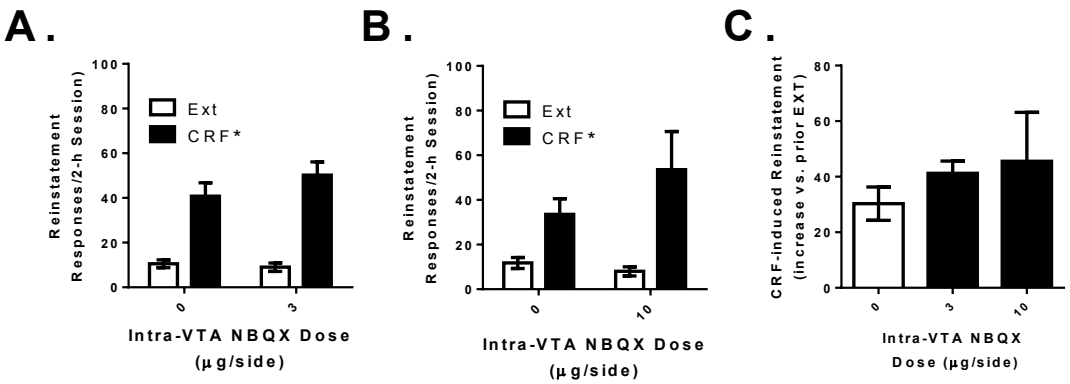
Figure 33: Intra-VTA NBQX failed to block footshock-induced reinstatement at all doses tested which included 1 µg/side (33A; n=4), 3 µg/side (33B; n=3), and 10 µg/side (33C; n=6). In all cases, significant reinstatement was observed in rats pretreated with vehicle or the AMPA receptor antagonists (*P<0.05 vs. Ext). At the 3 µg/side dose, NBQX increased reinstatement compared to vehicle controls (P=0.05). A significant interaction between footshock and intra-VTA NBQX pretreatment of 3 µg/side ($F_{1,5}$=9.627; P<0.05) was observed. A significant overall effect of reinstatement magnitude and NBQX delivery was not observed at the 1, 3, or 10 µg/side dose (33D).



Effects were assessed using 2-way ANOVA. In both cases, significant main effects of CRF were observed (3 µg/side dose: $F_{1,8}$=71.892; 10 µg/side dose: $F_{1,16}$=12.880; P<0.05 for each), but main effects of NBQX pretreatment or NBQX x CRF interactions were not. One-way ANOVA also failed to show that the

magnitude of intra-VTA CRF-induced reinstatement varied across AP-5 dose conditions. Although reinstatement magnitude tended to increase with NBQX dose, one-way ANOVA failed to show a statistically significant overall effect of intra-VTA NBQX pretreatment on the magnitude intra-VTA CRF-induced reinstatement (34C). Significant effects on previously inactive lever pressing during the reinstatement session were not observed (Table 2).

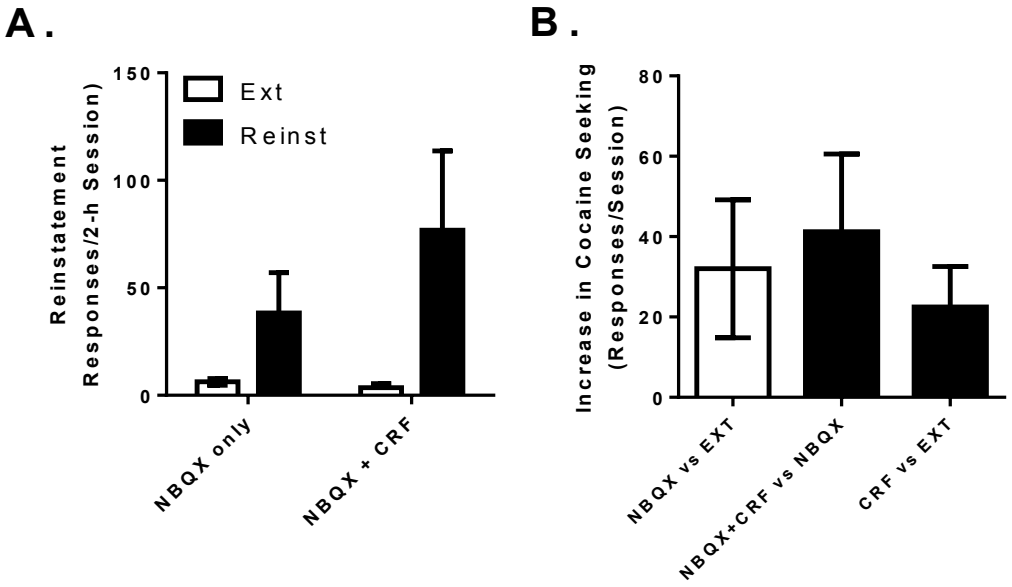
Figure 34: Intra-VTA NBQX failed to block CRF-induced reinstatement at all doses tested which included 3 µg/side (34A; n=4), and 10 µg/side (34B; n=9). For each dose tested, main effects of CRF were observed (3 µg/side dose: $F_{1,8}$=71.892; 10 µg/side dose: $F_{1,16}$=12.880; P<0.05 for each), but main effects of NBQX x CRF interactions were not. A statistically significant overall effect of intra-VTA NBQX pretreatment on the magnitude intra-VTA CRF-induced reinstatement was not observed at any dose tested (34C).



Since intra-VTA NBQX tended to increase reinstatement in response to either footshock or intra-VTA CRF, we hypothesized that NBQX, via AMPA receptor blockade in the VTA, may alone induce cocaine seeking. To test this hypothesis, we selected four rats that displayed high levels of reinstatement when tested for CRF-induced reinstatement following pretreatment with NBQX

and tested them for reinstatement in response to 10 µg/side intra-VTA NBQX by itself (Figure 35).

Figure 35: In the absence of footshock or CRF delivery NBQX (10 µg/side) increased mean active lever responses by 32, although the difference did not reach statistically significance (t_3=1.860; P=.160) (35A; n=4). Mean increases in responding by NBQX alone, NBQX and CRF, or CRF alone suggest that NBQX didn't prevent nor block reinstatement by CRF (35B).



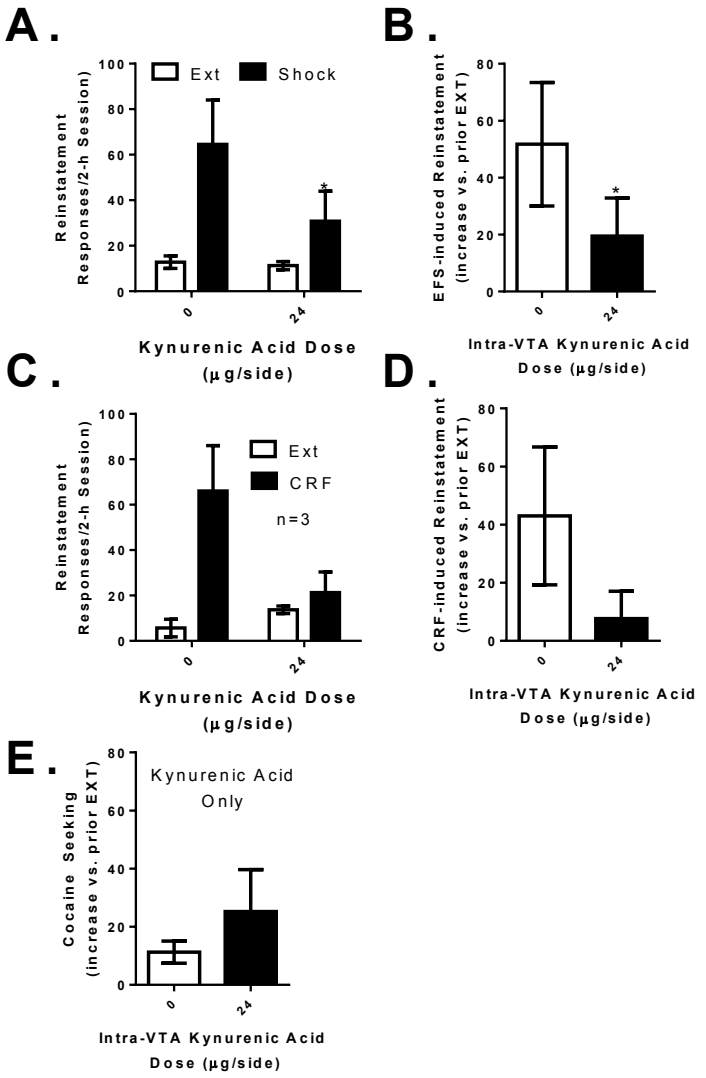
Although the difference was not statistically significant (t_3=1.860; P=.160), NBQX alone produced a mean increase in lever pressing of 32 responses in these rats (35A). Notably the mean increase in responding in these rats relative to extinction when they received NBQX prior to CRF was 73.25 responses and the mean difference in responding during the session prior to which they received NBQX/CRF and that prior to which they received only NBQX was 41.25 responses, a number that is comparable to the mean level of reinstatement observed when the same rats received only intra-VTA CRF delivery (35B). Thus, even though intra-VTA NBQX evoked cocaine seeking, CRF-induced cocaine

seeking was observed, suggesting that NBQX was neither preventing nor masking reinstatement.

To test the possibility that blockade of both AMPA and NMDA receptors in the VTA is necessary to reveal a role for glutamatergic neurotransmission in stress and CRF-induced cocaine seeking, we conducted a preliminary study in which we tested rats for the effects of intra-VTA kynurenic acid (KA) delivery on reinstatement. In addition to other pharmacological properties, KA antagonizes both AMPA and NMDA receptors. In fact, Wang et al (2005) have reported that intra-VTA delivery of KA can prevent reinstatement of cocaine seeking in response to both footshock and intra-VTA CRF administration. The effects of intra-VTA KA administration on reinstatement in response to footshock (36A and 36B) intra-VTA CRF (36C and 36D) are shown in Figure 36.

Effects of KA on footshock-induced reinstatement were tested in four rats using a 24 µg/side KA dose (Vorel et al., 2001). A two-way repeated measure KA pretreatment x footshock condition ANOVA showed a significant overall effect of intra-VTA KA pretreatment ($F_{1,3}$=20.461; P<0.05) but not shock on cocaine seeking and a significant KA pretreatment x shock condition interaction ($F_{1,3}$=11.788; P<0.05). Post-hoc testing showed that cocaine seeking following shock was significantly reduced following intra-VTA KA compared to intra-VTA vehicle control conditions (36A; P<0.05). Further, a paired t-test comparing the magnitude of shock-induced reinstatement in KA-pretreated rats with that in vehicle treated controls showed a significant KA-induced reduction (t_3=3.433; P<0.05; 36B).

Figure 36: Effects of intra-VTA injections of kynurenic acid (KA) on reinstatement by footshock stress and intra-VTA CRF delivery in LgA rats. Data represent the effects of bilateral injection of KA (24 µg/side) as a pretreatment to footshock stress (36A/B; n=4), intra-VTA CRF delivery (500 ng/side; 36C/D; n=3), or KA in absence of footshock or CRF (36E; n=4). Reinstatement by shock was significantly reduced following intra-VTA KA compared to intra-VTA vehicle control conditions (36A; $P<0.05$), and significantly reduced the magnitude of shock-induced reinstatement as compared to vehicle controls ($t_3=3.433$; $P<0.05$; 36B). KA attenuated CRF-induced cocaine seeking but failed to reach significance due to a small sample size (36C/D). KA alone produces activational instead of suppressive effects in active lever pressing in the absence of shock or CRF (36E).



The effects of 24 µg/side intra-VTA KA on reinstatement in response to intra-VTA CRF were tested in only three rats and are shown in 36C and 36D. Because of the small sample size, statistically significant effects were not observed. However, as was the case with footshock, KA attenuated CRF-induced cocaine seeking. Although preliminary studies investigating the effects of intra-VTA KA on sucrose-pellet reinforced responding suggest that this dose of KA may impair lever pressing, intra-VTA administration of KA alone in rats with a history of cocaine SA produced an increase in lever pressing, suggesting that it either reinstated cocaine seeking or produced an activational rather than suppressant effect (36E). Notably KA-induced increases in previously inactive lever pressing were observed (increase from 5.67 responses to 23). Nonetheless, we have begun testing for the effects of lower intra-VTA KA doses on stress- and CRF-induced cocaine seeking.

DISCUSSION

Stress during periods of drug abstinence contributes to relapse in cocaine-dependent individuals. In the rodent model of relapse, excessive cocaine use increases susceptibility to stressor-induced reinstatement of drug-seeking behavior. We previously reported that the reinstatement of extinguished cocaine seeking by a stressor (footshock) is corticotropin releasing factor (CRF) dependent, and is characterized by increased VTA dopamine neuron responsiveness during the reinstatement session. Furthermore, excessive cocaine use increases susceptibility to later stressor-induced relapse at least in

part by augmenting CRF-R1 receptor-dependent regulation of addiction-related neurocircuitry in the ventral tegmental area (VTA). The VTA represents a convergence point whereby dopamine, glutamate, GABA, and CRF neurons interact to regulate motivated behavior including stress-induced reinstatement.

Footshock stress can cause GABA release into the VTA and activate VTA GABA neurons (Tan et al., 2012, Jennings et al., 2013). Ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors are present in the VTA (Kalivas, 1993, Westerink et al., 1996, Westerink et al., 1998). $GABA_A$ receptors are located predominantly, but not exclusively, on GABA neurons while $GABA_B$ receptors are predominantly located on dopamine neurons (Churchill et al., 1992, Klitenick et al., 1992, Xi and Stein, 1998, Magreta-Mitrovic, 1999, Laviolette and van der Kooy, 2001, Laviolette et al., 2004). Therefore, GABAergic interneuron inhibition of VTA DA neurons is regulated by ionotropic $GABA_A$ receptors (Sugita et al., 1992, Kalivas, 1993), while direct GABA inhibition of dopamine neurons is regulated by metabotropic $GABA_B$ receptors (Xi and Stein, 1998, Margeta-Mitrovic et al., 1999). Additionally, $GABA_B$ receptors are located on presynaptic GABA and glutamate terminals in the VTA inhibiting neurotransmitter release (Bonci and Williams, 1997, Shen and Johnson, 1997, Manzoni and Williams, 1999, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010).

Intra-VTA $GABA_A$ receptors have been implicated in neuroadaptations regulating effects of stress and cocaine on midbrain dopamine neurons (Giorgetti et al., 1998, Tan et al., 2012). Moreover, intra-VTA $GABA_A$ receptors can regulate aversion, reward, and relapse (David et al., 1997, Ikemoto et al., 1997a,

Ikemoto et al., 1997b, Tan et al., 2012, Graziane et al., 2013). For these reasons we characterize the role of $GABA_A$ receptors in the LgA rodent model of relapse. Intra-VTA administration of the $GABA_A$ antagonist (1 and 10 ng/side) fails to significantly attenuate both footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine seeking. The initial dose of 30 ng/side was chosen based of primary literature (Sandner et al., 1996) but produced potent seizure like effects. The 10 ng/side dose is the highest concentration that didn't produce these effects and is therefore utilized. The potent effects of bicuculline are presumably due to powerful disinhibition of dopamine neurons through blocking $GABA_A$ receptors on resident VTA GABA interneurons (Churchill et al., 1992, Kalivas, 1993, Laviolette and van der Kooy, 2001, 2004); which control the excitability of VTA dopamine neurons (Grace and Bunney, 1979, Waszczak and Walters, 1980, Wirtshafter and Klitenick, 1989, Oakley et al., 1991, Westerink et al., 1996, Westerink et al., 1998, Xi and Stein, 1998, Laviolette and van der Kooy, 2001, 2004). These results are indicative that intra-VTA $GABA_A$ receptor activation is not necessary for footshock- and intra-VTA CRF-induced reinstatement of LgA cocaine seeking.

In contrast to $GABA_A$ receptors, $GABA_B$ receptors are functionally and anatomically preferentially expressed by VTA DA neurons (Xi and Stein, 1998, Margeta-Mitrovic et al., 1999, Laviolette and van der Kooy, 2001, Margolis et al., 2012). Therefore, in addition to the role of $GABA_A$ receptors we also characterize the role of $GABA_B$ receptors. This lead to the novel finding from this set of experiments. $GABA_B$ receptor antagonism (2-hydroxysaclofen; 2 µg/side) blocks

both footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine-seeking behavior.

Moreover, $GABA_B$ and CRF-R1 can have coordinated function on VTA dopamine neurons. We have previously shown that CRF-R1 activation in the VTA is necessary and sufficient for reinstatement of extinguished LgA cocaine seeking (Blacktop et al., 2011). Notably, others have found that intra-VTA CRF-R1 activation can facilitate $GABA_B$ inhibition of VTA dopamine neurons (Beckstead et al., 2009). This inhibition of VTA dopamine neurons occurs through $GABA_B$-receptor coupling to G protein activated inwardly rectifying potassium (GIRK) channels (Beckstead et al., 2009). CRF robustly increases $GABA_B$ activated GIRK-induced IPSCs on VTA dopamine neurons through CRF-R1 activation (Beckstead et al., 2009).

These findings are consistent with the notion that $GABA_B$ receptors, GIRK channels, CRF, and CRF-R1 receptors synergistically inhibit VTA dopamine neurons. This is one possible postsynaptic mechanism by which stress facilitates reinstatement of extinguished LgA cocaine-seeking behavior. However, under these conditions $GABA_B$ receptor blockade would be expected to increase VTA dopamine neuron activity not inhibit it. Although this is a possibility, it is not the only mechanism by which the $GABA_B$ receptor can regulate dopamine neuron excitation.

Presynaptic $GABA_B$ receptor activation can inhibit release of both glutamate and GABA in the VTA (cite) (Bonci and Williams, 1997, Manzoni and Williams, 1999, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010).

Repeated psychostimulant administration can augment the ability of $GABA_B$ receptors to inhibit postsynaptic dopamine neurons and recruit the ability of $GABA_B$ receptors to inhibit presynaptic glutamate release in the VTA (Giorgetti et al., 2002). This suggests that following drug exposure the $GABA_B$ receptor functions to inhibit VTA neuron excitation by decreasing glutamate input.

These previous findings suggest that $GABA_B$ receptor antagonism could be blocking reinstatement by: (1) decreasing inhibitory drive of the motive circuit by decreasing GIRK IPSCs on VTA dopamine neurons (Giorgetti et al., 2002, Beckstead et al., 2009), (2) increasing GABA and/or glutamate release in the VTA by decreasing $GABA_B$ mediated inhibition of neurotransmitter release on presynaptic GABA and/or glutamate neurons (Bonci and Williams, 1997, Shen and Johnson, 1997, Manzoni and Williams, 1999, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010), (3) or even coding for optimal phasic firing of VTA dopamine neurons (Beckstead et al., 2004, Ford et al., 2009). It is important to note that the mechanism may or may not involve coordinated actions of $GABA_B$ and CRF-R1. $GABA_B$ receptor blockade may be producing downstream effects independent of CRF dependent processes to regulate relapse. However, $GABA_B$ receptor activation is necessary for CRF actions in the VTA to produce reinstatement.

Repeated cocaine self-administration can recruit the ability for footshock stress and intra-VTA CRF to provide excitatory drive on VTA dopamine neurons through presynaptic glutamate release and subsequent ionotropic glutamate receptor conductance on dopamine neurons (Ungless et al., 2003, Wang et al.,

2005, Wanat et al., 2008, Hahn et al., 2009). Stress-induced CRF release in the VTA occurs in both drug-naïve and drug-experienced animals. However, only in drug-experienced animals has the recruitment of presynaptic glutamate and postsynaptic somatodendritic dopamine signaling been reported (Wang et al., 2005). Footshock-induced reinstatement and the recruited concurrent increases in somatodendritic dopamine but not glutamate concentrations can be blocked by administration of a nonspecific ionotropic glutamate receptor antagonist (Wang et al., 2005). For these reasons we also characterize the role of ionotropic glutamate receptor subtypes in reinstatement.

Intra-VTA administration of AP-5 or NBQX failed to significantly attenuate reinstatement to both footshock stress and direct VTA CRF delivery. Intra-VTA CRF receptor activation can excite both dopaminergic and GABAergic neurons in the VTA (Korotkova et al., 2006), both of which express NMDA and non-NMDA ionotropic glutamate receptors (Kalivas et al., 1989, Seutin et al., 1990, Wang and French, 1995). Moreover, glutamate can activate these receptors on both dopamine and GABA neurons (Christie et al., 1985, Sesack and Pickel, 1992, Steffensen et al., 1998). Perhaps the inability of NMDA or AMPA receptor antagonists to block reinstatement is due to an opposition between dopamine and GABA neurons both expressing NMDA and AMPA receptors. This would produce opposition not allowing for direct control of phenotypic excitatory drive within the VTA.

Alternatively, the inability to block cocaine seeking by AP-5 and NBQX may suggest that reinstatement is dependent on the coordinated action of both

AMPA and NMDA receptors consistent with magnesium block dependent neuroplasticity to regulate drug seeking. For these reasons we test the role of the nonspecific ionotropic glutamate receptor antagonist kynurenic acid (KA). A preliminary pilot study reports that KA (24 µg/side) significantly attenuates footshock stress-induced reinstatement and with more subjects will likely block intra-VTA CRF-induced reinstatement. This may suggest that the coordinated action of both NMDA and AMPA receptors are necessary. Alternatively, reinstatement may be dependent on kainate receptor activation.

Kainate ionotropic glutamate receptors are substantially understudied, present in the VTA, and known to regulate intra-VTA dopamine neuron activity (Wang and French, 1993a, Barrera et al., 2005). AP-5 and NBQX do not target the kainate receptor. In contrast, kynurenic acid (KA) blocks both the kainate receptor (Alt et al., 2004) and stress-induced reinstatement. Initial investigation of KA (24 µg/side) reports significant reductions in food reinforced responding in drug naïve rats trained in sucrose pellet reinforcement behavior (data not shown). However, KA (24 µg/side) does not affect the ability of drug-experienced animals to press the lever in the absence of drug, stress, or intra-VTA CRF. In fact, KA produced an activational effect not a suppressive one (Figure 36E).

Kynurenic acid, in addition to being an antagonist at the NMDA, AMPA, and kainate ionotropic glutamate receptors, is an antagonist at alpha(α)7 nicotinic receptors (Hilmas et al., 2001, Stone, 2007). Alpha 7 nicotinic receptors are present in the VTA and reported to regulate not only dopamine neuron excitation (Calabresi et al., 1989, Pidoplichko et al., 1997, Schilstrom et al., 2000) but also

drug self-administration (Corrigall and Coen, 1994). Therefore, the ability of KA to block reinstatement also holds the possibility to be regulated by alpha 7 nicotinic receptors.

When interpreting these findings, it is important to note that in contrast to our self-administration rats, sucrose trained rats have no history of cocaine intake and are food-restricted, possibly altering their sensitivity to NMDA, AMPA, kainate, or alpha 7 nicotinic receptor antagonism. This may suggest differential responses of drug naïve and drug-experienced animals to the same intra-VTA KA dose. Alternatively, KA may be inhibiting sucrose-seeking and drug-seeking through a similar motivational mechanism without altering their ability to press the lever. Moreover, it is still possible that the effects of KA on reinstatement are attributable to locomotor impairments.

Moreover, glutamate could be regulating reinstatement through metabotropic but not ionotropic receptors. Metabotropic glutamate receptor (mGluR) activation in the VTA has been shown to regulate cocaine-induced plasticity involving potentiation of excitatory input onto VTA dopamine neurons (Bellone and Luscher, 2006). Specifically, activation of mGluR receptors reverses cocaine-induced insertion of calcium permeable AMPA receptors into the membrane of VTA dopamine neurons; termed mGluR-LTD (Bellone and Luscher, 2005, 2006, Mameli et al., 2007, Luscher and Huber, 2010). In addition to regulating cocaine-induced AMPA neuroplasticity, mGluR current can be enhanced by CRF release in the VTA (Riegel and Williams, 2008). However, mGluR expression levels in the VTA have been reported to be unchanged

following extended access to cocaine (Ben-Shahar et al., 2009, Ghasemzadeh et al., 2011).

In summary, the findings from this series of experiments involving ionotropic glutamate receptors is difficult to interpret. To this end, the exact role of AMPA and NMDA receptors in stress-induced reinstatement of extinguished LgA cocaine-seeking remains unclear. Current studies are being conducted to look at the role of an NMDA/AMPA receptor specific antagonist cocktail (3 μg of both AP-5 and NBQX per side). There are possible roles for kainate, alpha 7 nicotinic, and metabotropic glutamate receptors in stress-induced reinstatement that were not addressed by the current set of experiments.

The current findings suggest that $GABA_B$ but not $GABA_A$, NMDA, or AMPA receptors by themselves are necessary for both footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine seeking. These results are surprising, it was hypothesized that both NMDA and AMPA antagonists would block reinstatement while $GABA_A$ and $GABA_B$ antagonists would augment it. However, only 2-hydroxysaclofen, the $GABA_B$ receptor antagonist, reliably blocked reinstatement by both footshock stress and intra-VTA CRF delivery. This does not suggest that $GABA_A$, NMDA, or AMPA receptors are not involved in stress-induced reinstatement, but rather likely reflects the inability to isolate neuronal phenotype specific activation by these receptors. However, the novel finding of this set of experiments is that $GABA_B$ receptor activation is necessary for both footshock- and intra-VTA CRF-induced reinstatement.

The ability of stressful life events to precipitate drug use through actions involving $GABA_B$ receptors in the VTA may or may not represent an emergent consequence of excessive cocaine use. Identification of the precise mechanisms behind the necessity of $GABA_B$ receptor activation in footshock- and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine seeking should provide important insight into how stress responsiveness in cocaine addicts promotes cocaine craving and relapse.

CHAPTER 5

GENERAL DISCUSSION: VENTRAL TEGMENTAL AREA REGULATION OF STRESS-INDUCED REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR

SUMMARY OF FINDINGS BY CHAPTER

This dissertation addresses key unknown mechanisms behind addiction-related drug-induced neuroplasticity and how such neuroplasticity gates the ability of stress to cause relapse. The high relapse rates in drug abstinent addicts have made preventing relapse central for the long-term management of drug addiction. In order to better understand how to prevent relapse, we must further understand the neurobiological processes that contribute to it. Primary questions of importance related to relapse are: 1) what are the primary triggers for relapse, 2) what systems of the brain regulate these triggers of relapse, and 3) what maintains the vulnerability for these triggers to cause relapse even following periods of prolonged drug abstinence? Stressful life events are unpredictable and unavoidable causes of relapse in human addicts. In this dissertation stress-induced relapse was modeled using the long-access cocaine self-administration/stress-induced reinstatement rodent model. General questions addressed in this dissertation are: 1) what are the primary mechanisms of stress-induced reinstatement, 2) what part of the brain regulates stress-induced reinstatement, and 3) what maintains the vulnerability for stress to trigger reinstatement even after extinction of drug seeking?

Very little is known about the neuromechanisms through stress contributes to the relapse process. Previous work from our laboratory has demonstrated that intake dependent neuroplasticity occurs with repeated cocaine use. This

neuroplasticity, in turn, interacts with the stress-related neuropeptide corticotropin-releasing factor (CRF) to facilitate stress-induced reinstatement of cocaine-seeking behavior. Importantly, stress-induced reinstatement only occurs in animals with a prior history of daily long-access but not short-access cocaine use (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). This suggests that stress-induced reinstatement in our animal model is dependent on cocaine intake-dependent neuroplasticity. The exact brain regions where this neuroplasticity and the neuropeptide CRF are interacting to regulate reinstatement are not fully defined. To this end, areas of convergence between motivational- and stress-related neurocircuitry provide opportunity for the study of the neuromechanisms by which stress facilitates relapse. A major area of convergence between motivational- and stress-related neurocircuitry examined in this dissertation is the ventral tegmental area (VTA). This area was examined because it represents a site where there is convergence of resident motivational-related dopamine neurons and inputs that release the stress-related neuropeptide corticotropin releasing factor (CRF) (Wise and Rompre, 1989, Korotkova et al., 2006, Tagliaferro and Morales, 2008, Wanat et al., 2008, Hahn et al., 2009, Almela et al., 2012, Wanat et al., 2013). Novel CRF-related neurobiological mechanisms within the ventral tegmental area that contribute to stressor-induced reinstatement of cocaine seeking are described.

CHAPTER 2

Chapter two characterizes whether CRF actions in the VTA represent a primary mechanism responsible for stress-induced reinstatement, and, if so, which CRF receptor CRF is acting through. This was accomplished using site-specific pharmacological manipulations within the VTA and the rodent reinstatement model of relapse. The hypotheses of chapter are that intra-VTA CRF administration is sufficient to reinstate drug seeking in high- (long-access) but not moderate-intake (short-access) animals and that CRF-R1, but not CRF-R2, activation in the VTA is necessary and sufficient for reinstatement.

Chapter two reports that CRF actions in the VTA do in fact represent a primary mechanism underlying stress-induced reinstatement. Intra-VTA CRF-administration is sufficient to reinstate drug seeking in a similar way to footshock stress and this is only observed following long-access self-administration. Footshock stress and intra-VTA CRF administration produces robust reinstatement following LgA but not ShA cocaine self-administration. Footshock stress- and intra-VTA CRF-induced reinstatement are both dependent on CRF-R1 receptor activation and not CRF-R2 receptor activation. Moreover, intra-VTA administration of a CRF-R1 but not CRF-R2 specific agonist is sufficient to reinstate drug seeking in a similar way to intra-VTA CRF administration. Therefore, intra-VTA CRF-R1 activation is necessary and sufficient for reinstatement of extinguished long-access cocaine-seeking behavior.

Altogether, the results of chapter two are indicative that excessive cocaine use increases the susceptibility to stressor-induced relapse at least in part by augmenting CRF-R1 dependent regulation of addiction-related neurocircuitry in the VTA. Importantly, these findings reveal a site specific neuromechanism that regulates stress-induced reinstatement of cocaine-seeking behavior. The findings of chapter 2 also suggest that CRF actions, by way of CRF-R1 activation in the VTA, may be one way through which stressful life events facilitate relapse in human addicts.

CHAPTER 3

Chapter three characterizes whether stressor-induced reinstatement of cocaine-seeking behavior involves an increase or a decrease in VTA dopamine neuron activation. This was done utilizing dual immunohistochemistry to detect stress-induced expression of an indicator of neuronal activation, c-Fos (Sagar et al., 1988), with tyrosine hydroxylase, a known marker for dopamine neurons in the VTA (Hokfelt, 1984). It was hypothesized that a significant increase in dopamine neuron activation would only be observed under conditions in which footshock stress reinstates cocaine seeking. Therefore, it was hypothesized that a significant increase in intra-VTA dopamine neurons that co-express c-Fos in response to footshock stress would occur in long-access but not short-access animals. This hypothesis was guided by previous findings from our laboratory reporting that footshock stress only reinstates drug-seeking in animals with a

history of long-access but not short-access cocaine self-administration (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011).

In contrast to previous findings, and the original hypotheses, it was found that 30% of short-access and 60% of long-access animals receiving footshock showed reinstatement of cocaine-seeking behavior. This is a disproportionately high percentage of ShA and low percentage of LgA rats relative to our previous reports. Independent of whether the animals received footshock or not animals with a history of short-access cocaine self-administration displayed increased, while animals with a history of long-access displayed decreased, VTA dopamine neuron activation. Surprisingly, the overall number and percent of dopamine neurons expressing Fos were found to be significantly increased in ShA but not LgA rats. Despite overall differences in dopamine (TH-positive) neuron activation (Fos positive) across groups, significant footshock stress-induced increases in dopamine cellular c-Fos reactivity were not observed in any cocaine self-administration access group.

Although, stress-induced reinstatement tends to be increased in LgA animals, an increase in c-Fos immunoreactivity was not observed under any condition. Interestingly, when LgA and ShA animals were combined, the percentage of TH-positive cells co-expressing c-Fos following stress-induced reinstatement was positively correlated with reinstatement magnitude. Further analysis of reinstatement and dopamine neuron activation was conducted on animals that did or did not reinstate in response to footshock (i.e. combining both ShA and LgA subjects). When examining across these groups and comparing to

non-stress, saline controls, and non-reinstating rats, the number and percentage of VTA Fos-expressing TH-positive cells was significantly increased in rats that displayed stress-induced reinstatement. In other words, footshock stress produces a significant increase in VTA dopamine neuron activation only in animals that reinstated. Importantly, this suggests that stress-induced reinstatement of cocaine seeking is correlated with increased VTA dopamine neuron activation and that individual differences in VTA dopamine neuron activation by stress may also contribute to stress-induced reinstatement. The findings of chapter three suggest that stressful life events may cause relapse in human addicts by increasing VTA dopamine neuron activation, and that individual differences in the stress response of the addict can regulate the susceptibility to relapse.

CHAPTER 4

Lastly, chapter 4 characterizes whether both stress- and intra-VTA CRF-induced reinstatement of extinguished cocaine-seeking behavior is dependent on excitatory or inhibitory receptor activation in the VTA. More specifically, the necessity of AMPA, NMDA, $GABA_A$, and $GABA_B$ receptor activation in this reinstatement was examined. This was also examined using site-specific pharmacological manipulations within the VTA and the rodent reinstatement model of relapse. It was hypothesized that both stress- and intra-VTA CRF-induced reinstatement of extinguished long-access cocaine-seeking behavior would be dependent on excitatory (glutamate) but not inhibitory (GABA) receptor

activation within the VTA. Therefore, it was more specifically hypothesized that both NMDA and AMPA antagonists would block while both $GABA_A$ and $GABA_B$ antagonists would augment both stress- and intra-VTA CRF-induced reinstatement in long-access animals. These hypotheses are supported by earlier reports implicating excitatory glutamatergic activation of VTA dopamine neurons in CRF-dependent stress-induced reinstatement of cocaine-seeking behavior (Wang et al., 2005, Wang et al., 2007).

In contrast to our original hypotheses, multiple doses of intra-VTA delivery of NMDA and AMPA receptor specific antagonists alone failed to block, while both $GABA_A$ and $GABA_B$ specific antagonists alone failed to augment reinstatement by either footshock stress or intra-VTA CRF delivery. Even more surprisingly, intra-VTA administration of a $GABA_B$ receptor specific antagonist alone blocked both footshock- and intra-VTA CRF-induced reinstatement in rats with a history of long-access self-administration in a similar way to CRF-R1 antagonism described in chapter 2. Not only is this finding contrary to our original hypotheses but it is novel and implicates inhibitory $GABA_B$ receptor signaling in reinstatement of extinguished LgA cocaine seeking. These findings may also suggest that stressful life events precipitate relapse by engaging activation of the $GABA_B$ receptor in the ventral tegmental area.

In summary the findings from chapters two through four suggest that: 1) stress is a primary trigger for relapse to cocaine use, 2) the CRF and dopamine systems within the ventral tegmental area regulate stress triggered relapse, 3) long-term vulnerability to stress-induced relapse involves drug-induced

neuroplasticity which in turn interacts with CRF and, possibly, dopamine systems within the VTA, 4) stress-induced relapse is correlated with increased activation of VTA dopamine neurons, and 5) stress-induced relapse is dependent on CRF-R1 and $GABA_B$ receptor activation within the VTA. The findings from this dissertation provide much needed insight into the cocaine-induced neuroadaptations that occur within the VTA and which systems interact with those neuroadaptations to regulate later stressor-induced relapse in cocaine addicts. The hope is that these findings will make current drug addiction therapies more effective or help with the development new drug therapies for the long-term management of cocaine addiction. The following chapter will discuss the significance of the findings from chapters two through four in further detail.

CRF RECEPTOR SUBTYPES AND THE VTA

CRF and both its receptors appear to be functionally expressed within the VTA (Ungless et al., 2003, Korotkova et al., 2006, Wanat et al., 2008). CRF-R1 mRNA has been found in both dopaminergic and GABAergic neurons of the VTA (Korotkova et al., 2006, Tagliaferro and Morales, 2008, Refojo et al., 2011). Although, the VTA does express CRF-R1 mRNA it does so at much lower levels than in other brain regions (Sauvage and Steckler, 2001). For this reason, CRF-R1 expression may be increased following LgA cocaine self-administration. Although not reported in this dissertation, a current collaborative study between our laboratory and the Seasholtz laboratory at the University of Michigan is being conducted. This study is utilizing double *in situ* hybridization for CRF-R1 or CRF-

BP mRNA in dopamine and GABA neurons in rats with a history of ShA or LgA self-administration and in saline controls. Preliminary reports show a greater proportion of CRF-R1 mRNA in dopamine neurons than in GABA neurons within the VTA.

Although the distribution of CRF receptor subtype mRNA expression has been extensively studied (Van Pett et al., 2000), the neurochemical identity of neurons expressing CRF-R1 protein remains largely unknown. A clear understanding of the localization of CRF receptor subtype receptors in the VTA awaits the availability of better antibodies for immunohistochemical characterization. This is largely due to difficulties generating CRF-R subtype specific antibodies (Chen et al., 2000, Campbell et al., 2003). Although, there are reports of CRF receptor subtype protein expression in the rat brain (Lukkes et al., 2011) the general consensus is that commercially available CRF-R1 antibodies are insufficient in sensitivity and specificity to detect endogenous physiological levels of CRF-R1 protein in the brain (Refojo et al., 2011). Unfortunately, this has made characterizing CRF-R1 protein expression in the VTA under conditions in which it regulates relapse difficult.

CRF RECEPTOR SIGNALING IN THE VTA AND STRESS

Potent anxiogenic effects have been reported following ventricular administration of CRF (Britton et al., 1982, Sutton et al., 1982, Gosnell et al., 1983, Veldhuis and De Wied, 1984, Eaves et al., 1985, Berridge and Dunn, 1986, Britton et al., 1986a, Britton et al., 1986b, Ruckebusch and Malbert, 1986,

Sherman and Kalin, 1986, Berridge and Dunn, 1987, Dunn and File, 1987, Ehlers and Chaplin, 1987, Sherman and Kalin, 1987, Swerdlow et al., 1989, Dunn and Berridge, 1990); see corticotropin releasing factor section in chapter one. In contrast to ventricular administration, the role of intra-VTA CRF has been under-characterized. However, early reports have implicated intra-VTA CRF in increased locomotor activity (Kalivas et al., 1987) with chronic cocaine administration increasing CRF binding, as measured using autoradiography, within the VTA (Goeders et al., 1990). These findings are consistent with indirect observations from our laboratory in which intra-VTA CRF administration in drug-experienced animals produces increased locomotor activity, anxiogenic like behaviors (i.e. increased grooming, agitation, and aggression), and reinstatement of cocaine seeking.

CRF-R1 SIGNALING IN THE VTA AND STRESS

The finding that CRF-R1 regulates stress-induced relapse is consistent with its primary role in the stress response of an organism. CRF-R1 expression is more abundant and consistently reported in the VTA as compared to CRF-R2 (Van Pett et al., 2000, Sauvage and Steckler, 2001, Refojo et al., 2011). Moreover, CRF has a tenfold higher affinity for CRF-R1 over CRF-R2 (Perrin et al., 1995), and is the receptor subtype that results in ACTH release and HPA-axis activation. CRF-R1 activation produces anxiogenic pro-stress effects (Bale and Vale, 2004) with CRF-R1 antagonists producing anxiolytic anti-stress effects (Schulz et al., 1996, Deak et al., 1999, Okuyama et al., 1999). Paradoxically,

selective deletion of CRF-R1 in midbrain dopaminergic neurons increases anxiety-like behavior and reduces dopamine release in the terminal fields of the VTA (Refojo et al., 2011). This suggests that CRF-R1 activation in the VTA can increase medial prefrontal dopamine concentrations and regulate stress-related behavior. This is consistent with stress-induced increases in mesocortical dopamine release (Thierry et al., 1976, Lavielle, 1978, Herman et al., 1982, Sorg and Kalivas, 1993). However, the finding that CRF-R1 activation in the VTA can be anxiolytic (Refojo et al., 2011) is not consistent with our findings that CRF-R1 antagonist administration into the VTA blocks footshock stress-induced reinstatement (Blacktop et al., 2011).

Similarly, the central function of the CRF-R2 receptor is not as well understood and has been implicated in both stress-protective and stress-coping effects (Bale et al., 2000, Valdez et al., 2002). Even less is known about the exact role of the CRF-R2 receptor within the ventral tegmental area. Therefore, the exact role of intra-VTA CRF release and activation of its receptors in anxiogenic and anxiolytic behavior in both drug naïve and drug-experienced animals needs to be investigated. It is possible that anxiogenic behavior induced by CRF administration into the VTA is augmented or even recruited following cocaine self-administration. Alternatively, anxiogenic effects of CRF may be mediated elsewhere in the CNS with intra-VTA CRF regulating other important aspects of stress reactivity (e.g., coping behaviors). These explanations are consistent with the emergent ability of intra-VTA CRF to cause reinstatement that is reported in chapter two.

LONG-ACCESS VERSUS SHORT-ACCESS COCAINE-INDUCED NEUROPLASTICITY

The amount of cocaine used is positively correlated with stress-induced cocaine craving in human addicts (Fox et al., 2005). Cocaine-induced neuroplasticity appears to involve increased stress responsiveness and cocaine craving (Sinha et al., 1999). In preclinical animal models of drug addiction and relapse, the long-access (LgA) approach has been utilized to examine drug-induced neuroplasticity (Ahmed and Koob, 1998, 1999, Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). Reinstatement is augmented in response to cocaine (Mantsch et al., 2004, Madayag et al., 2011), cocaine cues following long-access cocaine self-administration (Kippin et al., 2006), and stress (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011).

Reinstatement by footshock stress, ventricular CRF administration, and intra-VTA CRF administration appears to represent an emergent intake dependent phenomenon (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). Specifically, reinstatement by acute footshock stress exposure, ventricular CRF administration, and intra-VTA CRF administration is more reliably observed following high intake long-access (LgA; 14 x 6 hrs/day; ~70 mg/kg/day) but not short-access (ShA; 14 x 2 hrs/day; ~15 mg/kg/day) cocaine self-administration (Mantsch et al., 2008a, Blacktop et al., 2011, Graf et al., 2011). These findings suggest that the emergent ability of stress and CRF delivery to reinstate cocaine seeking is the likely consequence of cocaine-induced neuroplasticity in the circuitry of motivated behavior. This hypothesis is further supported by the

findings that CRF signaling (Richter and Weiss, 1999, Zorrilla et al., 2001, Specio et al., 2008, Zorrilla et al., 2012) is augmented following extended LgA cocaine self-administration. Altogether, this would suggest that the circuitry of motivated behavior becomes hypersensitized to CRF signaling following long-access cocaine self-administration.

ROLE OF CRF RECEPTOR SUBTYPES IN THE VTA IN STRESSOR- AND INTRA-VTA CRF-INDUCED REINSTATEMENT OF EXTINGUISHED LONG-ACCESS COCAINE-SEEKING BEHAVIOR

Excessive long-access cocaine self-administration increases the susceptibility to stressor-induced relapse at least in part by augmenting CRF-R1 dependent regulation of addiction-related neurocircuitry in the VTA (Blacktop et al., 2011). The chapter two findings that VTA CRF-R1 receptors are necessary and sufficient for reinstatement of cocaine seeking is further supported by previous studies which found that footshock stress-induced reinstatement is inhibited by systemic or ventricular delivery of CRF-R1 but not CRF-R2 receptor antagonists (Shaham et al., 1998, Lu et al., 2001). However, these findings are inconsistent with previous reports of Wise and colleagues suggesting the involvement of intra-VTA CRF-R2 instead of CRF-R1 receptors along with an unknown mechanism involving the CRF-binding protein (Wang et al., 2005, Wang et al., 2007).

Wise and colleagues reported that reinstatement, increased glutamate transmission, and increased somatodendritic dopamine signaling were all prevented by administration of a CRF-R2 antagonist and not a CRF-R1

antagonist (Wang et al., 2007). Furthermore, CRF receptor agonists found to be sufficient to cause reinstatement included ligands that bound to both the CRF-R1 and CRF-R2 receptors (Wang et al., 2007). However, every agonist that reinstated also bound to the CRF-BP while ineffective agonists did not (Wang et al., 2007, Wise and Morales, 2010). For these reasons, the authors hypothesized a distinct role for both the CRF-R2 receptor and the CRF-BP in intra-VTA CRF dependent reinstatement of cocaine seeking.

In contrast to Wang et al. (2007), chapter two reports that the CRF-R1 specific agonist, cortagine, was sufficient while the CRF-R2 specific agonist, rat Urocortin 2 (rUcn2), was insufficient to reinstate cocaine-seeking when administered into the VTA (Blacktop et al., 2011). Cortagine does not bind to the CRF-BP (Tezval et al., 2004) while rUcn2 does (Jahn et al., 2004). These findings suggest that the involvement of CRF-BP in reinstatement is yet another inconsistency between our findings reported in chapter two and those of Wise and colleagues.

DISPARATE FINDINGS & METHODOLOGIES: CHAPTER TWO AND WISE AND COLLEAGUES

The reason for the inconsistencies between our findings and those of others is unclear but may involve differential experimental methodologies. These include the mode of delivery of CRF and CRF antagonist/agonists (microinjection vs. reverse dialysis), CRF receptor antagonist/agonist doses (90 μM to 5.5 mM vs. 1 to 10 μM), different rat strains (Sprague Dawley vs. Long-Evans), differential experimental cocaine histories (6 hrs vs. 4 hrs), and most notably the

amount of total cocaine intake. The daily cocaine reported in our LgA animals was greater than 70 mg/kg compared to 33 mg/kg (Wang et al., 2007, Blacktop et al., 2011). It is possible that regulation of cocaine seeking by CRF-R1 receptors in VTA requires a prior history of very high levels of cocaine intake. In our hands excessive cocaine intake has been required for stress-induced reinstatement (Mantsch et al., 2008a). This is supported by previous findings reporting CRF-R1 only reduces cocaine self-administration following escalation in LgA rats (Specio et al., 2008).

The method of intra-VTA CRF delivery (microinjection vs. reverse dialysis) may have determined which CRF receptor was necessary for reinstatement. Agonist stimulation of CRF-R1 results in the desensitization of CRF-R1 signaling, as a consequence of both second messenger-dependent protein kinase activity and G protein-coupled receptor kinase phosphorylation (Oakley et al., 2007). This promotes β-arrestin recruitment to CRF-R1 facilitating endocytosis (Holmes et al., 2006, Oakley et al., 2007). Following CRF binding CRF-R1 receptors are internalized and degraded (Reyes et al., 2006, Reyes et al., 2008).

CRF has a tenfold higher affinity for CRF-R1 over CRF-R2 (Perrin et al., 1995) producing differential effects of β-arrestin recruitment and therefore internalization. Specifically, preferential CRF-R1 over CRF-R2 internalization occurs in the micromolar range (Oakley et al., 2007, Hauger et al., 2013), as utilized by Wise and colleagues during reverse dialysis. At this concentration there is only weak β-arrestin recruitment to the CRF-R2 receptor but strong recruitment to the CRF-R1 receptor (Oakley et al., 2007, Hauger et al., 2013).

Considering that reverse dialysis is a more extended approach it is possible that preferential internalization of the CRF-R1 receptor occurred. Although our dose of CRF (420 μM) is significantly greater than the 10 μM used by Wang et al., (2007) it was acutely administered. Therefore, our dose likely readily activated both receptors but likely did not cause internalization prior to the phase of reinstatement testing during which cocaine seeking was observed. In contrast, reverse dialysis at the 10 μM concentration may have caused preferential CRF-R1 internalization throughout the reinstatement session. In summary, endogenous CRF-R1 signaling was likely intact in our experiments and may not have been in others at the time of reinstatement. This may explain the CRF receptor subtype discrepancies between chapter two and Wise and colleagues.

CRF RECEPTOR G-PROTEIN COUPLING

The complexities of CRF receptor subtype-dependent behaviors is enhanced considering the promiscuity of their G-protein coupling. CRF receptors may change their downstream signaling pathway in the ventral tegmental area (VTA) following high intake of cocaine. This possible change in G-protein coupling could account for differences observed in which CRF-receptor subtype is regulating drug seeking in the VTA (Wang et al., 2007, Blacktop et al., 2011). Therefore, characterizing downstream signaling cascades for both CRF receptor subtypes following extended cocaine access may be informative as to how cocaine history is changing CRF responsiveness in the VTA. In support, CRF receptors are known to couple to multiple G-proteins including G_s, G_i, and G_q

proteins (Grammatopoulos et al., 1999, Grammatopoulos et al., 2000, Grammatopoulos et al., 2001, Blank et al., 2003, Wietfeld et al., 2004, Berger et al., 2006). Different receptor states or conformations can have different G-protein couplings and different G protein couplings can have different affinities for different conformational states (Kenakin, 2002). The active allosteric conformational states of the CRF-R1 receptor are thought to determine the G-protein coupling preference (Nielsen et al., 2000, Assil et al., 2001, Kenakin, 2002, Hoare et al., 2003, Hoare et al., 2004, Wietfeld et al., 2004, Berger et al., 2006).

It is well established that G_s activates PKA, G_i inhibits PKA, while G_q activates PKC all of which have different complex downstream signaling cascades. Chronic cocaine administration followed by acute withdrawal can produce a shift from CRF-R2 dependent G_s to G_q coupling in other brain regions (Liu et al., 2005). The promiscuous signaling of CRF receptors may help explain why CRF-R1, CRF-R2, PKA, and PKC have all been implicated in neuroplasticity of VTA dopamine neurons (Ungless et al., 2003, Wanat et al., 2008, Beckstead et al., 2009, Hahn et al., 2009). Therefore, there is the possibility that CRF-R1 may change its receptor coupling in the VTA following high cocaine intake. Future studies need to address the coupling and downstream signaling of the CRF-R1 receptor in the VTA in both cocaine naïve and long-access cocaine-experienced animals.

INTRACRANIAL CHEMICAL INJECTIONS: CHAPTERS TWO AND FOUR

Intracranial chemical injections (ICIs) play a critical role in the investigation and localization of neuromechanisms involved in drugs of abuse and relapse. However, there are significant caveats of the methodologies used that need to be taken into consideration. Pharmacological controls are necessary to assess non-receptor-mediated local actions of the drug, anatomical controls are necessary to rule out drug efflux to distal sites of action, and behavioral controls are necessary to separate the neuromechanism of interest from generalized activational or suppressive effects of the administered drugs (Wise and Hoffman, 1992).

The ventricular hypothesis involves the sphere of influence of ICI having and that sphere having the potential to spread from the site of ICI administration to the ventricular system (Routtenberg, 1972). Therefore, the anatomical region of interest along with spread must be considered when using site specific intracranial chemical injections. ICI material spreads through extracellular space (Bondareff and Pysh, 1968, Bondareff et al., 1970, Bondareff et al., 1971) with removal of ICI material occurring via blood vessels located near or at the injection site (Grossman and Stumpf, 1969).

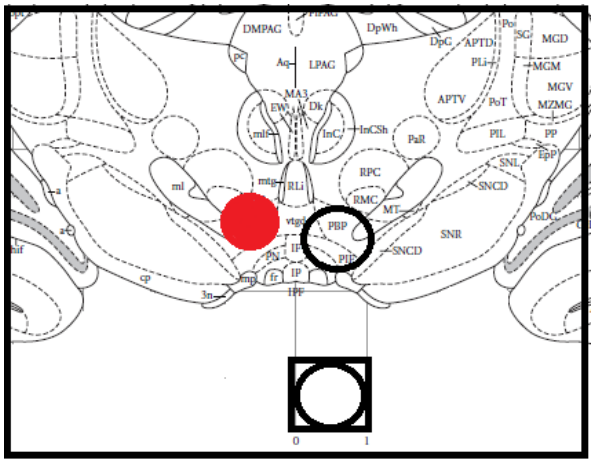
In fact, liquid application has less spread and more immediate effects than crystalline application of drug (Routtenberg and Olds, 1966, Stein and Levitt, 1971). However, liquid administration has its own challenges. For example, a large injection volume, such as 1 µl, produces damage at the injection site in the form of a large vacuole at the site of the cannula tip (Routtenberg and Olds,

1966, Routtenberg, 1972). This is not surprising since a 1 µl volume is occupying 1 mm^3 within the brain (Routtenberg, 1972). To circumvent this, others have tried using much smaller volumes (ex. 0.05 µl) which failed to produce repeatable results (Routtenberg, 1972). Therefore, a compromise was made setting the standard for ICI volume methodology. The compromise involved a volume with minimal damage yet repeatable administration which occurred at either 0.5 or 0.25 µl (Routtenberg and Simpson, 1971, Routtenberg, 1972). The spread of these smaller volumes was determined using dye and radiolabeled phosphorus with the same methodologies (ex. rate, cannula diameter, volume) and similar characteristics to the drug being administered (ex. pH, osmolarity, viscosity) (Maclean, 1957, Myers, 1966). These studies reported minimal restricted spread when 0.5 or 0.25 µl volumes are used. For these aforementioned reasons the 0.25 µl volume was used for this dissertation.

A 1 µl injection has an approximate spread radius of 0.6 mm producing a sphere of 1.2 mm in diameter (Lomax, 1966). Although 1 µl ICI showed considerable more spread than the 0.5 and 0.25 µl ICI (Routtenberg and Simpson, 1971, Routtenberg, 1972) it can be used as a conservative ratiometric estimate of spread for these smaller volumes. Using this volume to spread ratio a 0.25 µl ICI, as administered in this dissertation, can be calculated to have a spread radius of 0.15 mm and a sphere of 0.30 mm in diameter. Considering the VTA in the rat has a conservative bilateral radius of 0.5 mm (Paxinos, 2007) it is reasonable to assume that upon histological confirmation of an injection site the spread will be likely localized to the VTA, within reason. It is important to note

that without a certain amount of spread ICI's would be much less effective. The optimal amount of spread is necessary for three dimensional diffusion into the area of interest that has not been damaged upon insertion of cannula. In this way, diffusion can be an advantage and not a disadvantage for experimention.

Figure 37: Depiction of the area of spread by a microinjection in the VTA. The black circle is demonstrating a 1 mm^2 area representing the VTA area of interest, and the red circle is representing the calculated spread for a 0.25 µl injection of ~ 0.30 mm in diameter (Paxinos, 2007).



In addition to spread, there is damage to the site where the cannulae are placed within the brain. Tissue damage (e.g., reactive astrocytes, increased extracellular space) can, in turn, effect the neurochemistry of the injection site which can interact with the effects of the drugs being administered (Stavraky, 1961, Miller et al., 1964, Routtenberg, 1968, Weiss and Heller, 1969, Routtenberg, 1971). These effects can be minimized by using smaller gauge cannulae, using a dummy probes that are longer than the actual microinjector, and allowing ample recovery time from surgery.

To further minimize complications, the effect of injector cannula insertion by itself must be taken into account. The insertion of injector cannulae alone has been shown to effect behavior (Goddard, 1965). If this is suspected or of concern sham microinjections can be used as a control. In addition to cannula insertion, the rate of volume infusion can affect the rate of spread, amount of tissue damage, time course of the drug effect, and even the behavioral outcome (Booth, 1968, Routtenberg, 1971, Routtenberg and Simpson, 1971). A very slow rate of infusion (1 µl over 1-hr) and a very fast rate of infusion (1 µl over 1-minute; 0.5 µl over 10-sec) have been assessed for behavioral efficacy; with faster infusion rates producing most reliable results (Booth, 1968, Routtenberg, 1971, Routtenberg and Simpson, 1971). Therefore, the balance between latency of desired effect and possible tissue damage due to the rate of delivery must be considered. However, to reduce the possibility of ICI liquid from traveling back up the cannulae (i.e. path of least resistance) the injector cannula should be held in place for a period of time after the cessation of the injection. Repeatability of rate specific volumes can be applied using Hamilton syringes. For these reasons, 0.25 µl was infused over a period of 1-minute with a Hamilton syringe, with the injector cannula being left in for an additional minute past cessation of the injection.

Another factor that needs to be taken into consideration is the number of microinjections being applied to one injection site. The ideal number of intra-cranial microinjections is one (Olds et al., 1964, Routtenberg, 1972). However, one injection may become impractical under certain paradigms. This is especially

true for long-access cocaine self-administration followed by extinction, which requires reinstatement testing following both vehicle and pharmacological pre-treatments. To minimize damage to the injection site and the confound of this damage interacting with treatment-order bias, treatment conditions must be counter balanced with a maximum of 3-4 microinfusions per site. Alternatively, the experimenter can design between-group experimental methods at the expense of time and resources. In summary, the majority of methodological issues discussed cannot be entirely avoided but merely minimized. It is important to note that every method, when critically analyzed, will have its weaknesses.

However, it is important not to lose sight of the powerful advantages that ICI gives the experimenter. ICI avoids complications of first pass metabolism and the difficulty of the blood-brain barrier. ICI is reproducible and the properties of the liquid being applied can be tightly controlled (ex. pH, osmolarity, spread, rate, any other important properties of an aqueous solution) (Routtenberg, 1972). This includes dose-response testing that includes both very low and high concentrations of drug. Moreover, findings from different research groups can be compared with a certain amount of reproducibility. Intracranial injections give the experimenter a much needed, albeit blunt, instrument with the ability to access presynaptic, postsynaptic, and astrocytic targets. This gives the experimenter the ability to characterize neuromechanisms at the tripartite synapse in freely behaving animals *in vivo*. It is particularly useful in determining which brain sites and receptors regulate behavior, such as drug seeking.

Longer periods of abstinence can produce heightened responsiveness to stressors (Erb et al., 1996, Sorge and Stewart, 2005) and dopamine neuron activation by CRF is greatly affected by prior cocaine exposure and the stage of drug abstinence (Beckstead et al., 2009). Footshock-induced reinstatement of cocaine seeking is relatively suppressed in the first 24 hrs after withdrawal from cocaine in animals provided prolonged cocaine self-administration (Sorge and Stewart, 2005). The findings that footshock is relatively ineffective in inducing reinstatement in the early withdrawal period appears to be specific to animals with a history of prolonged drug exposure. Interestingly, early withdrawal is when the anxiogenic effects are the highest (Weddington et al., 1990, Markou and Koob, 1991, 1992, Miller et al., 1993, Barros and Miczek, 1996) – thus, there is a functional disconnect between cocaine seeking and withdrawal-induced anxiety. Suppressed responding to footshock 24 hrs after withdrawal from cocaine is not observed in animals that self-administer cocaine for only 2 h per day (Sorge and Stewart, 2005). This suggests that LgA animals are fundamentally different than ShA animals during early abstinence following extinction training.

Diminished footshock-induced reinstatement observed during early cocaine withdrawal normalizes within 48 hrs (Erb et al., 2004, Rudoy and Van Bockstaele, 2007). Furthermore, footshock-induced reinstatement can became progressively augmented over time (Sorge and Stewart, 2005) with stressor responsiveness being significantly greater in animals with prolonged cocaine

exposure (Sorge and Stewart, 2005). To this end, the amount of cocaine exposure is positively correlated with the magnitude of stress-induced reinstatement (active lever pressing) following forced abstinence and extinction training (Mantsch et al., 2008a). Augmented reinstatement magnitude in extended access cocaine self-administration animals was further supported by chapter two of this dissertation reporting augmented footshock- and intra-VTA CRF-induced reinstatement in LgA but not ShA animals.

C-FOS AND NEURON ACTIVATION

Due to insufficient antibodies for both the CRF-R1 and CRF-R2 receptors protein cannot be characterized in the VTA following LgA conditions. Therefore, we characterized dopamine neuron activation following footshock in the VTA following different cocaine self-administration histories (saline/cocaine naive, short-access cocaine, long-access cocaine; ± footshock). Characterizing c-Fos protein expression, a marker for neuronal activation, with tyrosine hydroxylase (TH), a marker for dopamine cells in the VTA, allowed us to determine dopamine neuron activation under conditions in which footshock does and does not reinstate cocaine seeking.

Immediate early genes (IEGs) are the first set of genes activated by external signals and do not require *de novo* protein synthesis (Sheng and Greenberg, 1990, Herrera and Robertson, 1996) making their induction both rapid and transient. IEGs are thought to function by encoding/activating transcription factors that will modify the expression of other genes, referred to as

target genes (TG) (Franza et al., 1988, Sheng and Greenberg, 1990). The most widely studied IEG in the CNS has been c-Fos (c-fos = mRNA, c-Fos = protein) (Herrera and Robertson, 1996, Kovacs, 2008). Importantly, in most cases, increased neuronal activity *in vivo* induces c-f/Fos expression in the nucleus of neurons (Curran et al., 1984, Dragunow and Robertson, 1987a, b, Hunt et al., 1987, Morgan et al., 1987). To this end, c-f/Fos is used as a generic marker for neuronal activation following a diverse array of different stimuli (Sagar et al., 1988, Cole et al., 1989, Dragunow and Faull, 1989). This provides a cellular method to label polysynaptically activated neurons within functional brain pathways (Sagar et al., 1988).

Fos is reported in the nuclei of neurons in both normal and pathological states (Dragunow and Robertson, 1987b, a, Hunt et al., 1987, Morgan et al., 1987) being transiently expressed in neurons after synaptic stimulation (Sagar et al., 1988). This makes the time course of Fos expression critical. The peak expression of c-fos mRNA is ½-1 hr after the cessation of the stimulus of interest, while the peak expression of c-Fos protein is 1-3 hr after cessation of the stimulus of interest (Sonnenberg et al., 1989, Chan et al., 1993, Imaki et al., 1993, Ding et al., 1994, Ikeda et al., 1994, Cullinan et al., 1995, Kovacs and Sawchenko, 1996a, b, Kovacs, 1998). Fos expression then gradually disappears from the nucleus after 4-6 hrs after the stimulus of interest (Sonnenberg et al., 1989, Chan et al., 1993, Imaki et al., 1993, Ding et al., 1994, Ikeda et al., 1994, Cullinan et al., 1995, Kovacs and Sawchenko, 1996a, b, Kovacs, 1998).

Neuronal c-f/Fos expression can be induced by neurotropic factors, neurotransmitters, depolarization, and an increase in intracellular Ca^{2+} either through influx or release from intra-cellular/nuclear stores (Greenberg and Ziff, 1984, Szekely et al., 1987, Didier et al., 1989, Morgan and Curran, 1989, Szekely et al., 1989, Doucet et al., 1990, Sheng and Greenberg, 1990, Vaccarino et al., 1992, Bading et al., 1993, Ghosh et al., 1994, Gaiddon et al., 1996). Mechanisms of c-f/Fos induction include PKA/cAMP/CaM Kinase/CRE activation (e.g., Gs-GPCRs) (Sassone-Corsi et al., 1988, Gonzalez and Montminy, 1989, Sheng and Greenberg, 1990, de Groot and Sassone-Corsi, 1993, Bito et al., 1996); PKC/ras/MAPK/ERK activation (ex. growth factors, Gq-GPCRs, and calcium detection via voltage dependent Ca2+ channel influx) (Treisman, 1992, Hill and Treisman, 1995), and ligand gated Ca^{2+} NMDAR/MAPK activation (Greenberg et al., 1986, Morgan and Curran, 1986, Bading et al., 1993, Ghosh et al., 1994).

Fos is the most widely used functional anatomical marker for activated neurons within the CNS for several reasons: 1) it is expressed at low levels in the intact brain under basal conditions (Curran, 1988, Kovacs, 2008), 2) it is typically induced in response to several extracellular signals, including ions, neurotransmitters, growth factors, and drugs (Kovacs, 2008), 3) the response is transient (Kovacs, 2008), and 4) detection of c-fos mRNA or c-Fos protein is simplistic (Kovacs, 2008).

However, the canonical mRNA and protein labelling technique used has low temporal resolution and does not provide information about the connectivity of the activated neuron *in vivo* (Kovacs, 2008). Neuronal activation can occur

without induction of IEGs and markers for neuronal activation such as c-f/Fos are not expressed in chronically activated neurons (Kovacs, 2008). Under basal conditions c-f/Fos levels are very low (Hughes et al., 1992, Fenelon et al., 1993, Kaczmarek and Chaudhuri, 1997, Kovacs, 1998). Moreover, different stimuli can activate the same IEGs at the same time (Kovacs, 2008). This suggests that it is much more likely that afferent inputs and/or changes in external stimuli induce c-f/Fos expression rather than tonic activation (Luckman et al., 1994). This suggests that c-f/Fos expression is very informative under the appropriate circumstances.

c-fos mRNA and its protein product c-Fos are reliable markers for identifying activated cells and central nervous system circuits that respond to: daily rhythm (Maywood et al., 1995, Recio et al., 1996, Duffield et al., 1998, Vuillez et al., 1998), sleep/wake cycle (Novak et al., 2000), oestrus (Funabashi et al., 1997, Hairston et al., 2003), mating (Tetel et al., 1993, Wersinger et al., 1993, Cameron et al., 2004), lactation (Fenelon et al., 1993, Hoffman et al., 1994, Pape et al., 1996), drug of abuse (Graybiel et al., 1990, Young et al., 1991, Curran et al., 1996, Rotllant et al., 2010, Fanous et al., 2011, Zhao-Shea et al., 2011), and various stressors.

C-FOS INDUCTION AND STRESSORS

The various stressors known to induce c-f/Fos include those involved in drug seeking paradigms such cold (Pacak and Palkovits, 2001), restraint (Cullinan et al., 1995, Imaki et al., 1995, Dayas et al., 2001, Viau and

Sawchenko, 2002), predator exposure (Chang et al., 2001, Bennett et al., 2002, Figueiredo et al., 2003), novelty (Emmert and Herman, 1999), forced swim (Duncan et al., 1993, Cullinan et al., 1995, Cullinan et al., 1996) and most notably, footshock (Campeau et al., 1991, Smith et al., 1992, Adolfsson et al., 1998, Bale et al., 2000, Morrow et al., 2001).

Stress-induced c-Fos activation can result from a complex interaction between catecholamines, glutamate, and CRF (Dragunow et al., 1990, Campeau et al., 1991, Arnold et al., 1992, Pezzone et al., 1992, Smith et al., 1992, Covenas et al., 1993, Harbuz et al., 1993, Imaki et al., 1993, Pezzone et al., 1993, Wan et al., 1993, Wan et al., 1994). Footshock stress has been shown to cause reinstatement of cocaine seeking and CRF release within the brain under conditions shown to increase c-Fos expression; an effect prevented by pre-treatment with a CRF-R antagonist (Arnold et al., 1992, Erb et al., 2005, Wang et al., 2005, Wang et al., 2007). This suggests that CRF receptor activation can induce c-Fos expression in the brain in response to a stressor. Cocaine and amphetamine exposure both induce c-Fos expression in the nucleus accumbens, prefrontal cortex, and VTA (Graybiel et al., 1990, Young et al., 1991, Colussi-Mas et al., 2007, Rotllant et al., 2010, Fanous et al., 2011). Moreover, previous cocaine exposure can increase footshock-induced c-Fos expression in the VTA (Morrow et al., 2001). Therefore, it is reasonable to hypothesize that intra-VTA CRF released via footshock stress will increase c-Fos expression in an intake dependent manner (i.e. short-access vs. long-access).

Stressful stimuli both induce c-Fos expression in (Deutch et al., 1991, Ma et al., 1993b, a) and increase firing of (Guarraci and Kapp, 1999, Anstrom and Woodward, 2005, Anstrom et al., 2009, Brischoux et al., 2009) VTA dopamine neurons. Importantly, c-Fos immunolabeling has been used to characterize footshock-induced neuronal activity (Van Pett et al., 2000) and reinstatement of drug seeking (Zhao et al., 2006). Therefore, it is possible that co-localization of c-Fos with VTA dopamine neurons in response to footshock stress will be significantly greater under LgA self-administration conditions, as compared to ShA conditions. This could be attributed to a functional change following LgA self-administration, whereby footshock stress and intra-VTA CRF can now reinstate cocaine-seeking behavior. If footshock stress-induced reinstatement involves increased dopamine neuron activity in the VTA, it is logical to hypothesize that an increase in c-Fos immunoreactivity in dopamine neurons by footshock stress will only occur in LgA animals.

FOOTSHOCK-INDUCED VTA DOPAMINE NEURON ACTIVATION

In chapter three a disproportionately high percent of short-access (ShA; 30%) and low percent of long-access (LgA; 60%) animals reinstated in response to footshock stress. This is in comparison to chapter two reporting negligible reinstatement in ShA rats. These discrepancies cannot be easily explained but likely reflect the possibility that stress-induced reinstatement does not only occur following LgA cocaine self-administration but rather that a much higher percentage of rats reinstate in response to stress with extended-access to

cocaine. Nonetheless, while prior intake is one predictor of stress-induced cocaine seeking, other factors may also dictate the ability of stress to activate VTA dopamine neurons. This is consistent with others reporting stress-induced reinstatement under ShA conditions (Erb et al., 1996, Shalev et al., 2000). However, some of these other reports utilized three 2-h sessions per day to reliably induce reinstatement by footshock. Therefore, it is still likely that cocaine intake is in fact positively correlated with stress-induced reinstatement magnitude.

Individual responsiveness to stress likely facilitated relapse in our short-access (ShA) animals instead of drug intake. The reason for this conclusion is that, there was not a correlation between cocaine intake and reinstatement magnitude in ShA rats. Although, the findings of chapter two indicate that prior intake is one predictor of stress-induced cocaine seeking, chapter three suggests that other factors (e.g., pre-existing individual differences in stress reactivity) can also dictate the ability of stress to activate VTA dopamine neurons. Future studies characterizing these individual differences may give further insight into the factors regulating the propensity to relapse as a result of stressor exposure following periods of abstinence in human cocaine addicts.

Chapter three also reports increased and decreased dopamine neuron activation in ShA and LgA animals, respectively. Surprisingly, overall number and percentage of dopamine neurons expressing c-Fos was significantly increased in ShA but not LgA rats relative to saline (Sal) controls. In fact, the c-Fos expression in TH-positive cells in LgA animals more closely resembled that of

Sal-treated animals. Despite overall differences in dopamine (TH-positive) neuron activation (Fos positive) across groups, significant footshock stress-induced increases in dopamine cellular c-Fos immunoreactivity was not observed in any self-administration access group (Sal, ShA, and LgA). These findings are surprising in that they suggest that LgA rats display less dopamine neuron activation in the self-administration environment as compared to ShA rats.

The basal levels of dopamine neuron activation in ShA versus LgA suggests that, in ShA animals, dopamine neurons show increased activation to the drug self-administration environment. Alternatively, in LgA animals there is either: (1) decreased activation to the drug self-administration environment, or there is an (2) extended up-regulation of an inhibitory tone imprint that can be removed by salient stressful stimuli in the drug self-administration context. ShA animals show increased, while LgA animals show decreased, dopamine neuron activation while in the drug self-administration context. This may also reflect a loss in dopamine neuron function in LgA animals. However, animals that reinstate in response to footshock stress exhibit increased VTA dopamine neuron activation. Moreover, the findings that inhibitory drive (i.e. $GABA_B$ receptor activation; Chapter 4) may be facilitating relapse is not consistent with increased c-Fos responsiveness during relapse in cocaine experienced animals unless it is coding for optimal phasic firing of VTA dopamine neurons (Beckstead et al., 2004, Ford et al., 2009) in response to salient drug environmental cues during stress.

Although, chapter three reports that LgA animals appear to be less responsive to the drug self-administration context than ShA animals (i.e., Fos expression in TH-positive cells in the VTA under stress-free conditions is lower), the self-administration context appears to be an important component for reinstatement of cocaine seeking (Shalev et al., 2000). Drug self-administration context cues have been shown to reinstate extinguished cocaine seeking and induce c-Fos expression in VTA dopamine neurons of ShA animals (Kufahl et al., 2009). Alternatively, LgA cocaine self-administration could be inhibiting the activation of dopamine cells in this brain region independent of the drug self-administration context. For example, in the reinstatement rodent model of relapse, extinction is necessary to measure increases in drug-seeking behavior by stress and may affect VTA dopamine neuron excitation differentially in LgA animals as compared to ShA animals. Extinction neurocircuitry is another factor to take into consideration when comparing long-access and short-access animals. Extinction training may have divergent effects on VTA dopamine neuron activation in response to stress in an intake-dependent manner. Alternatively, others have supported context-independent effects of extended access to cocaine in the absence of extinction training on mesocorticolimbic dopamine neurotransmission. Specifically, extended-access to cocaine has been found to be associated with decreased mesocorticolimbic dopamine system activity during protracted withdrawal independent of context (Weiss et al., 1992a, Weiss et al., 1992b).

In chapter three, when all rats were included in the analysis (i.e., short-access and long-access rats combined), a positive overall correlation between the number and percentage of TH-positive cells co-expressing c-Fos and reinstatement magnitude (active lever presses) was observed. Moreover, when reinstating rats were compared to non-stress, saline controls, and non-reinstating rats, the number and percentage of VTA TH-positive cells co-express c-Fos was significantly increased in rats that display stress-induced reinstatement. This suggests that footshock stress-induced reinstatement of cocaine-seeking behavior is positively correlated activation of VTA dopamine neurons.

COCAINE-INDUCED NEUROPLASTICITY AND VTA DOPAMINE NEURON ACTIVATION BY STRESS

Drug experience may recruit the ability of footshock stress to increase Fos expression in the VTA, which may be dependent on intact upstream extended amygdala neurocircuitry (Ahmadi et al., 2008). To this end, drug experience is likely necessary for stress-induced activation of VTA dopamine neurons by CRF (Wang et al., 2005, Wang et al., 2007). Accordingly, CRF- and glutamate-dependent somatodendritic release of dopamine has been reported to be dependent on cocaine experience in rats (Wang et al., 2005, Wang et al., 2007). However, the same studies found that footshock-induced CRF release was found to be comparable in both drug-naïve and drug-experienced animals (Wang et al., 2005). This suggests that VTA dopamine neurons are more readily activated by CRF following drug exposure. In contrast, evidence suggests that VTA CRF inputs express more CRF and are more active following drug exposure (Richter

and Weiss, 1999, Harris and Aston-Jones, 2003b, Zorrilla et al., 2012), these findings suggest that VTA neurons or downstream processes are likely more responsive to CRF as a result of drug-induced neuroplasticity.

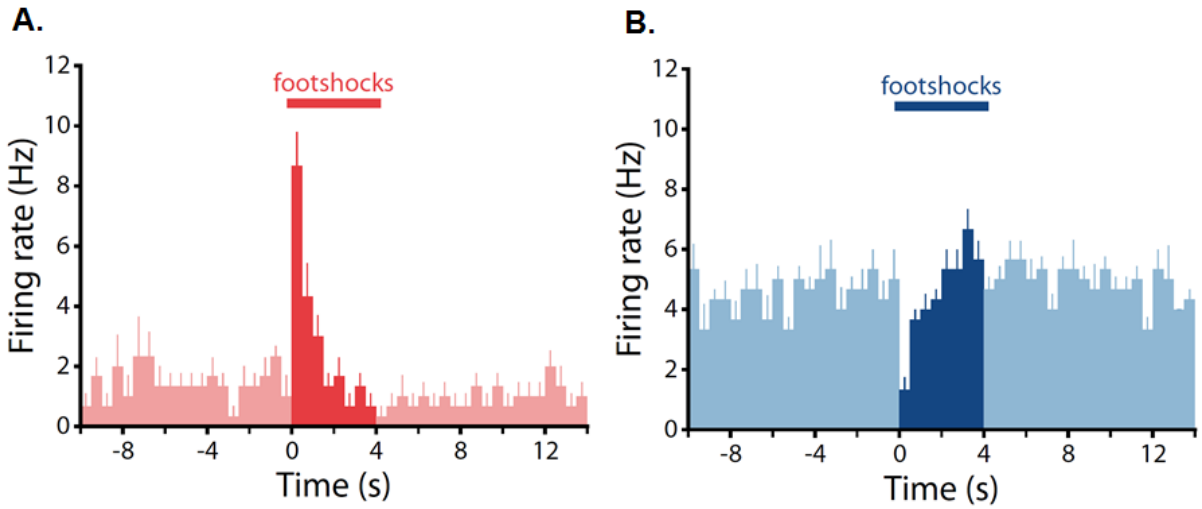
Fos immunoreactivity is significantly increased in the extended amygdala at the time of drug seeking during protracted withdrawal (Harris and Aston-Jones, 2003b). Following extended cocaine self-administration CRF immunoreactivity is also increased in the extended amygdala (Zorrilla et al., 2012), further supporting increased neuron activation by stress in upstream targets that release CRF into the VTA. Moreover, elevated anxiety during drug abstinence may reflect increased sensitivity to stress that is not evident in resting baseline conditions (Aston-Jones and Harris, 2004). This is congruent with elevated stress sensitivity in addiction (Kreek and Koob, 1998, Sinha et al., 1999, Mantsch et al., 2008a). In summary, the finding from chapter three that footshock-induced VTA dopamine neuron activation is positively correlated with reinstatement magnitude likely reflects increased VTA dopamine neuron activation by CRF. In support, chronic cocaine administration increases CRF binding in the VTA (as measured by autoradiography) (Goeders et al., 1990), preliminary reports from a collaborative effort with the Seasholtz lab suggest a greater proportion of CRF-R1 mRNA in dopamine neurons than in GABA neurons within the VTA (unpublished findings), cocaine experience increases the excitatory effects and decreases the inhibitory effects of CRF actions upon VTA dopamine neurons (Ungless et al., 2003, Wang et al., 2005, Korotkova et al., 2006, Wang et al., 2007, Wanat et al., 2008, Beckstead et al., 2009, Hahn et al., 2009), CRF antagonists inhibit evoked

mesolimbic (measured by *in vivo* microdialysis) dopamine release (Lodge and Grace, 2005), and selective deletion of the CRF-R1 gene in VTA dopamine neurons decreases dopamine release in the prefrontal cortex (Refojo et al., 2011).

HETEROGENEOUS VTA DOPAMINE NEURON POPULATIONS: RESPONSE TO FOOTSHOCK STRESS

Aversive events and stressors have often been associated with reductions in the activity of mesolimbic dopamine projections and increases in the activity of mesocortical dopamine projections (Thierry et al., 1976, Herman et al., 1982, Deutch et al., 1985, Roth et al., 1988, Deutch et al., 1990, Deutch et al., 1991, Ungless et al., 2004, Ungless et al., 2010, Wanat et al., 2013). VTA dopamine neurons can be activated or inhibited by footshock stress (Brischoux et al., 2009). Approximately, half of VTA dopamine neurons that are inhibited by footshock stress display excitation at the offset of the stimulus (Brischoux et al., 2009). This suggests the possibility that the offset of an aversive stimulus may excite VTA dopamine neurons contributing to the increase in dopamine neuron Fos expression seen in reinstatement of cocaine seeking reported in chapter 3.

Figure 38: Demonstration of different VTA dopamine responses to footshock stress from Brischoux et al., (2009); peristimulus time histogram averaged across 6 footshocks + SEM; 500-ms bins. A) Excitatory response to footshock stress B) Inhibitory response to footshock stress followed by a rebound in activity. Both A and B conditions could result in increased c-Fos in VTA dopamine neurons.



An increase in footshock-induced Fos immunoreactivity in VTA dopamine neurons could reflect an increase in activation by footshock stress or possibly removal of footshock stress. Behavioral reports suggest that the offset of an aversive stimulus can act as a reward (Tanimoto et al., 2004) and can excite dopamine neurons (Daw et al., 2002) especially at the onset of appetitive events. Therefore, removal of the footshock may result in activation of the same dopamine neuron subpopulation that is inhibited by footshock stress (Daw et al., 2002, Tanimoto et al., 2004, Brischoux et al., 2009, Ungless et al., 2010). Footshock removal-induced activation of VTA dopamine neurons as a key mechanism in Fos induction and reinstatement is much more likely if footshock is producing uniform inhibition of VTA dopamine neurons as reported by others (Ungless et al., 2004). However, this conclusion cannot be made without characterization of VTA dopamine neuron activation in response to footshock using high temporal resolution techniques (ex. voltammetry) in saline, ShA, and LgA animals.

The dysphoric effects of footshock stress in combination with the presentation of salient drug-associated cues (cocaine self-administration context) may produce overwhelming drug craving and motivation to use, in turn, resulting in reinstatement of drug seeking. Coinciding with the termination of footshock stress is both the extension of levers (both active and inactive) and the presentation of drug-associated cues (houselight), albeit extinguished cues, which may themselves activate VTA dopamine neurons in a manner that is dependent on prior cocaine self-administration history. Alternatively, a stressor, in the drug self-administration context, and in the presence of extinguished cocaine-assocaited cues may all be necessary for reinstatement of cocaine seeking that is characterized by increased Fos expression in VTA dopamine neurons.

Due to the low temporal resolution (1-3 hrs) of c-Fos immunoreactivity the increased in c-Fos in VTA dopamine neurons in animals that reinstate in response to footshock stress may be induced by: 1) the actual stressor, 2) removal of the stressor, 3) presentation of the active lever, 4) chamber light activation, or 5) any combination of these factors. Equally important, it is possible that all of these factors together are needed to get footshock-induced reinstatement, which in turn may contribute to c-Fos induction in VTA dopamine neurons.

COORDINATED ACTION OF CRF ON MESOLIMBIC AND MESOCORTICAL SYSTEMS: CRF AN OPPORTUNISTIC NEUROMODULATOR IN THE VTA

While the VTA sends dense dopaminergic projections to the NAc, dopamine neurons activated by stress preferentially project to the medial prefrontal cortex (Thierry et al., 1976, Tassin et al., 1980, Herman et al., 1982, Deutch et al., 1985, Lammel et al., 2011, Lammel et al., 2012). When measuring dopamine activation at the level of the VTA it is very difficult to determine which cell populations are regulated by CRF and contribute to stress-induced reinstatement of cocaine seeking. This is particularly important when considering the selective heterogeneous regulation (mesocortical over mesolimbic) of the mesocorticolimbic dopamine system by stress and CRF.

Dopamine is essential for information processing in the prefrontal cortex (Goldman-Rakic et al., 2000) and alterations of dopamine function within these systems have been implicated in drug addiction (Volkow et al., 1996, Volkow and Fowler, 2000, McFarland et al., 2004, Kalivas and Volkow, 2005, Peters et al., 2008). Optimal dopamine receptor signaling on both pyramidal and non-pyramidal cells within the mPFC is required for proper functional output (Penit-Soria et al., 1987, Vincent et al., 1993, Gaspar et al., 1995, Seamans et al., 1995, Fritts et al., 1998, Gulledge and Jaffe, 1998, Seamans et al., 1998, Gulledge and Jaffe, 2001, Wang and O'Donnell, 2001, Dong and White, 2003). When the PFC dopamine system signaling becomes dysregulated, a disruption of informational processing results (Sawaguchi and Goldman-Rakic, 1991, Williams and Goldman-Rakic, 1995, Zahrt et al., 1997, Seamans et al., 1998,

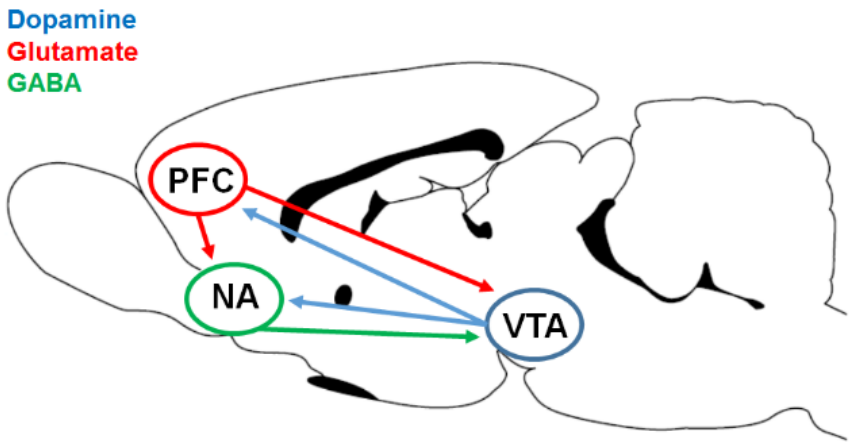
Seamans et al., 2001, Seamans and Yang, 2004) which has been implicated in stress-induced reinstatement of cocaine seeking (McFarland et al., 2004).

The mesocortical DA projection arises primarily from the ventral tegmental area (VTA) and terminates mainly on pyramidal neurons in deep layers V and VI of medial PFC (mPFC) (Berger et al., 1976, Bjorklund et al., 1978, Emson and Koob, 1978, Berger et al., 1991, Carr et al., 1999). These neurons project back to the VTA and to the nucleus accumbens, and as a result, mesocortical dopamine input can also modulate VTA dopamine neuronal activity and mesolimbic dopamine inputs and NAc MSN dopamine responsiveness (Sesack and Pickel, 1992, Taber et al., 1995, Carr and Sesack, 2000b, Geisler et al., 2007).

STRESS SELECTIVELY ACTIVATES MESOCORTICAL OVER MESOLIMBIC DOPAMINE NEURONS

Stress, by way of CRF release in the VTA, may be modulating the mesocorticolimbic circuit in such a way that, upon drug context informational processing, drug seeking is engaged (Mantsch et al., 2014). To accomplish this, intra-VTA CRF might be acting as a coordinator of mesocorticolimbic function silencing dopamine neuronal activity in the absence of drug-associated stimuli while opportunistically promoting signaling in the presence of drug-associated stimuli (Mantsch et al., 2014). This may take the form of increased mesocortical and decreased mesolimbic dopamine signaling during periods of stress and upon exposure to the drug self-administration context.

Figure 39: Mesocorticolimbic connectivity. Schematic demonstrating dopamine terminal fields in both the medial prefrontal cortex and nucleus accumbens, glutamate input to the nucleus accumbens from the medial prefrontal cortex, and GABAergic feedback from the nucleus accumbens to the VTA.



Exposure to certain stressors, notably footshock, selectively activate VTA dopamine neurons that project to the medial prefrontal cortex and not the nucleus accumbens (Thierry et al., 1976, Westerink and Korf, 1976, Fadda, 1978, Lavielle, 1978, Tissari et al., 1979, Blanc et al., 1980, Fadda et al., 1980, Tassin et al., 1980, Herman et al., 1982, Reinhard et al., 1982, Bannon and Roth, 1983, Deutch et al., 1985, Roth et al., 1988). Importantly, stress has been shown to selectively increase Fos expression in neurons of the VTA that project to the medial prefrontal cortex and not in the nucleus accumbens, as defined using retrograde tracers (Deutch et al., 1991). This is consistent with the hypothesis that stress preferentially targets the medial prefrontal cortex while dopamine neurons that project to the nucleus accumbens signal reward and salience

(Thierry et al., 1976, Tassin et al., 1980, Herman et al., 1982, Deutch et al., 1985, Lammel et al., 2011, Lammel et al., 2012).

The selective regulation of mesocortical versus mesolimbic dopamine neurons in the VTA is currently under investigation by numerous groups with the goal of functionally and anatomically characterizing how each cell type differentially responds to stress and reward. However, the current literature suggests the intra-VTA nuclei/region of origin for the various mesolimbic versus mesocortical dopamine neurons that are differentially activated by both stress and/or reward is very convoluted (Bannon and Roth, 1983, Murase et al., 1993a, Murase et al., 1993b, Schultz, 1998, Ungless et al., 2004, Brischoux et al., 2009, Lammel et al., 2011, Lammel et al., 2012). In summary, ther is currently no clear organization model that defines where these dopamine neurons originate. Nonetheless, there are dopamine neurons that are preferentially activated by stress that project to the medial prefrontal cortex and dopamine neurons that are preferentially activated by reward that project to the nucleus accumbens.

Figure 40: Differential effects of footshock stress on mesocortical versus mesolimbic neurotransmission. Schematic illustrating preferential activation of VTA dopamine neurons projecting to the medial prefrontal cortex and not the nucleus accumbens. *Magnitude is indicated by width of the arrow.* CRF-R1 is hypothesized to be on dopamine neurons projecting to the prefrontal cortex.

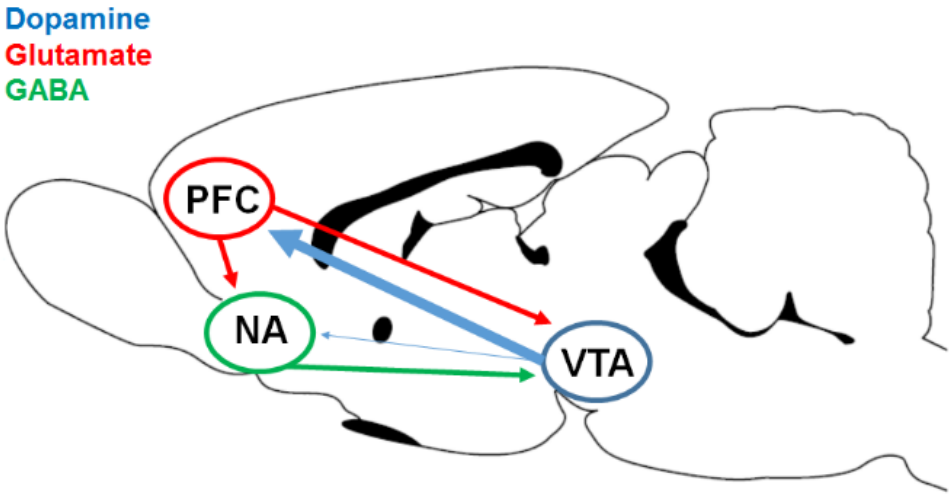
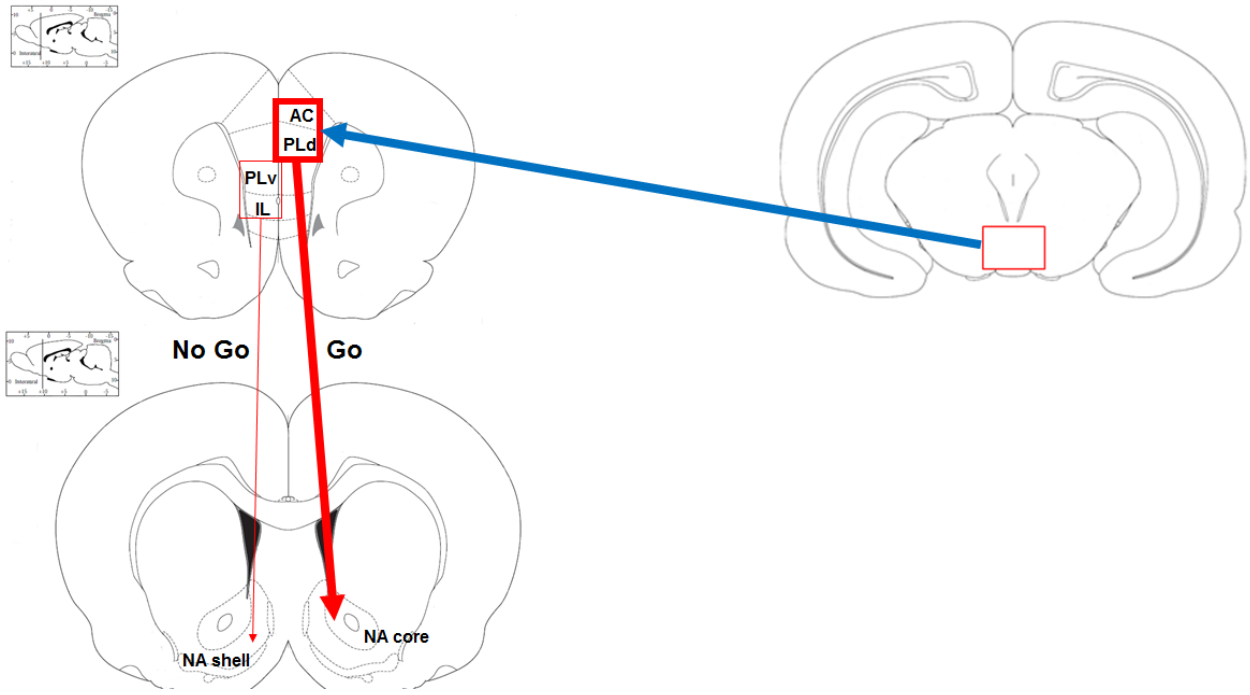


Figure 41: Effects of footshock stress on animals that reinstate. Proposed mechanism behind reinstatement and increased c-Fos in TH neurons by footshock in those animals. The dopamine neurons that are activated by footshock stress increase prelimbic cortex dopamine concentrations, providing excitatory glutamate input into the “Go” nucleus accumbens core circuit.



EFFECTS OF DRUG EXPOSURE ON STRESS-INDUCED MEDIAL PREFRONTAL DOPAMINE RELEASE

In drug-naïve animals, footshock stress increases dopamine levels in the medial prefrontal cortex (Sorg and Kalivas, 1993). However, prior daily pre-treatment with amphetamine or cocaine decreases footshock-induced dopamine release in the PFC (Robinson et al., 1985, Sorg and Kalivas, 1993). Importantly, the time course of the footshock can dictate whether mesocortical dopamine levels increase or decrease in drug experienced animals. Early on (5-10 min) after footshock onset dopamine signaling is increased, but decreases to baseline or even lower levels with continued footshock exposure (20-30 min) (Robinson et al., 1987, Kalivas and Duffy, 1989). This is in stark contrast to marked increases in prefrontal dopamine signaling in cocaine naïve animals receiving footshock for 20 minutes in duration (Thierry et al., 1976, Roth et al., 1988). Moreover, repeated footshock decreases cocaine challenge-induced increases in prefrontal dopamine concentrations (Sorg and Kalivas, 1993). These data suggest that repeated stress or cocaine exposure can induce tolerance in mesocortical dopamine release to footshock or a cocaine challenge, respectively (Sorg and Kalivas, 1993). This is important when considering that footshock stress in the experiments described in this dissertation involved cocaine-experienced rats, lasted 15 minutes, and, in some cases, was counterbalanced, and repeatedly delivered.

Fos data described in chapter 3 would suggest that footshock increases VTA dopamine activity only when reinstatement occurs and that reinstatement

may involve increases in VTA dopamine neuron activation. However, it is unclear if stress alone is activating dopamine cells or if it is enhancing the ability of the self-administration context to produce activation. To investigate this, the effect of footshock stress of VTA dopamine neuron activation in the absence of the drug context in both drug naïve and drug experienced animal's needs to be examined. Likewise, footshock-induced c-Fos induction in the prefrontal cortex needs to be characterized in drug-naïve and drug-experienced animals inside and outside the drug self-administration context.

Although, the exact effect of stress on mesolimbic dopaminergic neurotransmission is still up for debate, recent evidence suggests that VTA CRF signaling may decrease NAc dopamine (Wanat et al., 2013). This decreased mesolimbic response is likely signaling dysphoria, while increased dopamine in the prefrontal cortex and its regulation of glutamatergic projections to the nucleus accumbens core may be more important for drug craving and relapse (Figure 41). Decreased mesolimbic dopamine in parallel with increased mesocortical dopamine may regulate different aspects of the stress response, differentially contributing to relapse. Increased mesocortical dopamine originates from the VTA and terminates in both the prelimbic and infralimbic cortices in the rat (Berger et al., 1976, Van Eden et al., 1987). Mesocortical dopamine may also regulate the mesolimbic dopamine stress response. Depletion of dopaminergic signaling in the prefrontal cortex has been reported to both increase and decrease the mesolimbic dopamine response to stress (Deutch et al., 1990, Feenstra et al., 1992, King et al., 1997, Harden et al., 1998, Moghaddam, 2002).

Stress may be selectively activating mesocortical and inhibiting mesolimbic VTA dopamine projection neurons but how does CRF-R1 activation contribute? It has been reported that deletion of CRF-R1 in VTA dopaminergic neurons reduces stress-induced dopamine release selectively in the prefrontal cortex (Refojo et al., 2011). This suggests that in the VTA CRF-R1 may be selectively express in dopamine neurons that project to the prefrontal cortex. Therefore, CRF-R1-dependent stress-induced reinstatement of extinguished long-access cocaine-seeking behavior may be the result of increasing dopamine release in the medial prefrontal cortex via CRF-R1 activation in the VTA.

Figure 42: CRF-R1 antagonist effects upon footshock stress on mesocortical versus mesolimbic dopamine neurotransmission. CRF-R1 antagonism is hypothesized to block mesocortical increases in dopamine by stress, and increase mesolimbic dopamine levels.

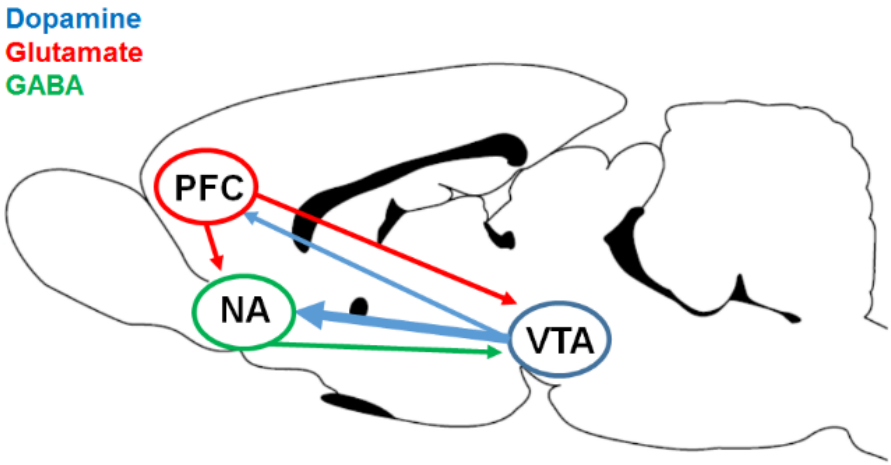
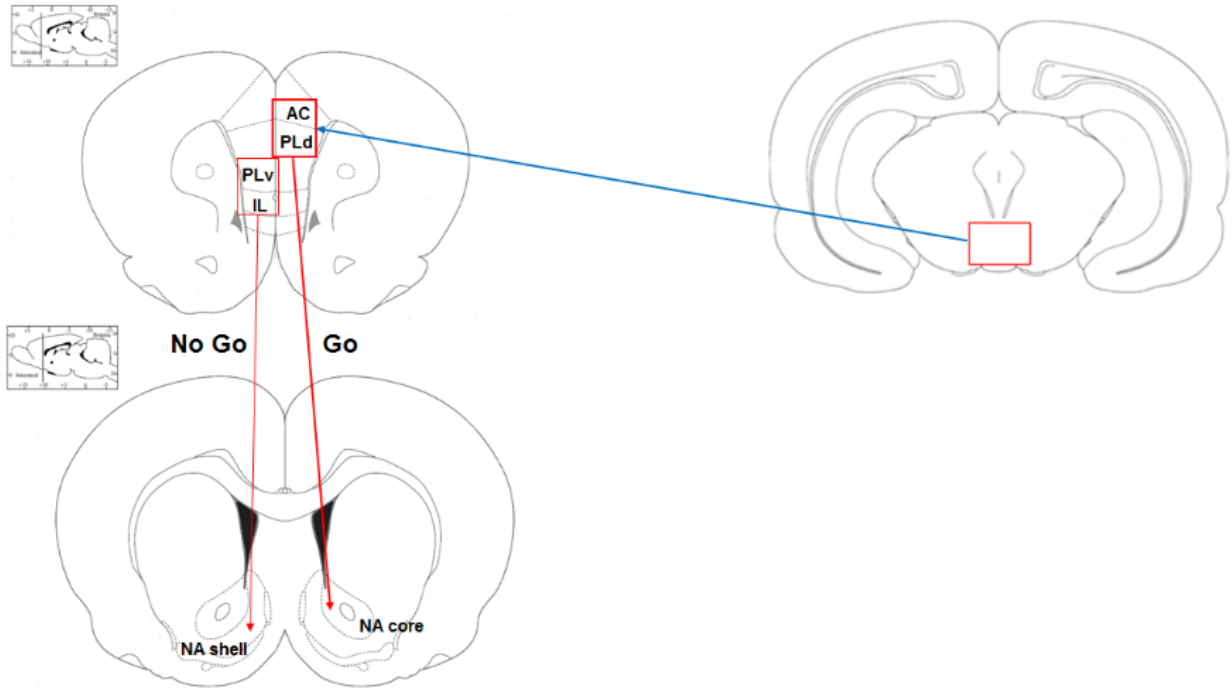


Figure 43: Proposed effects of CRF-R1 antagonism on mesocortical input. CRF-R1 antagonism is hypothesized to decrease stress-induced increases in mesocortical prelimbic dopamine concentrations, blocking relapse by inhibiting glutamate input into the nucleus accumbens core "Go" circuit.



In summary, there are increases and decreases in VTA dopamine neuron activation in ShA and LgA animals in the self-administration context in the absence of stress relative to sline control rats, respectively. Importantly, saline controls did not exhibit significant changes in VTA dopamine neuron activity in response to footshock stress. The data from chapters 2 and 3 suggest that footshock-induced reinstatement in either ShA or LgA animals is positively correlated with increased activation of VTA dopamine cells that may target the medial prefrontal cortex through a CRF-R1 dependent mechanism. The apparent differential regulation of dopamine neuronal activity by different cocaine self-administration histories (ShA versus LgA) upon exposure to the drug-taking

context is intriguing. This suggests the possibility that dopamine neuron activation in LgA animals is fundamentally different than in ShA animals. Previous studies have implicated the importance of context in cocaine experienced animals on both VTA dopamine neuron activation and footshock-induced reinstatement (Shalev et al., 2000, Kufahl et al., 2009). Future studies should characterize dopamine neuron activation by footshock stress outside of the drug self-administration context in both short-access and long-access cocaine experienced animals that have undergone extinction training.

REWARD AND DOPAMINE SIGNALING

Rewarding stimuli, including drugs of abuse, increase VTA dopamine cell firing, thereby increasing downstream dopamine release in the nucleus accumbens (Wise and Rompre, 1989, Wise, 1996, Moisan and Rompre, 1998, Hernandez and Shizgal, 2009). Increase dopamine in the nucleus accumbens is strongly associated with both reward perception and motivated behavior (Mogenson et al., 1980) such as drug seeking. Increased dopaminergic activity within the prefrontal cortex, especially for cocaine, is also associated with motivation (Gariano and Groves, 1988, Chang et al., 1998, Tzschentke, 2000), suggesting that burst firing of VTA dopamine neurons target both the nucleus accumbens and the prefrontal cortex (Gariano and Groves, 1988). The rewarding value of a stimulus is signaled by a shift from tonic to phasic burst firing of VTA dopamine neurons (Gonon, 1988, Schultz, 2007a). Burst firing activity of VTA dopamine neurons optimizes terminal field release of dopamine concentrations

that are significantly greater than those evoked by spikes within a non-bursting mode (Gonon, 1988, Nissbrandt et al., 1994).

VTA DOPAMINE NEURON BURST FIRING: REGULATION BY GLUTAMATE AND GABA

Ventral tegmental area dopamine neuron burst firing activity is regulated in an opposing manner by excitatory glutamatergic and inhibitory GABAergic inputs. Glutamatergic inputs increase (West et al., 2003) while inhibitory GABAergic inputs decrease (Di Chiara et al., 1979, Stanford and Lacey, 1996, Steffensen et al., 1998) VTA dopamine neuron burst firing activity. Glutamate-induced increases in burst firing are dependent on NMDAR and AMPAR activation while GABA-induced decreases in burst firing are dependent on $GABA_BR$ and $GABA_AR$ activation (Johnson et al., 1992, Klitenick et al., 1992, Overton and Clark, 1992, Chergui et al., 1993, Ikemoto et al., 1997a, Overton and Clark, 1997, Paladini et al., 1999b, Laviolette and van der Kooy, 2001, Erhardt et al., 2002, Georges and Aston-Jones, 2002, Zweifel et al., 2008, Deister et al., 2009, Tan et al., 2012).

In the VTA, glutamatergic nerve terminals synapse upon both dopamine and GABA neurons (Carr and Sesack, 2000b, Omelchenko et al., 2009, Dobi et al., 2010). NMDA and AMPA receptors are located on both VTA dopamine and GABA neurons, with NMDA and AMPA receptor agonists increasing dopamine and GABA neuron activation (Kalivas et al., 1989, Seutin et al., 1990, Mereu et al., 1991, Suaud-Chagny et al., 1992, Chergui et al., 1993, Wang and French, 1993b, a, Wang et al., 1994, Wang and French, 1995, Tong et al., 1996, White,

1996, Gronier and Rasmussen, 1998, Giorgetti et al., 2001, Schultz, 2007a). GABA interneuron activation in the VTA can in inhibit dopamine neurons (Grace et al., 2007a). In this way, glutamate regulates the output of VTA DA neurons through the ratio of direct excitatory and indirect inhibitory activity mediated by glutamate receptor activation on dopamine and GABA neurons, respectively.

THE VENTRAL TEGMENTAL AREA, GLUTAMATE, REWARD, AND DRUG SEEKING

A significant role for midbrain glutamate transmission in cocaine addiction-related process has been reported ranging from drug-induced neuroplasticity in the VTA, to reward, to the regulation of drug-seeking behavior in response to stress, cue, and drug (Boyson et al., Kalivas and Duffy, 1998, Ungless et al., 2001, Vorel et al., 2001, Carlezon and Nestler, 2002, Tzschentke and Schmidt, 2003, Dunn et al., 2005, Sun, 2005, Wang et al., 2005, Wang et al., 2007, Chen et al., 2008, Covington et al., 2008, Yap and Miczek, 2008, Wise, 2009, Nolan et al., 2010, Lane et al., 2011).

Glutamate stimulates intra-VTA DA cell firing acting through several ionotropic receptors including NMDA- and AMPA-receptors (Gonon, 1988, Seutin et al., 1990, Wang and French, 1993b, a, Ungless et al., 2001, Sziraki et al., 2002, Harris et al., 2004, Zweifel et al., 2009, Jalabert et al., 2011, Lammel et al., 2012). For example, these receptors meiate synaptic plasticity in the VTA in the form of LTP and LTD. The AMPA receptor regulates fast and short depolarization, while the NMDA receptor regulates longer lasting depolarization (Mereu et al., 1991, Johnson and North, 1992b).

Activation of both mesolimbic and mesocortical dopamine pathways are dependent on ionotropic glutamate receptor activation. *In vivo* glutamate and glutamate receptor agonist application into the VTA increases dopamine signaling in the nucleus accumbens and the medial prefrontal cortex (Kalivas et al., 1989, Suaud-Chagny et al., 1992, Chergui et al., 1993, Wang et al., 1994, Jedema and Moghddam, 1996, Karreman et al., 1996, Westerink et al., 1996, Schilstrom, 1998, Giorgetti et al., 2001, Tye et al., 2013) with increased dopamine in the NAc and mPFC being blocked by NMDA and AMPA receptor antagonism in the VTA (Westerink et al., 1996, Mathe et al., 1998, Schilstrom et al., 1998, Westerink et al., 1998, Giorgetti et al., 2001, Lammel et al., 2012).

Paradoxically, both activation and inhibition of VTA NMDA receptors can increase nucleus accumbens dopamine release (French et al., 1993, Karreman et al., 1996, Mathe et al., 1998, Kretschmer, 1999). Moreover, rats can be trained to self-administer the NMDA antagonist AP-5 into the VTA suggesting there are potential positive motivational properties associated with blocking the NMDA receptor in the VTA (Webb et al., 2012). Intra-VTA NMDAR antagonism has also been shown to increase reward signal via intracranial stimulation (Bergeron and Rompre, 2013, Ducrot et al., 2013). Therefore, NMDA receptor blockade in the VTA has been shown to be reinforcing by multiple groups.

Less is known about VTA AMPAR activation and reward. Intra-VTA AMPA receptor specific antagonism (NBQX) (Sheardown et al., 1990) can dose dependently reduce reward elicited by intracranial stimulation (Miliaressis et al., 1986, Ducrot et al., 2013). However, intra-VTA AMPA receptor antagonism has

been reported to increase operant responding for conditioned reinforcement, and induce conditioned place preference both of which reflect a positive motivational property (Harris and Aston-Jones, 2003a, Harris et al., 2004, Nolan et al., 2010). Increased reinforcement measures and operant responding by intra-VTA AMPA receptor blockade can occur (Ducrot et al., 2013).

NMDA, AMPA, AND COCAINE SEEKING

Glutamate release into the VTA appears to play a central role in cocaine seeking. In support, increases in extracellular glutamate concentrations in the VTA precedes and coincides with active lever pressing in rats responding during extinction (You et al., 2007), and intra-VTA administration of the glutamate receptor agonist NMDA can reinstate cocaine-seeking behavior (Vorel et al., 2001). Moreover, cocaine seeking in response to drug exposure can be attenuated by intra-VTA treatment with both an NMDA and AMPA receptor antagonist (You et al., 2007, Schmidt et al., 2009). Specifically, cocaine seeking can be significantly decreased by intra-VTA administration with AP5 (NMDA antagonist), CNQX (AMPA antagonist), both AP5 and CNQX simultaneously, and kynurenic acid a nonspecific ionotropic glutamate receptor antagonist (Vorel et al., 2001, Wang et al., 2005, You et al., 2007, Schmidt et al., 2009). This suggests that, under some circumstances, cocaine seeking may be dependent on stimulation of both NMDA and AMPA receptors in the VTA.

The hypothesis that intra-VTA AMPAR antagonism would decrease cocaine seeking has been previously tested by other groups. Our results that NMDA and AMPA receptor blockade failed to block intra-VTA CRF- and

footshock stress-induced of cocaine-seeking behavior failed to support this hypothesis. Moreover, our results suggest that AMPAR antagonism causes general activating effects, and may enhance reinstatement, similar to previously reported effects on the capacity of cocaine conditioned stimuli to function as conditioned reinforcers as reported by others (Nolan et al., 2010). The discrepancy between Nolan et al. (2010) and You et al. (2007) could be due to differences in methodology. You et al. (2007) administered drug to the VTA via reverse dialysis whereas Nolan et al., (2010) administered drug via microinjection. You et al. (2007) used CNQX, while Nolan et al. (2010) used NBQX. NBQX has been demonstrated to be a more effective blocker of AMPA receptors than CNQX (Yu and Miller, 1995), and CNQX, even though considered an AMPA receptor antagonist, has significant blocking action at the glycine site of NMDA receptors (Sheardown et al., 1990, Yu and Miller, 1995, Mead and Stephens, 1999). Lastly, You et al. (2007) had the majority of their cannulae sites at -5.6 mm from bregma, while Nolan et al. (2010) had most cannulae sites between -5.8 to -6.3 mm from bregma consistent with the location of high GABAergic populations (Perrotti et al., 2005, Olson and Nestler, 2007, Kaufling et al., 2009). Interestingly, methodologies and data from chapter 4 of this dissertation are most similar and consistent to Nolan et al. (2010), whose finding that AMPAR antagonism in the VTA enhanced cocaine seeking are similar to our own with stress- and intra-VTA CRF-induced reinstatement. Altogether, the current data suggest that reward is more complex than simple ionotropic glutamate receptor activation of mesolimbic dopamine neurons. This complexity

most certainly also applies to drug seeking induced by aversive events such as footshock stress.

IONOTROPIC GLUTAMATE RECEPTORS AND REINSTATEMENT

Exposure to drugs of abuse, such as cocaine, produces neuroplastic changes involving ionotropic glutamate receptor function in the VTA. This plasticity includes enhanced AMPA and NMDAR signaling (Ungless et al., 2001, Schilstrom et al., 2006). Moreover, CRF itself appears to also enhance ionotropic glutamate receptor signaling in cocaine-experienced animals (Ungless et al., 2003, Wang et al., 2005) suggesting that cocaine use can augment the ability of stress to facilitate ionotropic glutamate receptor function in the VTA.

The mechanism of action of intra-VTA CRF-R1 receptor-dependent reinstatement of extinguished cocaine seeking was hypothesized to involve augmented excitatory drive on VTA dopamine neurons either through enhancement of glutamate release and/or ionotropic glutamate receptor signaling on VTA DA neurons (Sun, 2005, Wang et al., 2005, Hahn et al., 2009). More specifically, it was hypothesized that if the reinstating effects of CRF in the VTA involve an increase in in mesocorticolimbic dopamine system by way of glutamatergic signaling, then blockade of either AMPA or NMDA ionotropic glutamate receptors should prevent reinstatement in response to footshock stress or intra-VTA delivery of CRF. To test this hypothesis, the necessity of intra-VTA NMDAR and AMPAR function in both footshock- and intra-VTA CRF-induced reinstatement of extinguished cocaine-seeking behavior was tested

using the ionotropic glutamate receptor antagonists reported in Chapter 4 of this dissertation.

Surprisingly, neither intra-VTA AMPA nor NMDA receptor blockade alone blocked reinstatement at the doses tested. It remains unclear if intra-VTA AMPAR- or NMDAR- receptor activation is involved in intra-VTA CRF-dependent stress-induced reinstatement of cocaine-seeking behavior. The findings from this series of experiments involving ionotropic glutamate receptors is difficult to interpret. To this end, the exact role of AMPA and NMDA receptors in stress-induced reinstatement of extinguished LgA cocaine-seeking remains unclear. It is possible that inactivation of both AMPA and NMDA receptors is necessary to block reinstatement. Current studies are being conducted to look at the ability of whether an NMDA/AMPA receptor specific antagonist cocktail (3 μg of both AP-5 and NBQX per side) can block reinstatement. Another possible interpretation of the inability of NMDA or AMPA receptor antagonists, by themselves, to block reinstatement may be due to an opposition between dopamine and GABA neurons both expressing NMDA and AMPA receptors. This would produce opposition inhibiting excitation of both dopamine and GABA neurons within the VTA. Alternatively, glutamate may be regulating reinstatement not through AMPA or NMDA receptors but through either kainate or metabotropic glutamate (mGluRs) receptors, both of which are not targeted by either AP-5 or NBQX.

OPPOSITION BETWEEN NEURONAL PHENOTYPES BOTH EXPRESSING NMDA AND AMPA RECEPTORS

Slice electrophysiology studies have demonstrated that intra-VTA CRF receptor activation can excite both dopaminergic and GABAergic neurons in the VTA (Korotkova et al., 2006), both of which express NMDA and AMPA ionotropic glutamate receptors (Kalivas et al., 1989, Seutin et al., 1990, Wang and French, 1995) and receive excitatory inputs (Christie et al., 1985, Sesack and Pickel, 1992, Steffensen et al., 1998, Lammel et al., 2012, Jennings et al., 2013). Therefore, one explanation for the unexpected inability of antagonists to block reinstatement using intra-VTA AMPAR or NMDAR antagonists is that the combined effects on both neuronal phenotypes resulted in opposing effects on behavior.

MESOLIMBIC AND MESOCORTICAL AFFERENT NEURONS BOTH EXPRESS NMDA AND AMPA RECEPTORS

Stress appears to specifically target the prefrontal cortex increasing both dopamine and glutamate concentrations (Thierry et al., 1976, Tassin et al., 1980, Herman et al., 1982, Deutch et al., 1985, Abercrombie et al., 1989, Cenci et al., 1992, Moghaddam, 1993, Moghaddam et al., 1994, Karreman and Moghaddam, 1996, Bagley and Moghaddam, 1997, Lammel et al., 2011, Lammel et al., 2012). Blockade of both NMDA and AMPA receptors in the VTA blocks stress-induced increases in medial prefrontal dopamine signaling (Kalivas et al., 1989, Jedema and Moghaddam, 1994, Takahata and Moghaddam, 2000).

The VTA is a major site for glutamatergic regulation of cortical dopamine increases in response to stress (Kalivas et al., 1989, Enrico et al., 1998, Takahata and Moghaddam, 1998, Wang et al., 2005). If reinstatement by footshock stress and intra-VTA CRF delivery is dependent on increased dopamine in the prefrontal cortex, then blockade of either the NMDA or AMPA receptor should block reinstatement. However, this is not what occurred in the studies described in chapter four. By blocking AMPA or NMDA receptors on all cell phenotypes inactivation of the whole mesocorticolimbic dopamine system (i.e. cell that project to multiple downstream regions) likely occurred. This stands in likely contrast to the selective regulation of the PFC by CRF in the VTA and stress and may prevent activation of circuits that constrain drug seeking as well as pathways that promote it. Paradoxically, reinstatement by both footshock and intra-VTA CRF delivery not only still still occurred following intra-VTA antagonist delivery, but in some cases it was augmented. Not only does this suggest that AMPA and NMDA receptor activation is not necessary for reinstatement but it may suggest that reinstatement involves selective inactivation of isolated components of the mesocorticolimbic system.

Elevations in both CRF and glutamate in the VTA by footshock stress have been found to be TTX-sensitive (Wang et al., 2005) suggesting that they are the consequence of afferent nerve terminal release. Activation of postsynaptic ionotropic glutamate receptors has been found to produce increases in somatodendritic dopamine release, an effect that is likely dependent on presynaptic CRF receptor activation, as it is blocked by CRF antagonist

administration into the VTA (Wang et al., 2005). These findings indicate that footshock-induced reinstatement is dependent on both CRF and ionotropic glutamate receptor activation in the VTA (Wang et al., 2005). CRF receptor blocked footshock-induced increases in glutamate but not dopamine suggesting that CRF receptor activation is increasing presynaptic glutamate release (Wang et al., 2005, Wang et al., 2007). However, CRF excites postsynaptic dopamine neurons (Ungless et al., 2003, Wanat et al., 2008, Hahn et al., 2009) suggesting that postsynaptic of dopamine neurons express CRF receptors. To this end, CRF antagonists would be expected to block stress-induced dopamine release in the terminal fields of the VTA. Importantly, stress-induced reinstatement and concomitant increases in dopamine but not glutamate concentrations were blocked by intra-VTA application of kynurenic acid, a nonspecific ionotropic glutamate receptor antagonist (Wang et al., 2005). Altogether, this seems to suggest that footshock-induced reinstatement of cocaine seeking is dependent on postsynaptic ionotropic glutamate receptor activation of VTA dopamine neurons as a result of CRF-dependent increases in VTA glutamate release.

KYNURENIC ACID BEHAVIORAL PHARMACOLOGY

Although a role for glutamatergic excitation of VTA dopamine neurons has been proposed, we report that neither NMDA (AP-5) nor AMPA (NBQX) receptor antagonists, across a wide range of doses, prevents stress- or intra-VTA CRF-induced reinstatement. Previously, Wang et al., (2005) showed that intra-VTA administration of the nonspecific ionotroptic glutamate receptors antagonist,

kynurenic acid (KA), was sufficient to block both stress- and intra-VTA CRF-induced reinstatement, along with concomitant increases in somatodendritic dopamine concentrations in the VTA (Wang et al., 2005).

Preliminary findings from chapter four suggest that KA (24 µg/.25µl per side) significantly attenuates both footshock- and intra-VTA CRF-induced reinstatement of extinguished cocaine-seeking behavior. These findings replicate those reported by Wang et al., (2005). The ability of KA but not AP-5 or NBQX to prevent reinstatement is unexpected but may be attributed to the unique pharmacological properties of KA. Kynurenic acid (KA) is referred to as a broad spectrum antagonist of ionotropic glutamate receptors with a preferential selectivity at the strychnine-insensitive glycine site of the NMDA receptor (IC_{50} =15 µM) (Birch et al., 1988, Danysz et al., 1989, Kessler et al., 1989, Stone, 1993) over the NMDA recognition site (Szalardy et al., 2012). In addition, the compound is an antagonist at the glutamate recognition-site of the NMDA receptor at moderate concentrations (IC_{50} = 200-500 µM) (Kessler et al., 1989). Kynurenic acid also blocks the AMPA receptor at high concentrations (micromolar to millimolar range), but at lower concentrations (nanomolar to micromolar) it facilitates AMPA receptor signaling (Stone, 1993, Prescott et al., 2006, Rozsa et al., 2008). In addition to blocking the NMDA and AMPA receptor it also blocks the ionotropic kainate receptor at moderate doses (IC_{50} = 200 to 600 µM) (Alt et al., 2004). Moreover, kynurenic acid at low concentrations, (micromolar or submicromolar) is also a competitive antagonist at α7 nicotinic receptors (IC_{50} ~ 7 µM) (Hilmas et al., 2001, Stone, 2007) and therefore may also

prevent excitatory regulation of VTA DA cells via ACh. Stimulation of the α7 nicotinic receptor in the VTA increases the firing of VTA dopamine neurons (Schilstrom et al., 2003). Lastly, in addition to inhibiting the α7 nicotinic receptor kynurenic acid also inhibits $GABA_A$ receptors at very high concentrations with an IC_{50} of 3 mM (Bruijnzeel et al., 2009).

Prior reports that reverse dialysis of kynurenic acid blocks stress-induced reinstatement of cocaine seeking and somatodendritic dopamine release (Wang et al., 2005) used at a concentration of 1 mM at which kynurenic acid is likely blocking all three ionotropic glutamate receptors (NMDA, AMPA, and Kainate). In chapter four we report (24 µg/side; 454 mM) bilateral microinjections of KA blocks stress-induced reinstatement. Although this dose is extremely high, it is only roughly twice the concentration previously reported to block reinstatement of cocaine-seeking induced by ventral subiculum stimulation (Vorel et al., 2001). Microinfusions inherently have higher concentrations than those used for microdialysis due to the fact that they are an acute bolus injection. Nonetheless, smaller doses are currently being tested for their ability to block reinstatement.

Notably, the 24 µg/side intra-VTA KA dose that blocked reinstatement also produced substantial nonspecific motor impairments in drug naïve animals trained to lever press unde a schedule of food pellet reinforcement (data not shown). Side effects consisted of head weaving, turning behavior, and ataxia. These effects were observed all the way down to an 11 mM concentration (0.6 µg/side). Our finding that kynurenic acid dose dependently reduces food

reinforced responding is consistent with previous studies that injected kynurenic acid (3.2 and 5.6 µg/0.5 µl; 34 and 59 mM) into the VTA (Sun, 2005).

In contrast, the 24 µg/0.25µl per side dose of kynurenic acid did not affect the ability of cocaine SA experienced animals to lever press during reinstatement conditions (i.e. responding did not drop below extinction levels). This suggests that perhaps drug experience can change how an animal responds to intra-VTA KA delivery through changes in ionotropic glutamate receptor signaling. Alternatively, KA could decrease sucrose-seeking in a similar way to cocaine-seeking independent of motor impairments.

Although we tested for the effects of intra-VTA AP-5 and NBQX delivery individually on reinstatement, we never tested for the effects of combinated VTA AMPAR and NMDAR blockate. Activation of both AMPA and NMDA receptors may be necessary for reinstatement. Due to the nonspecific pharmacological effects of KA along with the inability of AP-5 and NBQX to individually block reinstatement current studies are underway to characterize the role of a AP-5/NBQX (3 µg of each drug/side) cocktail to examine the potential contribution of a coordinated action of these receptors in the VTA to stress-induced cocaine seeking. However, unreported data from a pilot study suggests that a very low dose of 0.5 µg per side of both AP-5 and NBQX (cocktail) fails to block reinstatement by both footshock and intra-VTA CRF delivery.

Altogether, the inability of NMDA or AMPA receptor antagonists to block both footshock- and intra-VTA CRF-induced reinstatement is not supportive of excitatory drive on VTA DA neurons through AMPA or NMDA glutamate

receptors alone as a mechanism for intra-VTA CRF-dependent reinstatement. However, it also does not rule this possiblility out. It is clear that additional experiement are needed.

ALTERNATIVE EXCITATORY MECHANISMS OF INTRA-VTA CRF

Kynurenic acid is commonly referred to as a nonspecific excitatory ionotropic receptor antagonist (Birch et al., 1988). Kynurenic acid is an antagonist for kainate ionotropic glutamate receptors (Alt et al., 2004) and alpha 7 nicotinic receptors (Hilmas et al., 2001, Stone, 2007) in addition to the AMPA and NMDA ionotropic glutamate receptors. For this reason and the finidng that kynurenic acid appears to block stress-induced reinstatement while antagonist's specific for NMDA (AP-5) and AMPA (NBQX) receptors do not potentially reveals another mechanism through which CRF and stress regulate VTA function. The possibility that kynurenic acid may be blocking reinstatement through excitatory mechanisms mediated by either kainate ionotropic glutamate receptors and/or alpha 7 nicotinic acetylcholine receptors should be considered and represent another area for further investigation.

KAINATE RECEPTOR REGULATION OF VTA DA NEURON EXCITABILITY

Ionotropic glutamate receptors are named after their agonists NMDA, AMPA, and kainate (Watkins and Evans, 1981, Monaghan et al., 1989, Young and Fagg, 1990). NMDA receptors mediate slow excitatory responses while AMPA and kainate receptors mediate fast excitatory responses (Collingridge and Lester, 1989, Cossart et al., 1998). Although, kainate ionotropic glutamate

receptors are widely expressed throughout the brain (Bettler et al., 1990, Egebjerg et al., 1991, Werner et al., 1991, Herb et al., 1992, Wisden and Seeburg, 1993, Bahn et al., 1994, Feldmeyer and Cull-Candy, 1994) and known to regulate excitatory neurotransmission (Chittajallu et al., 1996, Castillo et al., 1997), they are substantially understudied, and their role in brain neurophysiology is largely unknown (Feldmeyer and Cull-Candy, 1994). Kainate receptors are present in the VTA and known to regulate intra-VTA dopamine neuron activity as well as dopamine release (Mayer et al., 1984, Kalivas and Stewart, 1991, Wang and French, 1993a, White, 1996, Westerink et al., 1998, Barrera et al., 2005, Ye et al., 2005). This suggests that the ability of kynurenic acid to block reinstatement may be regulated by kainate ionotropic glutamate receptor activation.

ACH RECEPTOR REGULATION OF VTA DA NEURON EXCITABILITY

In the brain, alpha 7 nicotinic acetylcholine receptors (α7-nAChRs) can excite neurons at both pre- and postsynaptic sites by increasing calcium permeability (Berg and Conroy, 2002). α7-nAChRs are present in the VTA at somatodendritic sites on dopamine and GABA neurons as well as on presynaptic glutamate terminals (Jones and Wonnacott, 2004). In support, VTA α7-nAChRs can facilitate LTP by enhancing release from presynaptic glutamatergic terminals (Mansvelder and McGehee, 2000). α7-nAChRs not only regulate VTA dopamine neuron excitation (Calabresi et al., 1989, Pidoplichko et al., 1997, Mansvelder and McGehee, 2000, Schilstrom et al., 2000, Mansvelder et al., 2002) but also

drug self-administration (Corrigall and Coen, 1994). Therefore, the ability of kynurenic acid to block reinstatement may also be regulated mediated by α7-nAChRs.

MGLUR RECEPTOR REGULATION OF VTA DA NEURON EXCITABILITY

Alternatively, glutamate may still be an integral part of intra-VTA CRF-dependent reinstatement of cocaine seeking but may be regulating dopamine cells through metabotropic instead of ionotropic receptors. Group 1 metabotropic glutamate receptors (mGluR1 and mGluR5) (Conn and Pin, 1997) are expressed on dopamine neurons in the VTA (Kane et al., 2005). Group 1 metabotropic glutamate receptor activation in the VTA can facilitate and inhibit burst firing of VTA dopamine neurons (Fiorillo and Williams, 1998, Zheng and Johnson, 2002), producing both excitatory and inhibitory effects on VTA dopamine neurons by way of group 1 mGluR stimulation. Whether or not group 1 mGluR activation is excitatory or inhibitory depends on both pattern and frequency of afferent input (Fiorillo and Williams, 1998).

MGLUR RECEPTOR INVOLVEMENT IN DRUG-INDUCED NEUROPLASTICITY

Group 1 mGluRs have been shown to regulate cocaine-induced plasticity involving potentiation of excitatory input onto VTA dopamine neurons (Bellone and Luscher, 2006). Specifically, activation of group 1 mGluR receptors reverses cocaine-induced insertion of calcium permeable AMPA receptors into the membrane of VTA dopamine neurons; a process termed mGluR-LTD (Bellone

and Luscher, 2005, 2006, Mameli et al., 2007, Luscher and Huber, 2010). In addition to regulating cocaine-induced AMPA neuroplasticity, group 1 mGluR currents can be enhanced by CRF release into the VTA (Riegel and Williams, 2008). However, mGluR expression levels in the VTA have been reported to be unchanged following extended access to cocaine for SA (Ben-Shahar et al., 2009, Ghasemzadeh et al., 2011).

GABA RECEPTORS AND VTA DOPAMINE SIGNALING

Burst firing patterns of intra-VTA dopamine neurons optimize and potentiate release of dopamine at both terminal and somatodendritic sites (Gonon, 1988, Wightman and Zimmerman, 1990, Nissbrandt et al., 1994). In this way, somatodendritic dopamine elevation in the VTA is indicative of burst firing of midbrain dopamine neurons (Bjorklund and Lindvall, 1975, Geffen et al., 1976, Kalivas and Duffy, 1991, Rice et al., 1997, Jaffe et al., 1998, Adell and Artigas, 2004). This burst firing is essential for reward perception, reward seeking, reward expectancy, and salience (Nishino et al., 1987, Schultz et al., 1997, Berridge and Robinson, 1998, Tsai et al., 2009, Zweifel et al., 2009).

As previously mentioned, VTA DA neuron burst firing is primarily by both excitatory glutamatergic NMDA receptor activation and inhibitory $GABA_B$-receptor activation. NMDA receptor activation stimulates burst firing while $GABA_B$ receptor activation inhibits burst firing (Overton and Clark, 1992, Overton and Clark, 1997, Erhardt et al., 2002). Therefore, the burst firing of midbrain dopamine neurons is tightly controlled by the coordinated actions of NMDA and $GABA_B$ receptors

(Erhardt and Engberg, 2002). Since, in our hands, NMDA receptor blockade, which would be expected to inhibit VTA burst firing, did not affect reinstatement, the next candidates that may contribute to the control of dopamine neuron burst firing are GABA receptors. In addition to $GABA_B$ receptors, VTA dopamine neuron activity can be inhibited upon activation of $GABA_A$ receptors on dopamine neurons (Tan et al., 2012, Graziane et al., 2013) and excited by $GABA_A$ receptors on GABAergic interneurons that provide tonic inhibition of dopamine cells and, thereby also regulate phasic firing (Kalivas et al., 1990, Johnson and North, 1992a, Xi and Stein, 1998).

INTRA-VTA GABA MECHANISMS AND REINSTATEMENT OF DRUG SEEKING

VTA synaptic plasticity implicated in stress-induced reinstatement of cocaine seeking includes both long term potentiation (LTP) and long term depression (LTD) of GABAergic synapses onto VTA DA neurons that emerges following repeated drug exposure (Nugent et al., 2007, Pan et al., 2008, Dacher and Nugent, 2011, Dacher et al., 2013). Recent findings suggest that LTP and LTD at GABAergic synapses upon VTA DA neurons display Hebbian characteristics effecting postsynaptic dopamine neurons in the VTA (Nugent et al., 2007, Nugent et al., 2009, Dacher et al., 2013, Graziane et al., 2013, Kodangattil et al., 2013).

GABAERGIC DRUG-INDUCED NEUROPLASTICITY

Acute exposure to nicotine, ethanol, cocaine, or stress has been shown to block LTP at GABAergic synapses on VTA dopamine neurons, thereby removing

an inhibitory brake on the dopaminergic system (Morita et al., Nugent et al., 2007, Nugent et al., 2009, Niehaus et al., 2010). However, this mechanism is dependent on postsynaptic NMDA receptor activation which produces nitric oxide which undergoes retrograde diffusion, and initiates presynaptic GABA release, thereby activating $GABA_A$ receptors on postsynaptic VTA dopamine neurons (Nugent et al., 2007, Nugent et al., 2009). Stress appears to remove this inhibitory brake on dopamine neurons by releasing dynorphin and activating the kappa opioid receptor in the VTA to block LTP GABA and facilitate relapse (Graziane et al., 2013). Blockade of the kappa receptor with Nor-BNI can block stress-induced reinstatement and rescue LTP GABA (Graziane et al., 2013). This mechanism is acute and appears to synergize with increased glutamatergic drive of the circuit. However, it is unlikely that this is the mechanism we are observing with $GABA_B$ receptor blockade. Chapter four reports that antagonism of both NMDA and $GABA_A$ receptors fail to inhibit or augment both footshock stress- and intra-VTA CRF-induced reinstatement in LgA animals. Moreover, blockade of $GABA_B$ receptor signaling is most likely removing an inhibitory break instead of rescuing one to block relapse.

CONVERGENCE OF CRF-R1 AND $GABA_B$ IN DRUG-INDUCED NEUROPLASTICITY

CRF-R1 activation can facilitate both excitatory NMDA and AMPA receptor signaling as well as inhibitory $GABA_B$ signaling (Beckstead et al., 2009, Hahn et al., 2009) in VTA dopamine neurons. Following repeated cocaine exposure, the excitatory effects of CRF are augmented (Hahn et al., 2009) and

the inhibitory effects of CRF are diminished (Beckstead et al., 2009), likely resulting in a net shift toward excitatory CRF-R1 receptor-mediated regulation of VTA dopamine neurons. Therefore, it was originally hypothesized that, if the reinstating effects of CRF involves decreased inhibitory drive through GABA receptor signaling, then blockade of GABA receptors should augment cocaine seeking in response to both footshock and intra-VTA CRF administration. In contrast to this hypothesis, $GABA_B$ receptor antagonism blocked reinstatement in response to both footshock and intra-VTA CRF delivery. The only antagonist other than CRF-R1 antagonists (Antalarmin and CP-376395) to reliably block reinstatement without producing secondary locomotor effects was the $GABA_B$ receptor antagonist, 2-hydroxysaclofen. This suggests that $GABA_B$ receptor activation is regulating the neurocircuitry of the VTA in such a way to facilitate stress-induced reinstatement of cocaine-seeking. It also suggests that reinstatement involves an increase and not a decrease in GABAergic signaling in the VTA and supports reports in the literature demonstrating inhibition of VTA dopamine neurons by aversive stimuli (Schultz and Romo, 1987, Mantz et al., 1989, Mirenowicz and Schultz, 1996, Guarraci and Kapp, 1999, Ungless et al., 2010).

$GABA_B$ AS A PHARMACOLOGICAL TARGET TO TREAT ADDICTION

Paradoxically, $GABA_B$ receptor agonists and other compounds that activate inhibitory G protein-coupled inwardly-rectifying potassium (GIRK) channels have been proposed as therapeutic candidates to help treat alcoholism and drug addiction (Brebner et al., 2002, Kobayashi et al., 2004, Walker and

Koob, 2007). For example, $GABA_B$ receptor agonists decrease the reinforcing effects of cocaine (Roberts et al., 1996, Roberts and Koob, 1997, Brebner et al., 1999), self-administration of cocaine as measured using self-administration, (Brebner et al., 1999, Brebner et al., 2000, Backes and Hemby, 2008), and increases in terminal field dopamine levels (Kalivas et al., 1990, Klitenick et al., 1992, Westerink et al., 1996). The finding that $GABA_B$ antagonism blocks both footshock- and intra-VTA CRF is consistent with the idea that $GABA_B$ antagonists rather than $GABA_B$ agonists could serve as potential medications. It should be noted that we tested for the ability of the $GABA_B$ receptor agonist (baclofen; 2 µg/side) injected into the VTA to cause relapse in the absence of stress or CRF. The result was substantial motor impairment, consistent with global neuronal inhibition, both pre- and postsynaptically as predicted (personal communication with John T. Williams). However, conclusions cannot be deduced from one subject and lower doses of baclofen should be tested due to its potent effects before drawing firm conclusions regarding the effects of $GABA_B$ receptor activation in the VTA.

POSSIBLE MECHANISMS OF VTA $GABA_B$ ANTAGONISM

In a set of elegant studies, Westerink et al., (1996, 1998) characterized intra-VTA pharmacological manipulations on downstream mesolimbic and mesocortical dopamine concentrations using dual-probe microdialysis in drug naïve animals. Examination of effects on the mesocortical system revealed that intra-VTA administration of a $GABA_B$ agonist decreased dopamine concentrations in the PFC, while a $GABA_B$ antagonist increased dopamine

concentrations in the PFC (Westerink et al., 1998). Likewise, examination of effects on the mesolimbic system revealed that intra-VTA administration of a $GABA_B$ agonist decreased nucleus accumbens dopamine concentrations (Westerink et al., 1996), while a $GABA_B$ antagonist did not significantly change dopamine concentrations in the nucleus accumbens (Westerink et al., 1996). This suggests that intra-VTA $GABA_B$ receptor antagonism preferentially increases dopamine in the prefrontal cortex in drug naïve rats (Westerink et al., 1996, Westerink et al., 1998). This conclusion should be met with some caution because the function of intra-VTA $GABA_B$ receptor blockade in drug naïve animals may or may not be similar to SA-experienced animals who've undergone extinction, and are exposed to stress with an environment associated with cocaine availability.

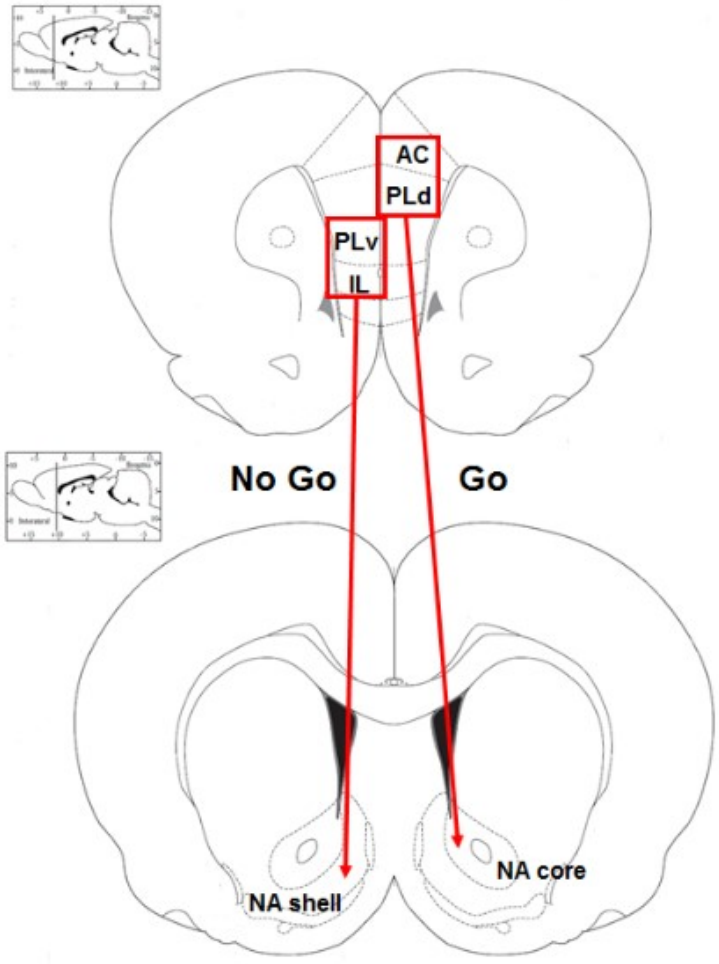
$GABA_B$ ANTAGONISM IN THE VTA SELECTIVELY TARGETS MESOCORTICAL PROJECTING NEURONS

Since $GABA_B$ receptor antagonism was the only manipulation that likely preferentially targeted the mesocortical dopamine neurons (Westerink et al., 1996, Westerink et al., 1998), it should not be surprising that it was the only drug sufficient to block stress-induced reinstatement. Considering that $GABA_B$ receptor antagonism increases (not decreases) dopamine levels in the medial prefrontal cortex (mPFC), how can it be that this manipulation blocks stress-induced reinstatement, which is likely dependent on increased mPFC dopamine release? A possible explanation is related to the anatomical organization of the medial prefrontal cortex.

As previously mentioned in Chapter 1, reinstatement is driven by an increase in prelimbic medial prefrontal cortex glutamate input into the nucleus accumbens core (Cornish et al., 1999, Cornish and Kalivas, 2000, McFarland and Kalivas, 2001, McFarland et al., 2003, McFarland et al., 2004, Kalivas and Volkow, 2005, LaLumiere and Kalivas, 2008). By contrast, glutamate projections from the ventromedial prefrontal cortex to the nucleus accumbens shell are thought to suppress drug-seeking behavior following extinction (Fuchs et al., 2008, Peters et al., 2008, Peters et al., 2009).

Projections from mPFC to the NA are organized into a dorsal-ventral pattern. The dorsal mPFC projects predominantly to the nucleus accumbens core and is a final common pathway for reinstatement of drug seeking, while the ventral mPFC projects to the nucleus accumbens shell and suppresses reinstatement of drug seeking (Cornish et al., 1999, Heidbreder and Groenewegen, 2003, Voorn et al., 2004, Peters et al., 2008, LaLumiere et al., 2010, LaLumiere et al., 2012). Interestingly, the target region for dialysis in Westerink et al., (1998) is consistent with the ventral medial prefrontal cortex. Therefore, increasing dopamine in infralimbic cortex via intra-VTA $GABA_B$ receptor antagonism would be hypothesized increase dopamine in the infralimbic cortex and therefore block reinstatement of drug-seeking by both footshock and intra-VTA CRF delivery.

Figure 44: Corticolimbic subregion specific neurociruitry. Illustration of the “Go” circuit of the glutamatergic projection from the prelimbic cortex to the nucleus accumbens core that is associated with reinstatement, and the “No Go” circuit of the glutamatergic projection from the infralimbic cortex to the nucleus accumbens shell that is associated with extinction/reinstatement prevention.



INTRA-VTA GABA$_B$ ANTAGONISM INCREASES PFC DOPAMINE: POSSIBLE COMPETITIVE ROLE INHIBITING COCAINE-SEEKING

The prelimbic as well as the infralimbic cortices both receive extensive VTA dopaminergic inputs (Berger et al., 1976, Bjorklund et al., 1978, Emson and Koob, 1978, Van Eden et al., 1987, Westerink et al., 1998, Carr et al., 1999).

Dopamine inputs into the prelimbic cortex are essential for stress-induced reinstatement of cocaine-seeking behavior to occur; D1 receptor blockade in the prelimbic cortex blocks footshock-induced reinstatement (Capriles et al., 2003, Sanchez et al., 2003, McFarland et al., 2004). Pathways involved in the inhibition (infralimbic cortex) of drug seeking (Peters et al., 2008, Peters et al., 2009) can inhibit pathways involved in the activation (prelimbic) of drug seeking (McFarland and Kalivas, 2001, McFarland et al., 2003, McFarland et al., 2004) through a feedforward mechanism (Ferrante et al., 2009). To this end, evidence suggests that activation of the infralimbic cortex inhibits prelimbic cortex output (Ji and Neugebauer, 2012). Since $GABA_B$ antagonism increases dopamine in the infralimbic cortex, it may inhibit prelimbic output. Perhaps the ability of 2-hydroxysaclofen to block both footshock and intra-VTA CRF-induced reinstatement of extinguished LgA cocaine-seeking is related to its ability to increase dopamine in the infralimbic cortex which competitively inhibits the glutamatergic input to the nucleus accumbens core from the prelimbic cortex. With this in mind, it is also important to note that activation of both the prelimbic and infralimbic cortices also regulate ventral tegmental area dopamine neuron activity (Patton et al., 2013).

Figure 45: Schematic representing competitive signaling between prelimbic and infralimbic cortices in drug-seeking behavior. Activation of the infralimbic can inhibit pyramidal neuron output in the prelimbic cortex which is essential for drug-seeking behavior. In this way infralimbic cortex activation may inhibit prelimbic cortex output and facilitate extinction while prelimbic cortex activation may inhibit infralimbic cortex output and facilitate reinstatement.

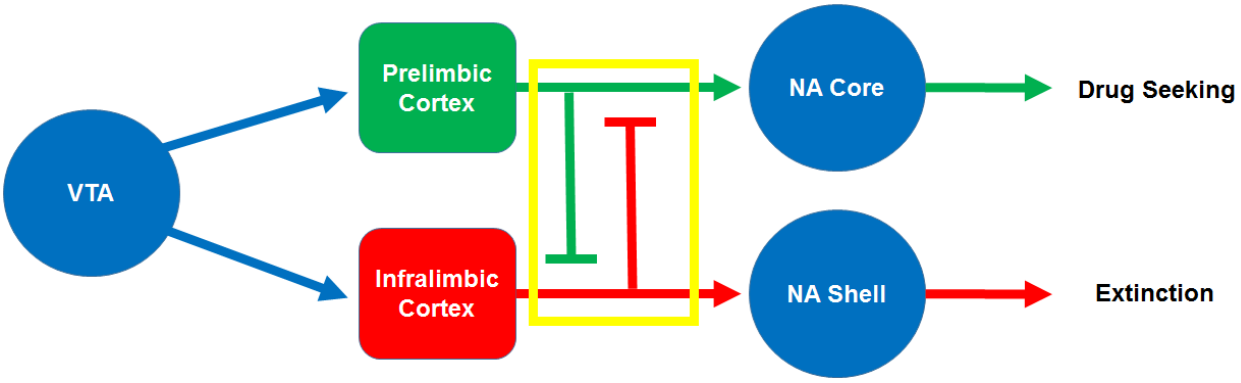
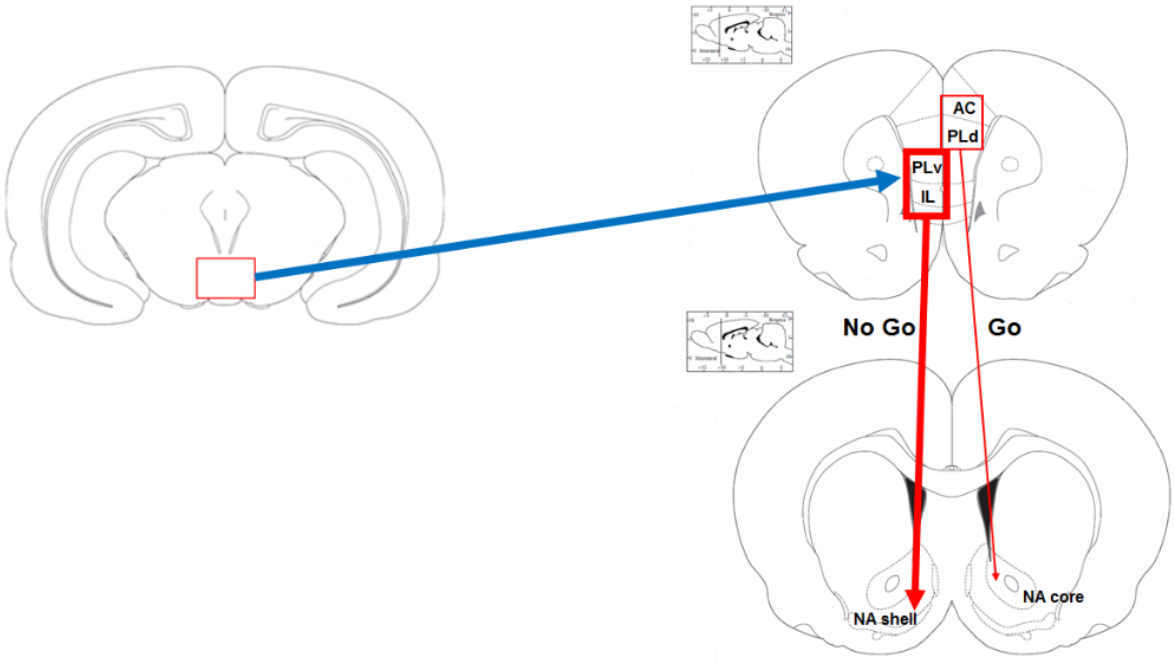


Figure 46: Proposed effects of $GABA_B$ antagonism on mesocortical input. $GABA_B$ antagonism may block reinstatement by increasing mesocortical dopamine input into the infralimbic cortex, increasing glutamate input into the nucleus accumbens shell “No Go” circuit.



$GABA_B$ AND G PROTEIN-COUPLED INWARDLY RECTIFYING POTASSIUM CHANNELS

$GABA_B$ receptors are thought to localize primarily to VTA DA neurons (Xi and Stein, 1998, Margeta-Mitrovic et al., 1999, Wu et al., 1999, Laviolette and

van der Kooy, 2001, Wirtshafter and Sheppard, 2001, Giorgetti et al., 2002, Beckstead et al., 2004, Laviolette et al., 2004, Laviolette and van der Kooy, 2004, Labouebe et al., 2007, Beckstead et al., 2009, Margolis et al., 2012). However, there is emerging evidence that $GABA_B$ receptors can also be expressed on GABA neurons where they can induce inhibitory currents (Cruz et al., 2004, Padgett et al., 2012). In the VTA, $GABA_B$ receptors are also expressed presynaptically on GABAergic and glutamatergic neuronal terminals (Bonci and Williams, 1997, Manzoni and Williams, 1999, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010). Presynaptic $GABA_B$ receptors can decrease presynaptic glutamate and GABA release in the rat VTA by inhibiting Ca^{2+} conductance (Olpe et al., 1977, Pinnock, 1984, Lacey et al., 1988, Seabrook et al., 1990, Bonci and Williams, 1997, Shen and Johnson, 1997, Wu et al., 1999, Giorgetti et al., 2002, Michaeli and Yaka, 2010).

$GABA_B$ RECEPTOR-MEDIATED GIRK CHANNEL CONDUCTANCE IN THE VTA

One prominent inhibitory signaling mechanism that is regulated by $GABA_B$ receptors is G protein-coupled inwardly rectifying potassium (GIRK/Kir3) channels (Johnson and North, 1992b, Beckstead et al., 2004). GIRK channels can be activated through $GABA_B$-receptor activation of $G_{i/o}$ G-proteins which increases membrane conductance, thereby producing inhibitory postsynaptic currents (IPSCs) through potassium ion efflux (Misgeld et al., 1995, Watts et al., 1996) and inhibiting neuronal activity (Dascal, 1997, Beckstead et al., 2004, Cruz et al., 2004, Ford et al., 2006, Labouebe et al., 2007).

$GABA_B$ RECEPTOR-MEDIATED GIRK CHANNEL REGULATION OF VTA DOPAMINE NEURON EXCITABILITY

$GABA_B$ activation of GIRK conductance hyperpolarizes dopamine neurons on a millisecond time scale (Beckstead et al., 2004) which is consistent with its role in channel conductance. Moreover, it contributes to the pause of DA excitation following action potential bursts, thereby strongly inhibiting midbrain dopamine cell firing (Lacey et al., 1987, Pucak and Grace, 1994, Davila et al., 2003, Beckstead et al., 2004, Koyrakh et al., 2005). $GABA_B$ receptor-regulated GIRKs not only play an inhibitory role but also a facilitatory role in phasic burst firing of VTA dopamine neurons. Specifically, $GABA_B$ receptor-regulated GIRKs allow for optimal coding of phasic bursts by inducing the pause before and after the burst (Beckstead et al., 2004, Ford et al., 2009). Therefore, $GABA_B$ receptors may be necessary for optimal phasic bursts at the time of stress-induced relapse. This suggests that $GABA_B$ receptors could be inhibiting phasic firing or paradoxically optimizing phasic firing at the time of stress-induced reinstatement.

DRUG-INDUCED NEUROPLASTICITY AND $GABA_B$ RECEPTOR-MEDIATED GIRK SIGNALING IN THE VTA

Inhibitory Gi/o G protein-coupled receptors (GPCRs), such as the $GABA_B$ receptor, have been implicated in both acute and chronic effects of drug-induced neuroadaptations (Nestler et al., 1990, Filip and Frankowska, 2007, Vlachou and Markou, 2010). Psychostimulant administration has been shown to decrease $GABA_B$ receptor-regulated GIRK channel conductance on both VTA DA and GABA neurons leading to enhanced dopamine signaling or GABA signaling,

respectively (Giorgetti et al., 2002, Beckstead et al., 2009, Arora et al., 2011, Padgett et al., 2012).

$GABA_B$ receptor mRNA expression doesn't appear to change as a result of cocaine exposure (Nestler et al., 1990, Arora et al., 2011). In contrast, decreased GIRK expression in conjunction with decreased $GABA_B$ receptor-mediated inhibition has been reported at somatodendritic sites of VTA dopamine neurons (Arora et al., 2011). Moreover, this inhibition of $GABA_B$ signaling is blocked by intracellular calcium chelation, independently from NMDA and AMPA receptor calcium entry (Malenka and Bear, 2004, Beckstead and Williams, 2007). Therefore, decreased $GABA_B$ GIRK conductance in VTA neurons is a drug-induced neuroadaptation that is independent of ionotropic glutamate receptor calcium entry (Beckstead et al., 2004).

Notably, neuroadaptations decreasing $GABA_B$ regulated inhibition of VTA dopamine neurons have only been observed during acute withdrawal from non-contingent drug administration (Beckstead et al., 2009, Padgett et al., 2012). These studies utilized *ex vivo* slice electrophysiology in post mortem brain slices where neuronal circuitry is not intact and in the presence of numerous drugs to isolate $GABA_B$ receptor function (Beckstead et al., 2009, Padgett et al., 2012). However, psychostimulant-induced changes in $GABA_B$ receptor coupling and neurotransmission have also been reported *in vivo*.

Diminished functional coupling of the $GABA_B$ receptor to $G_{i/o}$ G proteins has been observed during times of inhibited $GABA_B$ signaling *in vivo* following repeated cocaine administration (Striplin and Kalivas, 1992, 1993, Kushner,

2001). Importantly, both $GABA_B$ signaling and G protein-coupling recovers within two weeks of the last exposure to cocaine (Striplin and Kalivas, 1992, 1993, Arora et al., 2011). Our ability to block stress-induced relapse with a $GABA_B$ receptor antagonist within at least 14 days since the last cocaine exposure is more consistent with intact $GABA_B$ regulation of dopamine neuron excitability.

One of the only studies characterizing drug-induced neuroplastic effects of intra-VTA $GABA_B$ signaling *in vivo* was done using intra-VTA microdialysis in animals that had received noncontingent repeated amphetamine administration (Giorgetti et al., 2002). Repeated amphetamine administration was reported to produce an increase, rather than a decrease, in $GABA_B$ inhibitory tone in the VTA (Giorgetti et al., 2002). Specifically, intra-VTA $GABA_B$ receptor antagonism produced a dose-dependent increase in somatodendritic dopamine release without affecting glutamate concentrations in drug-naïve animals (Giorgetti et al., 2002). In contrast, $GABA_B$ antagonism in animals receiving repeated amphetamine displayed an augmented ability to increase somatodendritic dopamine release, and an emergent ability for it to presynaptically increase presynaptic release (Giorgetti et al., 2002). This suggests that somatodendritic dopamine release in the VTA is normally under tonic inhibition by $GABA_B$ receptors and that presynaptic inhibition of glutamate release in the VTA by $GABA_B$ can be recruited by drugs of abuse. These results are opposite of expected based on our findings that $GABA_B$ receptor antagonism blocked intra-VTA CRF-induced reinstatement and reports of the effects of CRF receptor antagonism in the VTA in response to repeated contingent cocaine self-

administration. Wang et al., (2005) reported that CRF receptor blockade prevents a recruited increase in both presynaptic glutamate release and somatodendritic dopamine release, inconsistent with a potential $GABA_B$ receptor-mediated mechanism for our studies.

In summary, the net effect of intra-VTA $GABA_B$ receptor blockade on dopaminergic neurotransmission likely involves increased presynaptic release of both GABA and glutamate, increased somatodendendritic dopamine release, and increased dopamine release in the prefrontal cortex but not the nucleus accumbens (Westerink et al., 1996, Shen and Johnson, 1997, Westerink et al., 1998, Giorgetti et al., 2002, Beckstead et al., 2009). The exact role of $GABA_B$ receptor activation during stress-induced reinstatement still remains undetermined. However, emerging evidence from chapter four suggests that $GABA_B$ receptor function in the VTA can regulate cocaine-seeking, particularly during stress.

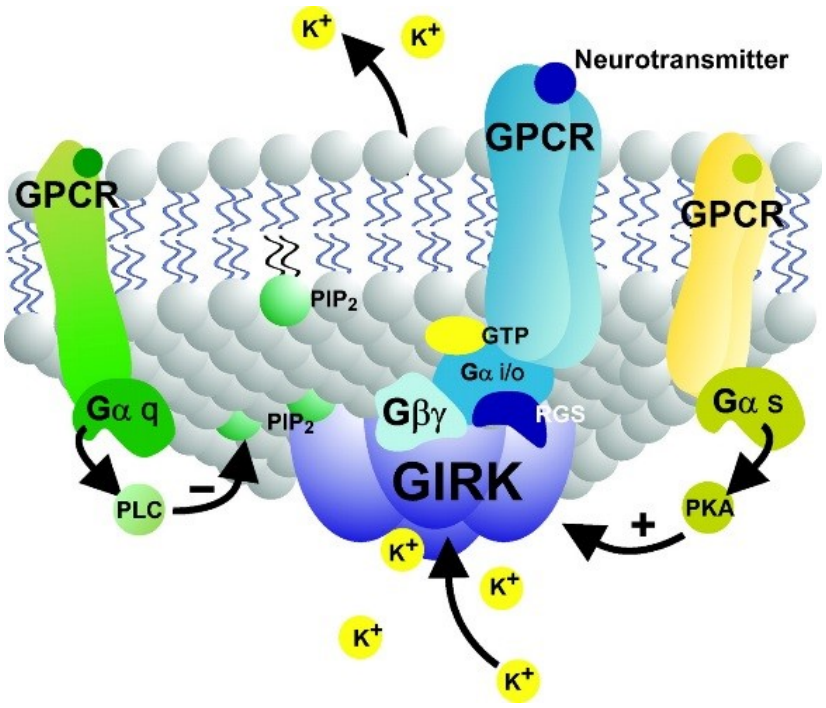
POSSIBLE CRF AND $GABA_B$ INTERACTIONS IN THE VTA

Importantly, coordinated function between CRF-R1 and $GABA_B$ receptors has been proposed in the regulation of VTA dopamine neuron activity. This coordinated function may involve both presynaptic and postsynaptic mechanisms. In terms of a presynaptic mechanism, the $GABA_B$ receptor shares a common sushi domain structure with the N-terminus of the CRF-R1 receptor suggesting these receptors may interact to augment inhibition of presynaptic release (Blein et al., 2004, Perez-Garci et al., 2006, Perrin et al., 2006, Grace et al., 2007b). These sushi repeats on the N-terminus of the $GABA_B$ receptor are

important determinants of the formation of heterocomplexes (Vigot et al., 2006). Although, molecular biologists suggest the possibility, there still remains lack of evidence for a coordinated inhibition of presynaptic release between CRF-R1 and $GABA_B$ receptors.

There is also evidence of a coordinated postsynaptic mechanism implicating both $GABA_B$ and CRF-R1 receptors. Specifically, intra-VTA CRF signaling can act postsynaptically on CRF-R1 receptors to enhance $GABA_B$ regulated GIRK conductance on DA neurons (Beckstead et al., 2009). This enhancement likely occurs through CRF-R1 G_s activation of PKA (Sadja et al., 2001, Raveh et al., 2009, Grammatopoulos, 2012). An increase in GIRK channel activity can be induced by protein kinase A (PKA) following the activation of a GPCR linked to $G_{\alpha s}$ G-proteins (Figure 38). CRF-R1 receptors appear to be necessary for CRF actions on GIRK currents whereas CRF-R2 receptors appear not to be (Beckstead et al., 2009).

Figure 47: The G protein-coupled potassium (GIRK) channel signaling complex viewed from the intracellular side of the membrane. GIRK channels are gated following the activation of GPCRs associated with G proteins $G_{i/o}$ (*pertusses* toxin-sensitive) that release $G_{\beta\gamma}$ dimers to gate the channel directly (blue). A reduction in membrane activity can be induced by activation G_q coupled receptors (green). In contrast, an increase in GIRK channel activity can be induced by activation of $G_{\alpha s}$ dependent protein kinase A (PKA) (yellow), which is the predominant coupling for CRF receptors in the CNS (Grammatopoulos, 2012). Both PLC and PKA can be soluble not having to be directly associated with the GIRK channel complex to affect conductance (Sadja et al., 2001, Raveh et al., 2009).



It is interesting to speculate that reinstatement under LgA conditions involves an increase in CRF-R1 and $GABA_B$ receptor coupling. As intriguing as this possibility is, there is no direct evidence that CRF-R1 and $GABA_B$ receptors form a heterocomplex to regulate motivated behavior. The observation that both $GABA_B$ antagonism (G_i coupled) and CRF-R1 antagonism (G_s coupled) block reinstatement suggests it is not simplay a case of convergence of a specific G-protein signaling cascade. Considering the complexities of CRF-R1 and $GABA_B$ receptor-mediated secondary messaging both Gs and Gi are good candidates to provide augmentation of GIRK conductance on dopamine neurons. Unlike CRF receptors (Liu et al., 2005) $GABA_B$ receptor coupling is unlikely to change within the VTA as a result of drug abuse (Zhang et al., 2007). However, it is more likely that CRF-R1 G_s activation of PKA activity is facilitating GIRK conductance, since

G_s coupling is the predominant coupling for CRF receptors in the CNS (Grammatopoulos, 2012).

In summary, CRF-R1 and $GABA_B$ receptor antagonists block footshock- and intra-VTA CRF-induced reinstatement of cocaine-seeking behavior following LgA SA. The finding that CRF-R1 is necessary and sufficient for reinstatement suggests that intra-VTA CRF-R1 signaling is recruited following LgA cocaine self-administration. This may take the form of increased CRF release, increased CRF receptor responsiveness, increased CRF receptor expression, or even changes in G protein-coupling. The ability for $GABA_B$ antagonism to block footshock- and intra-VTA CRF-induced reinstatement implicates inhibitory GABA regulation of the circuit. This may take the form of increased inhibition of postsynaptic dopamine neurons by GIRK IPSCs, inhibiting presynaptic glutamate or GABA release, or even changes in $GABA_B$/GIRK expression.

As mentioned in chapter one, increasing GABA input or intra-VTA GABA neuron activity produces aversive/anxiogenic behavioral responses, while increasing glutamatergic input or disinhibiting VTA dopamine neurons both produce reward/anxiolytic behavioral responses (Lammel et al., 2012, Jennings et al., 2013). Aversive stimuli target the medial prefrontal cortex while rewarding stimuli targets the nucleus accumbens (Lammel et al., 2011). Following drug-induced neuroplasticity, CRF signaling is likely recruited at both CRF-glutamatergic and CRF-GABAergic synapses (Tagliaferro and Morales, 2008). These inputs are hypothesized to provide complex and highly coordinated regulation of the VTA in response to stress. In this way, CRF acting as a

neuromodulator is likely setting the stage for glutamatergic and GABAergic inputs to manage activity of the mesocorticolimbic system in such a way to facilitate relapse.

Notably, the majority of studies reporting excitatory regulation of VTA dopamine neurons by CRF receptors have involved animals under conditions that most resemble our ShA animals. It is possible that, under ShA SA conditions, CRF blockade may prevent excitatory drive on VTA dopamine neurons to prevent relapse. Alternatively, under LgA SA conditions, GABA signaling may be recruited to combat excessive cocaine-induced excitatory drive of the circuit. This inhibitory tone may be present into the protracted abstinence phase. Prevention of overwhelming drug craving and motivation to use which results in reinstatement of drug seeking may be prevented by blocking aversive drive of the motive circuit, via CRF-R1 and $GABA_B$ receptors, when in the self-administration context and under stress.

CONCLUDING REMARKS

Importantly, both a decrease and an increase in dopamine signaling can precipitate relapse in human addicts (Volkow et al., 2006, Laskowitz et al., 2012). Addiction involves a transition from chasing drug-induced euphoria (positive reinforcement) to eventually using the drug to avoid dysphoria (negative reinforcement) (Solomon and Corbit, 1974, Gawin and Kleber, 1986, Koob et al., 2004, DSM-V, 2013). Future studies are needed to further characterize the intricacies of excitatory and inhibitory regulation of mesocortical versus mesolimbic circuits, how these circuits are changed by drug-induced

neuroplasticity, and how these changes promote relapse. It is my hope that the findings and discussions reported in this dissertation will help with the understanding and eventual long-term management of stressor-induced relapse in abstinent cocaine addicts.

BIBLIOGRAPHY

Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989) Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem 52:1655-1658.

Abercrombie ED, Keller RW, Jr., Zigmond MJ (1988) Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. Neuroscience 27:897-904.

Ackerman TF, Lamonte N, Bodnar RJ (2003) Lack of intersite GABA receptor subtype antagonist effects upon mu opioid receptor agonist-induced feeding elicited from either the ventral tegmental area or nucleus accumbens shell in rats. Physiol Behav 79:191-198.

Adell A, Artigas F (2004) The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. Neurosci Biobehav Rev 28:415-431.

Adolfsson PI, Dahle LO, Berg G, Svensson SP (1998) Characterization of alpha2-adrenoceptor subtypes in pregnant human myometrium. Gynecol Obstet Invest 45:145-150.

Agnati LF, Zoli M, Stromberg I, Fuxe K (1995) Intercellular communication in the brain: wiring versus volume transmission. Neuroscience 69:711-726.

Ahmadi J, Kampman K, Dackis C, Sparkman T, Pettinati H (2008) Cocaine withdrawal symptoms identify "Type B" cocaine-dependent patients. Am J Addict 17:60-64.

Ahmed SH, Koob GF (1997) Cocaine- but not food-seeking behavior is reinstated by stress after extinction. Psychopharmacology (Berl) 132:289-295.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Ahmed SH, Koob GF (1999) Long-lasting increase in the set point for cocaine self-administration after escalation in rats. Psychopharmacology (Berl) 146:303-312.

Ahmed SH, Koob GF (2004) Changes in response to a dopamine receptor antagonist in rats with escalating cocaine intake. Psychopharmacology (Berl) 172:450-454.

Ahmed SH, Lin D, Koob GF, Parsons LH (2003) Escalation of cocaine self-administration does not depend on altered cocaine-induced nucleus accumbens dopamine levels. J Neurochem 86:102-113.

Albanese A, Minciacchi D (1983) Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. J Comp Neurol 216:406-420.

Alheid GF (2003) Extended amygdala and basal forebrain. Ann N Y Acad Sci 985:185-205.

Alheid GF, Heimer L (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27:1-39.

Almela P, Navarro-Zaragoza J, Garcia-Carmona JA, Mora L, Hidalgo J, Milanes MV, Laorden ML (2012) Role of corticotropin-releasing factor (CRF) receptor-1 on the catecholaminergic response to morphine withdrawal in the nucleus accumbens (NAc). PLoS One 7:e47089.

Alt A, Weiss B, Ogden AM, Knauss JL, Oler J, Ho K, Large TH, Bleakman D (2004) Pharmacological characterization of glutamatergic agonists and antagonists at recombinant human homomeric and heteromeric kainate receptors in vitro. Neuropharmacology 46:793-806.

Anstrom KK, Miczek KA, Budygin EA (2009) Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. Neuroscience 161:3-12.

Anstrom KK, Woodward DJ (2005) Restraint increases dopaminergic burst firing in awake rats. Neuropsychopharmacology 30:1832-1840.

Arai M, Assil IQ, Abou-Samra AB (2001) Characterization of three corticotropin-releasing factor receptors in catfish: a novel third receptor is predominantly expressed in pituitary and urophysis. Endocrinology 142:446-454.

Argilli E, Sibley DR, Malenka RC, England PM, Bonci A (2008) Mechanism and time course of cocaine-induced long-term potentiation in the ventral tegmental area. J Neurosci 28:9092-9100.

Arnold FJ, De Lucas Bueno M, Shiers H, Hancock DC, Evan GI, Herbert J (1992) Expression of c-fos in regions of the basal limbic forebrain following intracerebroventricular corticotropin-releasing factor in unstressed or stressed male rats. Neuroscience 51:377-390.

Arora D, Hearing M, Haluk DM, Mirkovic K, Fajardo-Serrano A, Wessendorf MW, Watanabe M, Lujan R, Wickman K (2011) Acute cocaine exposure weakens GABA(B) receptor-dependent G-protein-gated inwardly rectifying K+ signaling in dopamine neurons of the ventral tegmental area. J Neurosci 31:12251-12257.

Assil IQ, Qi LJ, Arai M, Shomali M, Abou-Samra AB (2001) Juxtamembrane region of the amino terminus of the corticotropin releasing factor receptor type 1 is important for ligand interaction. Biochemistry 40:1187-1195.

Aston-Jones G, Harris GC (2004) Brain substrates for increased drug seeking during protracted withdrawal. Neuropharmacology 47 Suppl 1:167-179.

Axelrod J, Reisine TD (1984) Stress hormones: their interaction and regulation. Science 224:452-459.

Back SE, Hartwell K, DeSantis SM, Saladin M, McRae-Clark AL, Price KL, Moran-Santa Maria MM, Baker NL, Spratt E, Kreek MJ, Brady KT (2010) Reactivity to laboratory stress provocation predicts relapse to cocaine. Drug Alcohol Depend 106:21-27.

Backes EN, Hemby SE (2008) Contribution of ventral tegmental GABA receptors to cocaine self-administration in rats. Neurochem Res 33:459-467.

Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1998) Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: modulation by environmental novelty. J Neurosci 18:10579-10593.

Bading H, Ginty DD, Greenberg ME (1993) Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 260:181-186.

Badrinarayan A, Wescott SA, Vander Weele CM, Saunders BT, Couturier BE, Maren S, Aragona BJ (2012) Aversive stimuli differentially modulate real-time dopamine transmission dynamics within the nucleus accumbens core and shell. J Neurosci 32:15779-15790.

Bagley J, Moghaddam B (1997) Temporal dynamics of glutamate efflux in the prefrontal cortex and in the hippocampus following repeated stress: effects of pretreatment with saline or diazepam. Neuroscience 77:65-73.

Bahn S, Volk B, Wisden W (1994) Kainate receptor gene expression in the developing rat brain. J Neurosci 14:5525-5547.

Bakshi VP, Smith-Roe S, Newman SM, Grigoriadis DE, Kalin NH (2002) Reduction of stress-induced behavior by antagonism of corticotropin-releasing hormone 2 (CRH2) receptors in lateral septum or CRH1 receptors in amygdala. J Neurosci 22:2926-2935.

Baldwin HA, Britton, K.T., and Koob, G.F. (ed.) (1990) Behavioral effects of corticotropin-releasing factor. Berlin & Heidelberg: Springer-Verlag.

Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee KF (2000) Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat Genet 24:410-414.

Bale TL, Picetti R, Contarino A, Koob GF, Vale WW, Lee KF (2002) Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. J Neurosci 22:193-199.

Bale TL, Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. Annu Rev Pharmacol Toxicol 44:525-557.

Bannon MJ, Roth RH (1983) Pharmacology of mesocortical dopamine neurons. Pharmacol Rev 35:53-68.

Bannon MJ, Wolf ME, Roth RH (1983) Pharmacology of dopamine neurons innervating the prefrontal, cingulate and piriform cortices. Eur J Pharmacol 92:119-125.

Bannon MJEPJ, Alpert, J.E., Goedert, M., Iverson, S.D., and Iverson, L.L. (1983) Selective activation of mesocortical dopamine neurons by stress: The role of substance P afferents demonstrated using in vivo application of substance P monoclondal antibody. Nature 306:791-792.

Barbour B, Hausser M (1997) Intersynaptic diffusion of neurotransmitter. Trends Neurosci 20:377-384.

Barrera G, Echevarria DJ, Poulin JF, Laforest S, Drolet G, Morilak DA (2005) One for all or one for one: does co-transmission unify the concept of a brain galanin "system" or clarify any consistent role in anxiety? Neuropeptides 39:289-292.

Barros HM, Miczek KA (1996) Withdrawal from oral cocaine in rate: ultrasonic vocalizations and tactile startle. Psychopharmacology (Berl) 125:379-384.

Bassareo V, De Luca MA, Di Chiara G (2002) Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. J Neurosci 22:4709-4719.

Basso AM, Spina M, Rivier J, Vale W, Koob GF (1999) Corticotropin-releasing factor antagonist attenuates the "anxiogenic-like" effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats. Psychopharmacology (Berl) 145:21-30.

Beckstead MJ, Gantz SC, Ford CP, Stenzel-Poore MP, Phillips PE, Mark GP, Williams JT (2009) CRF enhancement of GIRK channel-mediated transmission in dopamine neurons. Neuropsychopharmacology 34:1926-1935.

Beckstead MJ, Grandy DK, Wickman K, Williams JT (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. Neuron 42:939-946.

Beckstead MJ, Williams JT (2007) Long-term depression of a dopamine IPSC. J Neurosci 27:2074-2080.

Beckstead RM, Domesick VB, Nauta WJ (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res 175:191-217.

Bedi G, Preston KL, Epstein DH, Heishman SJ, Marrone GF, Shaham Y, de Wit H (2011) Incubation of cue-induced cigarette craving during abstinence in human smokers. Biol Psychiatry 69:708-711.

Behan DP, De Souza EB, Lowry PJ, Potter E, Sawchenko P, Vale WW (1995) Corticotropin releasing factor (CRF) binding protein: a novel regulator of CRF and related peptides. Front Neuroendocrinol 16:362-382.

Behan DP, Grigoriadis DE, Lovenberg T, Chalmers D, Heinrichs S, Liaw C, De Souza EB (1996a) Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders. Mol Psychiatry 1:265-277.

Behan DP, Khongsaly O, Ling N, De Souza EB (1996b) Urocortin interaction with corticotropin-releasing factor (CRF) binding protein (CRF-BP): a novel mechanism for elevating "free' CRF levels in human brain. Brain Res 725:263-267.

Bellone C, Luscher C (2005) mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. Eur J Neurosci 21:1280-1288.

Bellone C, Luscher C (2006) Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. Nat Neurosci 9:636-641.

Ben-Shahar O, Obara I, Ary AW, Ma N, Mangiardi MA, Medina RL, Szumlinski KK (2009) Extended daily access to cocaine results in distinct alterations in Homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. Synapse 63:598-609.

Bennett AL, Greco B, Blasberg ME, Blaustein JD (2002) Response to male odours in progestin receptor- and oestrogen receptor-containing cells in female rat brain. J Neuroendocrinol 14:442-449.

Berg DK, Conroy WG (2002) Nicotinic alpha 7 receptors: synaptic options and downstream signaling in neurons. J Neurobiol 53:512-523.

Berger B, Gaspar P, Verney C (1991) Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. Trends Neurosci 14:21-27.

Berger B, Thierry AM, Tassin JP, Moyne MA (1976) Dopaminergic innervation of the rat prefrontal cortex: a fluorescence histochemical study. Brain Res 106:133-145.

Berger H, Heinrich N, Wietfeld D, Bienert M, Beyermann M (2006) Evidence that corticotropin-releasing factor receptor type 1 couples to Gs- and Gi-proteins through different conformations of its J-domain. Br J Pharmacol 149:942-947.

Bergeron S, Rompre PP (2013) Blockade of ventral midbrain NMDA receptors enhances brain stimulation reward: a preferential role for GluN2A subunits. Eur Neuropsychopharmacol 23:1623-1635.

Berridge CW, Dunn AJ (1986) Corticotropin-releasing factor elicits naloxone sensitive stress-like alterations in exploratory behavior in mice. Regul Pept 16:83-93.

Berridge CW, Dunn AJ (1987) A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. Horm Behav 21:393-401.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. Curr Opin Pharmacol 9:65-73.

Berton O, Covington HE, 3rd, Ebner K, Tsankova NM, Carle TL, Ulery P, Bhonsle A, Barrot M, Krishnan V, Singewald GM, Singewald N, Birnbaum S, Neve RL, Nestler EJ (2007) Induction of deltaFosB in the periaqueductal gray by stress promotes active coping responses. Neuron 55:289-300.

Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M, Heinemann S (1990) Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. Neuron 5:583-595.

Beyermann M, Sasse, A.E., Fechner, K., Furkert, J., Heinrich, N., Berger, H., Kaupp, U.B., and Bienert, M. (1997) CRF-receptor ligands: Insights into the mode of receptor-peptide interaction. European Neuropsychopharmacology 7:S88.

Bilezikjian LM, Vale WW (1987) Regulation of ACTH secretion from corticotrophs: the interaction of vasopressin and CRF. Ann N Y Acad Sci 512:85-96.

Birch PJ, Grossman CJ, Hayes AG (1988) Kynurenate and FG9041 have both competitive and non-competitive antagonist actions at excitatory amino acid receptors. Eur J Pharmacol 151:313-315.

Bito H, Deisseroth K, Tsien RW (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. Cell 87:1203-1214.

Bittencourt JC, Sawchenko PE (2000) Do centrally administered neuropeptides access cognate receptors?: an analysis in the central corticotropin-releasing factor system. J Neurosci 20:1142-1156.

Bjorklund A, Divac I, Lindvall O (1978) Regional distribution of catecholamines in monkey cerebral cortex, evidence for a dopaminergic innervation of the primate prefrontal cortex. Neurosci Lett 7:115-119.

Bjorklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci 30:194-202.

Bjorklund A, Lindvall O (1975) Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. Brain Res 83:531-537.

Blacktop JM, Seubert C, Baker DA, Ferda N, Lee G, Graf EN, Mantsch JR (2011) Augmented Cocaine Seeking in Response to Stress or CRF Delivered into the Ventral Tegmental Area Following Long-Access Self-Administration Is Mediated by CRF Receptor Type 1 But Not CRF Receptor Type 2. J Neurosci 31:11396-11403.

Blanc G, Herve D, Simon H, Lisoprawski A, Glowinski J, Tassin JP (1980) Response to stress of mesocortico-frontal dopaminergic neurones in rats after long-term isolation. Nature 284:265-267.

Blank T, Nijholt I, Grammatopoulos DK, Randeva HS, Hillhouse EW, Spiess J (2003) Corticotropin-releasing factor receptors couple to multiple G-proteins to activate diverse intracellular signaling pathways in mouse hippocampus: role in neuronal excitability and associative learning. J Neurosci 23:700-707.

Blein S, Ginham R, Uhrin D, Smith BO, Soares DC, Veltel S, McIlhinney RA, White JH, Barlow PN (2004) Structural analysis of the complement control protein (CCP) modules of GABA(B) receptor 1a: only one of the two CCP modules is compactly folded. J Biol Chem 279:48292-48306.

Bocklisch C, Pascoli V, Wong JC, House DR, Yvon C, de Roo M, Tan KR, Luscher C (2013) Cocaine Disinhibits Dopamine Neurons by Potentiation of GABA Transmission in the Ventral Tegmental Area. Science 341:1521-1525.

Boehm SL, 2nd, Piercy MM, Bergstrom HC, Phillips TJ (2002) Ventral tegmental area region governs GABA(B) receptor modulation of ethanol-stimulated activity in mice. Neuroscience 115:185-200.

Bohm A, Gaudet R, Sigler PB (1997) Structural aspects of heterotrimeric G-protein signaling. Curr Opin Biotechnol 8:480-487.

Bolanos CA, Perrotti LI, Edwards S, Eisch AJ, Barrot M, Olson VG, Russell DS, Neve RL, Nestler EJ (2003) Phospholipase Cgamma in distinct regions of the ventral tegmental area differentially modulates mood-related behaviors. J Neurosci 23:7569-7576.

Bonci A, Williams JT (1997) Increased probability of GABA release during withdrawal from morphine. J Neurosci 17:796-803.

Bondareff W, Narotzky R, Routtenberg A (1971) Intrastriatal spread of catecholamines in senescent rats. Journal of gerontology 26:163-167.

Bondareff W, Pysh JJ (1968) Distribution of the extracellular space during postnatal maturation of rat cerebral cortex. The Anatomical record 160:773-780.

Bondareff W, Routtenberg A, Narotzky R, McLone DG (1970) Intrastriatal spreading of biogenic amines. Exp Neurol 28:213-229.

Boorse GC, Crespi EJ, Dautzenberg FM, Denver RJ (2005) Urocortins of the South African clawed frog, Xenopus laevis: conservation of structure and function in tetrapod evolution. Endocrinology 146:4851-4860.

Booth DA (1968) Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. J Pharmacol Exp Ther 160:336-348.

Bourdy R, Barrot M (2012) A new control center for dopaminergic systems: pulling the VTA by the tail. Trends Neurosci 35:681-690.

Boutillier AL, Sassone-Corsi P, Loeffler JP (1991) The protooncogene c-fos is induced by corticotropin-releasing factor and stimulates proopiomelanocortin gene transcription in pituitary cells. Mol Endocrinol 5:1301-1310.

Bouton ME (2002) Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psychiatry 52:976-986.

Bouton ME, Swartzentruber, D. (1991) Sources of relapse after extinction in Pavlovian and instrumental learning. Clinical Psychology Review 11:123-140.

Bowers MS, Chen BT, Bonci A (2010) AMPA receptor synaptic plasticity induced by psychostimulants: the past, present, and therapeutic future. Neuron 67:11-24.

Boyson CO, Miguel TT, Quadros IM, Debold JF, Miczek KA Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA. Psychopharmacology (Berl).

Bradbury MJ, Strack AM, Dallman MF (1993) Lesions of the hippocampal efferent pathway (fimbria-fornix) do not alter sensitivity of adrenocorticotropin to feedback inhibition by corticosterone in rats. Neuroendocrinology 58:396-407.

Bradley BP, Phillips G, Green L, Gossop M (1989) Circumstances surrounding the initial lapse to opiate use following detoxification. Br J Psychiatry 154:354-359.

Brebner K, Childress AR, Roberts DC (2002) A potential role for GABA(B) agonists in the treatment of psychostimulant addiction. Alcohol Alcohol 37:478-484.

Brebner K, Froestl W, Andrews M, Phelan R, Roberts DC (1999) The GABA(B) agonist CGP 44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and a discrete trials procedure. Neuropharmacology 38:1797-1804.

Brebner K, Phelan R, Roberts DC (2000) Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. Pharmacol Biochem Behav 66:857-862.

Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, Goodman JM, Kantor HL, Gastfriend DR, Riorden JP, Mathew RT, Rosen BR, Hyman SE (1997) Acute effects of cocaine on human brain activity and emotion. Neuron 19:591-611.

Briand LA, Vassoler FM, Pierce RC, Valentino RJ, Blendy JA (2010) Ventral tegmental afferents in stress-induced reinstatement: the role of cAMP response element-binding protein. J Neurosci 30:16149-16159.

Brischoux F, Chakraborty S, Brierley DI, Ungless MA (2009) Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A 106:4894-4899.

Britton DR, Koob GF, Rivier J, Vale W (1982) Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci 31:363-367.

Britton KT, Lee G, Dana R, Risch SC, Koob GF (1986a) Activating and 'anxiogenic' effects of corticotropin releasing factor are not inhibited by blockade of the pituitary-adrenal system with dexamethasone. Life Sci 39:1281-1286.

Britton KT, Lee G, Vale W, Rivier J, Koob GF (1986b) Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in the rat. Brain Res 369:303-306.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68:815-834.

Brown MR, Fisher LA, Spiess J, Rivier C, Rivier J, Vale W (1982a) Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism. Endocrinology 111:928-931.

Brown MR, Fisher LA, Spiess J, Rivier J, Rivier C, Vale W (1982b) Comparison of the biologic actions of corticotropin-releasing factor and sauvagine. Regul Pept 4:107-114.

Brown ZJ, Nobrega JN, Erb S (2011) Central injections of noradrenaline induce reinstatement of cocaine seeking and increase c-fos mRNA expression in the extended amygdala. Behav Brain Res 217:472-476.

Brown ZJ, Tribe E, D'Souza N A, Erb S (2009) Interaction between noradrenaline and corticotrophin-releasing factor in the reinstatement of cocaine seeking in the rat. Psychopharmacology (Berl) 203:121-130.

Bruijnzeel AW, Prado M, Isaac S (2009) Corticotropin-releasing factor-1 receptor activation mediates nicotine withdrawal-induced deficit in brain reward function and stress-induced relapse. Biol Psychiatry 66:110-117.

Brunson KL, Grigoriadis DE, Lorang MT, Baram TZ (2002) Corticotropin-releasing hormone (CRH) downregulates the function of its receptor (CRF1) and induces CRF1 expression in hippocampal and cortical regions of the immature rat brain. Exp Neurol 176:75-86.

Buffalari DM, Baldwin CK, Feltenstein MW, See RE (2012) Corticotrophin releasing factor (CRF) induced reinstatement of cocaine seeking in male and female rats. Physiol Behav 105:209-214.

Buffalari DM, See RE (2009) Footshock stress potentiates cue-induced cocaine-seeking in an animal model of relapse. Physiol Behav 98:614-617.

Burbach J, and deWied, D. (1993) Brain Function of Neuropeptides. A current View: The Parthenon Publ. Group Ltd.

Burchfield SR (1979) The stress response: a new perspective. Psychosom Med 41:661-672.

Burrows HL, Nakajima M, Lesh JS, Goosens KA, Samuelson LC, Inui A, Camper SA, Seasholtz AF (1998) Excess corticotropin releasing hormone-binding protein in the hypothalamic-pituitary-adrenal axis in transgenic mice. J Clin Invest 101:1439-1447.

Calabresi P, Lacey MG, North RA (1989) Nicotinic excitation of rat ventral tegmental neurones in vitro studied by intracellular recording. Br J Pharmacol 98:135-140.

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1992) Long-term Potentiation in the Striatum is Unmasked by Removing the Voltage-dependent Magnesium Block of NMDA Receptor Channels. Eur J Neurosci 4:929-935.

Cameron DL, Williams JT (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J Neurosci 14:6763-6767.

Cameron NM, Ha GK, Erskine MS (2004) Fos expression after mating in noradrenergic cells of the A1 and A2 areas of the medulla is altered by adrenalectomy. J Neuroendocrinol 16:750-757.

Campbell RE, Grove KL, Smith MS (2003) Distribution of corticotropin releasing hormone receptor immunoreactivity in the rat hypothalamus: coexpression in neuropeptide Y and dopamine neurons in the arcuate nucleus. Brain Res 973:223-232.

Campeau S, Hayward MD, Hope BT, Rosen JB, Nestler EJ, Davis M (1991) Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Res 565:349-352.

Capriles N, Rodaros D, Sorge RE, Stewart J (2003) A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 168:66-74.

Cardinal RN, Everitt BJ (2004) Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. Curr Opin Neurobiol 14:156-162.

Carlezon WA, Jr., Haile CN, Coppersmith R, Hayashi Y, Malinow R, Neve RL, Nestler EJ (2000) Distinct sites of opiate reward and aversion within the midbrain identified using a herpes simplex virus vector expressing GluR1. J Neurosci 20:RC62.

Carlezon WA, Jr., Nestler EJ (2002) Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? Trends Neurosci 25:610-615.

Carr DB, O'Donnell P, Card JP, Sesack SR (1999) Dopamine terminals in the rat prefrontal cortex synapse on pyramidal cells that project to the nucleus accumbens. J Neurosci 19:11049-11060.

Carr DB, Sesack SR (2000a) GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse 38:114-123.

Carr DB, Sesack SR (2000b) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. J Neurosci 20:3864-3873.

Carter BL, Tiffany ST (1999) Meta-analysis of cue-reactivity in addiction research. Addiction 94:327-340.

Castillo PE, Malenka RC, Nicoll RA (1997) Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature 388:182-186.Catania CA (ed.) (1992) Learning. Englewood Cliffs, NJ: Prentice Hall.

Cenci MA, Kalen P, Mandel RJ, Bjorklund A (1992) Regional differences in the regulation of dopamine and noradrenaline release in medial frontal cortex, nucleus accumbens and caudate-putamen: a microdialysis study in the rat. Brain Res 581:217-228.

Chalmers DT, Lovenberg TW, De Souza EB (1995) Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J Neurosci 15:6340-6350.

Chan-Palay V, and Palay, S.L. (ed.) (1984) Coexistence of neuroactive substances in neurons. New York: Wiley.

Chan RK, Brown ER, Ericsson A, Kovacs KJ, Sawchenko PE (1993) A comparison of two immediate-early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry. J Neurosci 13:5126-5138.

Chan RK, Vale WW, Sawchenko PE (2000) Paradoxical activational effects of a corticotropin-releasing factor-binding protein "ligand inhibitor" in rat brain. Neuroscience 101:115-129.

Chang CP, Pearse RV, 2nd, O'Connell S, Rosenfeld MG (1993) Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11:1187-1195.

Chang JY, Janak PH, Woodward DJ (1998) Comparison of mesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. J Neurosci 18:3098-3115.

Chang YM, Kelliher KR, Baum MJ (2001) Maternal odours induce Fos in the main but not the accessory olfactory bulbs of neonatal male and female ferrets. J Neuroendocrinol 13:551-560.

Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, Ferguson D, Tsai HC, Pomeranz L, Christoffel DJ, Nectow AR, Ekstrand M, Domingos A, Mazei-Robison MS, Mouzon E, Lobo MK, Neve RL, Friedman JM, Russo SJ, Deisseroth K, Nestler EJ, Han MH (2013) Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature 493:532-536.

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron 59:288-297.

Chen C, Wilcoxen KM, Huang CQ, Xie YF, McCarthy JR, Webb TR, Zhu YF, Saunders J, Liu XJ, Chen TK, Bozigian H, Grigoriadis DE (2004) Design of 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1 ,5-a]pyrimidine (NBI 30775/R121919) and structure--activity relationships of a series of potent and orally active corticotropin-releasing factor receptor antagonists. J Med Chem 47:4787-4798.

Chen R, Lewis KA, Perrin MH, Vale WW (1993) Expression cloning of a human corticotropin-releasing-factor receptor. Proc Natl Acad Sci U S A 90:8967-8971.

Chen Y, Brunson KL, Muller MB, Cariaga W, Baram TZ (2000) Immunocytochemical distribution of corticotropin-releasing hormone receptor type-1 (CRF(1))-like immunoreactivity in the mouse brain: light microscopy analysis using an antibody directed against the C-terminus. J Comp Neurol 420:305-323.

Chen Y, Phillips K, Minton G, Sher E (2005) GABA(B) receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. Br J Pharmacol 144:926-932.

Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH, Chouvet G (1993) Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. Eur J Neurosci 5:137-144.

Chergui K, Suaud-Chagny MF, Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. Neuroscience 62:641-645.

Childress AR, Ehrman, R., Rohsenow, D.J., Robbins, S.J., O'Brien, C.P. (1992) Classically conditioned factors in drug dependence. In: Substance abuse: a comprehensive textbook (Lowinson J.H., R. P., Millman R.B., ed), pp 56-69 Baltimore: Williams and Wilkins.

Chiodo LA, Bannon MJ, Grace AA, Roth RH, Bunney BS (1984) Evidence for the absence of impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. Neuroscience 12:1-16.

Chittajallu R, Vignes M, Dev KK, Barnes JM, Collingridge GL, Henley JM (1996) Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. Nature 379:78-81.

Chiueh CC, Kopin IJ (1978) Centrally mediated release by cocaine of endogenous epinephrine and norepinephrine from the sympathoadrenal medullary system of unanesthetized rats. J Pharmacol Exp Ther 205:148-154.

Christie MJ, Bridge S, James LB, Beart PM (1985) Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. Brain Res 333:169-172.

Churchill L, Dilts RP, Kalivas PW (1992) Autoradiographic localization of gamma-aminobutyric acidA receptors within the ventral tegmental area. Neurochem Res 17:101-106.

Ciccocioppo R, Sanna PP, 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. Proc Natl Acad Sci U S A 98:1976-1981.

Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N (2012) Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature 482:85-88.

Cole AJ, Saffen DW, Baraban JM, Worley PF (1989) Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. Nature 340:474-476.

Collingridge GL, Lester RA (1989) Excitatory amino acid receptors in the vertebrate central nervous system. Pharmacol Rev 41:143-210.

Colussi-Mas J, Geisler S, Zimmer L, Zahm DS, Berod A (2007) Activation of afferents to the ventral tegmental area in response to acute amphetamine: a double-labelling study. Eur J Neurosci 26:1011-1025.

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205-237.

Cooper DC (2002) The significance of action potential bursting in the brain reward circuit. Neurochem Int 41:333-340.

Cooper J, Bloom, F., and Roth, R. (ed.) (1991) The Biochemical Basis of Neuropharmacology. New York: Oxford University Press.

Cornish JL, Duffy P, Kalivas PW (1999) A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. Neuroscience 93:1359-1367.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J Neurosci 20:RC89.

Cornish JL, Nakamura M, Kalivas PW (2001) Dopamine-independent locomotion following blockade of N-methyl-D-aspartate receptors in the ventral tegmental area. J Pharmacol Exp Ther 298:226-233.

Corrigall WA, Coen KM (1994) Nicotine self-administration and locomotor activity are not modified by the 5-HT3 antagonists ICS 205-930 and MDL 72222. Pharmacol Biochem Behav 49:67-71.

Cortright DN, Nicoletti A, Seasholtz AF (1995) Molecular and biochemical characterization of the mouse brain corticotropin-releasing hormone-binding protein. Mol Cell Endocrinol 111:147-157.

Cossart R, Esclapez M, Hirsch JC, Bernard C, Ben-Ari Y (1998) GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. Nat Neurosci 1:470-478.

Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, Hollis JH, Murray SE, Hill JK, Pantely GA, Hohimer AR, Hatton DC, Phillips TJ, Finn DA, Low MJ, Rittenberg MB, Stenzel P, Stenzel-Poore MP (2000) Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. Nat Genet 24:403-409.

Covenas R, de Leon M, Cintra A, Bjelke B, Gustafsson JA, Fuxe K (1993) Coexistence of c-Fos and glucocorticoid receptor immunoreactivities in the

CRF immunoreactive neurons of the paraventricular hypothalamic nucleus of the rat after acute immobilization stress. Neurosci Lett 149:149-152.

Covington HE, 3rd, Tropea TF, Rajadhyaksha AM, Kosofsky BE, Miczek KA (2008) NMDA receptors in the rat VTA: a critical site for social stress to intensify cocaine taking. Psychopharmacology (Berl) 197:203-216.

Crombag HS, Shaham Y (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 116:169-173.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C (2004) Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7:153-159.

Cullen MJ, Ling N, Foster AC, Pelleymounter MA (2001) Urocortin, corticotropin releasing factor-2 receptors and energy balance. Endocrinology 142:992-999.

Cullinan WE, Helmreich DL, Watson SJ (1996) Fos expression in forebrain afferents to the hypothalamic paraventricular nucleus following swim stress. J Comp Neurol 368:88-99.

Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ (1995) Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience 64:477-505.

Curran EJ, Akil H, Watson SJ (1996) Psychomotor stimulant- and opiate-induced c-fos mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. Neurochem Res 21:1425-1435.

Curran T (ed.) (1988) The fos oncogene. Amsterdam: Elsevier.

Curran T, Miller AD, Zokas L, Verma IM (1984) Viral and cellular fos proteins: a comparative analysis. Cell 36:259-268.

Dacher M, Gouty S, Dash S, Cox BM, Nugent FS (2013) A-kinase anchoring protein-calcineurin signaling in long-term depression of GABAergic synapses. J Neurosci 33:2650-2660.

Dacher M, Nugent FS (2011) Morphine-induced modulation of LTD at GABAergic synapses in the ventral tegmental area. Neuropharmacology 61:1166-1171.

Dahlstrom A, Fuxe, K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of

monoamine in the cell bodies of brain stem neurons. Acta Physiol Scand 62 (Suppl. 232):1-55.

Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N (1987) Regulation of ACTH secretion: variations on a theme of B. Recent Prog Horm Res 43:113-173.

Danysz W, Fadda E, Wroblewski JT, Costa E (1989) Kynurenate and 2-amino-5-phosphonovalerate interact with multiple binding sites of the N-methyl-D-aspartate-sensitive glutamate receptor domain. Neurosci Lett 96:340-344.

Dascal N (1997) Signalling via the G protein-activated K+ channels. Cellular signalling 9:551-573.

Dautzenberg FM, Hauger RL (2002) The CRF peptide family and their receptors: yet more partners discovered. Trends Pharmacol Sci 23:71-77.

Dautzenberg FM, Higelin J, Brauns O, Butscha B, Hauger RL (2002) Five amino acids of the Xenopus laevis CRF (corticotropin-releasing factor) type 2 receptor mediate differential binding of CRF ligands in comparison with its human counterpart. Mol Pharmacol 61:1132-1139.

David V, Durkin TP, Cazala P (1997) Self-administration of the GABAA antagonist bicuculline into the ventral tegmental area in mice: dependence on D2 dopaminergic mechanisms. Psychopharmacology (Berl) 130:85-90.

Davila V, Yan Z, Craciun LC, Logothetis D, Sulzer D (2003) D3 dopamine autoreceptors do not activate G-protein-gated inwardly rectifying potassium channel currents in substantia nigra dopamine neurons. J Neurosci 23:5693-5697.

Davis M (1992a) The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. Trends Pharmacol Sci 13:35-41.

Davis M (1992b) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15:353-375.

Davis M, Walker DL, Miles L, Grillon C (2010) Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology 35:105-135.

Davis TP, Konings PN (1993) Peptidases in the CNS: formation of biologically active, receptor-specific peptide fragments. Crit Rev Neurobiol 7:163-174.

Daw ND, Kakade S, Dayan P (2002) Opponent interactions between serotonin and dopamine. Neural Netw 15:603-616.

Dayas CV, Buller KM, Crane JW, Xu Y, Day TA (2001) Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. Eur J Neurosci 14:1143-1152.

de Groot RP, Sassone-Corsi P (1993) Hormonal control of gene expression: multiplicity and versatility of cyclic adenosine 3',5'-monophosphate-responsive nuclear regulators. Mol Endocrinol 7:145-153.

de Lima MS, de Oliveira Soares BG, Reisser AA, Farrell M (2002) Pharmacological treatment of cocaine dependence: a systematic review. Addiction 97:931-949.

De Souza EB (1987) Corticotropin-releasing factor receptors in the rat central nervous system: characterization and regional distribution. J Neurosci 7:88-100.

De Souza EB, Insel TR, Perrin MH, Rivier J, Vale WW, Kuhar MJ (1985) Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. J Neurosci 5:3189-3203.

De Souza EB, Liaw, C.W., and Grigoriadis, D.E. (1997) Corticotropin releasing factor receptors and its binding protein: molecular, pharmacological and localization studies. European Neuropsychopharmacology 7:S87.

de Wit H (1996) Priming effects with drugs and other reinforcers. Experimental and Clinical Psychopharmacology 4(1):5-10.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75:134-143.

Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF, Licinio J, Wong ML, Chrousos GP, Webster E, Gold PW (1999) The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. Endocrinology 140:79-86.

Deister CA, Teagarden MA, Wilson CJ, Paladini CA (2009) An intrinsic neuronal oscillator underlies dopaminergic neuron bursting. J Neurosci 29:15888-15897.

Denver RJ (2009) Structural and functional evolution of vertebrate neuroendocrine stress systems. Ann N Y Acad Sci 1163:1-16.

Deroche-Gamonet V, Martinez A, Le Moal M, Piazza PV (2003) Relationships between individual sensitivity to CS- and cocaine-induced reinstatement in the rat. Psychopharmacology (Berl) 168:201-207.

Deroche V, Marinelli M, Le Moal M, Piazza PV (1997) Glucocorticoids and behavioral effects of psychostimulants. II: cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. J Pharmacol Exp Ther 281:1401-1407.

Deroche V, Piazza PV, Deminiere JM, Le Moal M, Simon H (1993) Rats orally self-administer corticosterone. Brain Res 622:315-320.

Deutch AY, Clark WA, Roth RH (1990) Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. Brain Res 521:311-315.

Deutch AY, Lee MC, Gillham MH, Cameron DA, Goldstein M, Iadarola MJ (1991) Stress selectively increases fos protein in dopamine neurons innervating the prefrontal cortex. Cereb Cortex 1:273-292.

Deutch AY, Roth RH (1990) The determinants of stress-induced activation of the prefrontal cortical dopamine system. Prog Brain Res 85:367-402; discussion 402-363.

Deutch AY, Tam SY, Roth RH (1985) Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Res 333:143-146.

Devinsky O, Morrell MJ, Vogt BA (1995) Contributions of anterior cingulate cortex to behaviour. Brain 118 (Pt 1):279-306.

Di Chiara G (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 38:95-137.

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 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. Proc Natl Acad Sci U S A 85:5274-5278.

Di Chiara G, Loddo P, Tanda G (1999) Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. Biol Psychiatry 46:1624-1633.

Di Chiara G, Porceddu ML, Morelli M, Mulas ML, Gessa GL (1979) Substantia nigra as an out-put station for striatal dopaminergic responses: role of a GABA-mediated inhibition of pars reticulata neurons. Naunyn-Schmiedeberg's archives of pharmacology 306:153-159.

Di Ciano P, Everitt BJ (2001) Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology 25:341-360.

Di Fabio R, St-Denis Y, Sabbatini FM, Andreotti D, Arban R, Bernasconi G, Braggio S, Blaney FE, Capelli AM, Castiglioni E, Di Modugno E, Donati D, Fazzolari E, Ratti E, Feriani A, Contini S, Gentile G, Ghirlanda D, Provera S, Marchioro C, Roberts KL, Mingardi A, Mattioli M, Nalin A, Pavone F, Spada S, Trist DG, Worby A (2008) Synthesis and pharmacological characterization of novel druglike corticotropin-releasing factor 1 antagonists. J Med Chem 51:7370-7379.

Didier M, Roux P, Piechaczyk M, Verrier B, Bockaert J, Pin JP (1989) Cerebellar granule cell survival and maturation induced by K+ and NMDA correlate with c-fos proto-oncogene expression. Neurosci Lett 107:55-62.

Ding JM, Carver WC, Terracio L, Buggy J (1994) Proto-oncogene c-fos and the regulation of vasopressin gene expression during dehydration. Brain Res Mol Brain Res 21:247-255.

Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J Neurosci 13:3839-3847.

Dobi A, Margolis EB, Wang HL, Harvey BK, Morales M (2010) Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. J Neurosci 30:218-229.

Dobrakovova M, Kvetnansky R, Torda T, Murgas K (1982) Changes of plasma and adrenal catecholamines and corticosterone in stressed rats with septal lesions. Physiol Behav 29:41-45.

Doherty M, Gratton A (2007) Differential involvement of ventral tegmental GABA(A) and GABA(B) receptors in the regulation of the nucleus accumbens dopamine response to stress. Brain Res 1150:62-68.

Dole VP, Nyswander ME, Kreek MJ (1966) Narcotic blockade. Arch Intern Med 118:304-309.

Dong Y, White FJ (2003) Dopamine D1-class receptors selectively modulate a slowly inactivating potassium current in rat medial prefrontal cortex pyramidal neurons. J Neurosci 23:2686-2695.

Doucet JP, Squinto SP, Bazan NG (1990) Fos-jun and the primary genomic response in the nervous system. Possible physiological role and pathophysiological significance. Mol Neurobiol 4:27-55.

Dragunow M, Faull R (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Methods 29:261-265.

Dragunow M, Faull RL, Jansen KL (1990) MK-801, an antagonist of NMDA receptors, inhibits injury-induced c-fos protein accumulation in rat brain. Neurosci Lett 109:128-133.

Dragunow M, Robertson HA (1987a) Generalized seizures induce c-fos protein(s) in mammalian neurons. Neurosci Lett 82:157-161.

Dragunow M, Robertson HA (1987b) Kindling stimulation induces c-fos protein(s) in granule cells of the rat dentate gyrus. Nature 329:441-442.

Drummond DC, Tiffany, S.T., Glautier, S., and Remington, R. (ed.) (1995) Addictive behavior: Cue exposure theory and practice. New York: Wiley.

DSM-V (2013) Diagnostic and Statistical Manual of Mental Disorders (Fifth ed.). Arlington, VA: American Psychiatric Publishing.

Ducrot C, Fortier E, Bouchard C, Rompre PP (2013) Opposite modulation of brain stimulation reward by NMDA and AMPA receptors in the ventral tegmental area. Frontiers in systems neuroscience 7:57.

Dudish-Poulsen S, Hatsukami DK (2000) Acute abstinence effects following smoked cocaine administration in humans. Exp Clin Psychopharmacol 8:472-482.

Duffield GE, Hastings MH, Ebling FJ (1998) Investigation into the regulation of the circadian system by dopamine and melatonin in the adult Siberian hamster (Phodopus sungorus). J Neuroendocrinol 10:871-884.

Duncan GE, Johnson KB, Breese GR (1993) Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. J Neurosci 13:3932-3943.

Dunn AJ, Berridge CW (1990) Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Brain Res Rev 15:71-100.

Dunn AJ, File SE (1987) Corticotropin-releasing factor has an anxiogenic action in the social interaction test. Horm Behav 21:193-202.

Dunn JM, Inderwies BR, Licata SC, Pierce RC (2005) Repeated administration of AMPA or a metabotropic glutamate receptor agonist into the rat ventral tegmental area augments the subsequent behavioral hyperactivity induced by cocaine. Psychopharmacology (Berl) 179:172-180.

Duvarci S, Bauer EP, Pare D (2009) The bed nucleus of the stria terminalis mediates inter-individual variations in anxiety and fear. J Neurosci 29:10357-10361.

Dworkin SI, Mirkis S, Smith JE (1995) Response-dependent versus response-independent presentation of cocaine: differences in the lethal effects of the drug. Psychopharmacology (Berl) 117:262-266.

Eaves M, Thatcher-Britton K, Rivier J, Vale W, Koob GF (1985) Effects of corticotropin releasing factor on locomotor activity in hypophysectomized rats. Peptides 6:923-926.

Edwards G (1986) The alcohol dependence syndrome: a concept as stimulus to enquiry. Br J Addict 81:171-183.

Egebjerg J, Bettler B, Hermans-Borgmeyer I, Heinemann S (1991) Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. Nature 351:745-748.

Ehlers CL, Chaplin RI (1987) Chronic ethanol exposure potentiates the locomotor-activating effects of corticotropin-releasing factor (CRF) in rats. Regul Pept 19:345-353.

Ellinwood EH (ed.) (1977) Psychopharmacology in the Practice of Medicine. New York: Appleton-Century-Crofts.

Emmert MH, Herman JP (1999) Differential forebrain c-fos mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways. Brain Res 845:60-67.

Emson PC, Koob GF (1978) The origin and distribution of dopamine-containing afferents to the rat frontal cortex. Brain Res 142:249-267.

Engberg G, Kling-Petersen T, Nissbrandt H (1993) GABAB-receptor activation alters the firing pattern of dopamine neurons in the rat substantia nigra. Synapse 15:229-238.

Enrico P, Bouma M, de Vries JB, Westerink BH (1998) The role of afferents to the ventral tegmental area in the handling stress-induced increase in the release of dopamine in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. Brain Res 779:205-213.

Erb S, Funk D, Borkowski S, Watson SJ, Akil H (2004) Effects of chronic cocaine exposure on corticotropin-releasing hormone binding protein in the central nucleus of the amygdala and bed nucleus of the stria terminalis. Neuroscience 123:1003-1009.

Erb S, Funk D, Le AD (2003) Prior, repeated exposure to cocaine potentiates locomotor responsivity to central injections of corticotropin-releasing factor (CRF) in rats. Psychopharmacology (Berl) 170:383-389.

Erb S, Funk D, Le AD (2005) Cocaine pre-exposure enhances CRF-induced expression of c-fos mRNA in the central nucleus of the amygdala: an effect that parallels the effects of cocaine pre-exposure on CRF-induced locomotor activity. Neurosci Lett 383:209-214.

Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y, Stewart J (2000) Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. Neuropsychopharmacology 23:138-150.

Erb S, Kayyali H, Romero K (2006a) A study of the lasting effects of cocaine pre-exposure on anxiety-like behaviors under baseline conditions and in response to central injections of corticotropin-releasing factor. Pharmacol Biochem Behav 85:206-213.

Erb S, Petrovic A, Yi D, Kayyali H (2006b) 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 (Berl) 187:112-120.

Erb S, Salmaso N, Rodaros D, Stewart J (2001) A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 158:360-365.

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology (Berl) 128:408-412.

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. J Neurosci 18:5529-5536.

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. J Neurosci 19:RC35.

Erhardt S, Andersson B, Nissbrandt H, Engberg G (1998) Inhibition of firing rate and changes in the firing pattern of nigral dopamine neurons by gamma-hydroxybutyric acid (GHBA) are specifically induced by activation of GABA(B) receptors. Naunyn-Schmiedeberg's archives of pharmacology 357:611-619.

Erhardt S, Engberg G (2002) Increased phasic activity of dopaminergic neurones in the rat ventral tegmental area following pharmacologically elevated levels of endogenous kynurenic acid. Acta Physiol Scand 175:45-53.

Erhardt S, Mathe JM, Chergui K, Engberg G, Svensson TH (2002) GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. Naunyn-Schmiedeberg's archives of pharmacology 365:173-180.

Ericson M, Lof E, Stomberg R, Chau P, Soderpalm B (2008) Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. J Pharmacol Exp Ther 326:76-82.

Ersche KD, Roiser JP, Robbins TW, Sahakian BJ (2008) Chronic cocaine but not chronic amphetamine use is associated with perseverative responding in humans. Psychopharmacology (Berl) 197:421-431.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481-1489.

Fadda F, Argiolas A, Melis MR, Serra G, Gessa GL (1980) Differential effect of acute and chronic ethanol on dopamine metabolism in frontal cortex, caudate nucleus and substantia nigra. Life Sci 27:979-986.

Fadda F, Argiolas, A., Melis, M.R., Tissari, A., Onali, R., and Gessa, G.L. (1978) Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in N. accumbens: Reversal by diazepam. Life Sci 23:2219-2224.

Fadda P, Scherma, M., Fresu, A., Collu, M., and Fratta, W. (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 50.

Falck RS, Wang J, Carlson RG (2008) Among long-term crack smokers, who avoids and who succumbs to cocaine addiction? Drug Alcohol Depend 98:24-29.

Fallon JH (1981) Collateralization of monoamine neurons: mesotelencephalic dopamine projections to caudate, septum, and frontal cortex. J Neurosci 1:1361-1368.

Fallon JH, Koziell DA, Moore RY (1978) Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J Comp Neurol 180:509-532.

Fallon JH, Moore RY (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545-580.

Famous KR, Schmidt HD, Pierce RC (2007) When administered into the nucleus accumbens core or shell, the NMDA receptor antagonist AP-5 reinstates cocaine-seeking behavior in the rat. Neurosci Lett 420:169-173.

Fanous S, Lacagnina MJ, Nikulina EM, Hammer RP, Jr. (2011) Sensitized activation of Fos and brain-derived neurotrophic factor in the medial prefrontal cortex and ventral tegmental area accompanies behavioral sensitization to amphetamine. Neuropharmacology 61:558-564.

Feenstra MG, Kalsbeek A, van Galen H (1992) Neonatal lesions of the ventral tegmental area affect monoaminergic responses to stress in the medial prefrontal cortex and other dopamine projection areas in adulthood. Brain Res 596:169-182.

Fekete EM, Zhao Y, Li C, Sabino V, Vale WW, Zorrilla EP (2009) Social defeat stress activates medial amygdala cells that express type 2 corticotropin-releasing factor receptor mRNA. Neuroscience 162:5-13.

Fekete EM, Zorrilla EP (2007) Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: ancient CRF paralogs. Front Neuroendocrinol 28:1-27.

Feldmeyer D, Cull-Candy S (1994) Neurotransmitters. Elusive glutamate receptors. Current biology : CB 4:82-84.

Feltenstein MW, See RE (2006) Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. Behav Brain Res 174:1-8.

Fenelon VS, Poulain DA, Theodosis DT (1993) Oxytocin neuron activation and Fos expression: a quantitative immunocytochemical analysis of the effect of lactation, parturition, osmotic and cardiovascular stimulation. Neuroscience 53:77-89.

Ferrante M, Migliore M, Ascoli GA (2009) Feed-forward inhibition as a buffer of the neuronal input-output relation. Proc Natl Acad Sci U S A 106:18004-18009.

Fields HL, Hjelmstad GO, Margolis EB, Nicola SM (2007) Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 30:289-316.

Figueiredo HF, Bodie BL, Tauchi M, Dolgas CM, Herman JP (2003) Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. Endocrinology 144:5249-5258.

Filip M, Frankowska M (2007) Effects of GABA(B) receptor agents on cocaine priming, discrete contextual cue and food induced relapses. Eur J Pharmacol 571:166-173.

Fiorillo CD, Williams JT (1998) Glutamate mediates an inhibitory postsynaptic potential in dopamine neurons. Nature 394:78-82.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968-973.

Florio V, Longo VG (1972) Electroencephalographic effects of bicuculline. Physiol Behav 9:283-285.

Ford CP, Mark GP, Williams JT (2006) Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. J Neurosci 26:2788-2797.

Ford CP, Phillips PE, Williams JT (2009) The time course of dopamine transmission in the ventral tegmental area. J Neurosci 29:13344-13352.

Forster GL, Pringle RB, Mouw NJ, Vuong SM, Watt MJ, Burke AR, Lowry CA, Summers CH, Renner KJ (2008) Corticotropin-releasing factor in the dorsal raphe nucleus increases medial prefrontal cortical serotonin via type 2 receptors and median raphe nucleus activity. Eur J Neurosci 28:299-310.

Fox HC, Talih M, Malison R, Anderson GM, Kreek MJ, Sinha R (2005) Frequency of recent cocaine and alcohol use affects drug craving and associated responses to stress and drug-related cues. Psychoneuroendocrinology 30:880-891.

Francesconi W, Berton F, Repunte-Canonigo V, Hagihara K, Thurbon D, Lekic D, Specio SE, Greenwell TN, Chen SA, Rice KC, Richardson HN, O'Dell LE, Zorrilla EP, Morales M, Koob GF, Sanna PP (2009) Protracted withdrawal from alcohol and drugs of abuse impairs long-term potentiation of intrinsic excitability in the juxtacapsular bed nucleus of the stria terminalis. J Neurosci 29:5389-5401.

Frankle WG, Laruelle M, Haber SN (2006) Prefrontal cortical projections to the midbrain in primates: evidence for a sparse connection. Neuropsychopharmacology 31:1627-1636.

Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA, O'Brien CP, Childress AR (2002) Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. Biol Psychiatry 51:134-142.

Franza BR, Jr., Rauscher FJ, 3rd, Josephs SF, Curran T (1988) The Fos complex and Fos-related antigens recognize sequence elements that contain AP-1 binding sites. Science 239:1150-1153.

French ED, Mura A, Wang T (1993) MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists. Synapse 13:108-116.

Fritts ME, Asbury ET, Horton JE, Isaac WL (1998) Medial prefrontal lesion deficits involving or sparing the prelimbic area in the rat. Physiol Behav 64:373-380.

Fu Y, Pollandt S, Liu J, Krishnan B, Genzer K, Orozco-Cabal L, Gallagher JP, Shinnick-Gallagher P (2007) Long-term potentiation (LTP) in the central amygdala (CeA) is enhanced after prolonged withdrawal from chronic cocaine and requires CRF1 receptors. J Neurophysiol 97:937-941.

Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 30:296-309.

Fuchs RA, Ramirez DR, Bell GH (2008) Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 200:545-556.

Funabashi T, Jinnai K, Kimura F (1997) Fos expression by naloxone in LHRH neurons of the mediobasal hypothalamus and effects of pentobarbital sodium in the proestrous rat. J Neuroendocrinol 9:87-92.

Fuxe K, and Agnati, L.F. (ed.) (1991) Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission: Raven Press.

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145-177.

Gaiddon C, Loeffler JP, Larmet Y (1996) Brain-derived neurotrophic factor stimulates AP-1 and cyclic AMP-responsive element dependent transcriptional activity in central nervous system neurons. J Neurochem 66:2279-2286.

Gardi J, Biro E, Sarnyai Z, Vecsernyes M, Julesz J, Telegdy G (1997) Time-dependent alterations in corticotropin-releasing factor-like immunoreactivity in different brain regions after acute cocaine administration to rats. Neuropeptides 31:15-18.

Gariano RF, Groves PM (1988) Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. Brain Res 462:194-198.

Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. J Neurosci 14:6084-6093.

Gaspar P, Bloch B, Le Moine C (1995) D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. Eur J Neurosci 7:1050-1063.

Gaszner B, Csernus V, Kozicz T (2004) Urocortinergic neurons respond in a differentiated manner to various acute stressors in the Edinger-Westphal nucleus in the rat. J Comp Neurol 480:170-179.

Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251:1580-1586.

Gawin FH, Byck R, Kleber HD (1986) Desipramine augmentation of cocaine abstinence: initial results. Clin Neuropharmacol 9 Suppl 4:202-204.

Gawin FH, Ellinwood EH, Jr. (1988) Cocaine and other stimulants. Actions, abuse, and treatment. The New England journal of medicine 318:1173-1182.

Gawin FH, Kleber HD (1984) Cocaine abuse treatment. Open pilot trial with desipramine and lithium carbonate. Arch Gen Psychiatry 41:903-909.

Gawin FH, Kleber HD (1985) Cocaine use in a treatment: population: patterns and diagnostic distinctions. NIDA Res Monogr 61:182-192.

Gawin FH, Kleber HD (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry 43:107-113.

Gawin FH, Kleber HD (1988) Evolving conceptualizations of cocaine dependence. Yale J Biol Med 61:123-136.

Geffen LB, Jessell TM, Cuello AC, Iversen LL (1976) Release of dopamine from dendrites in rat substantia nigra. Nature 260:258-260.

Geisler S, Derst C, Veh RW, Zahm DS (2007) Glutamatergic afferents of the ventral tegmental area in the rat. J Neurosci 27:5730-5743.

Geisler S, Zahm DS (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. J Comp Neurol 490:270-294.

Georges F, Aston-Jones G (2001) Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. J Neurosci 21:RC160.

Georges F, Aston-Jones G (2002) Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. J Neurosci 22:5173-5187.

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055-1061.

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285-320.

Ghasemzadeh MB, Vasudevan P, Giles C, Purgianto A, Seubert C, Mantsch JR (2011) Glutamatergic plasticity in medial prefrontal cortex and ventral tegmental area following extended-access cocaine self-administration. Brain Res 1413:60-71.

Ghitza UE, Gray SM, Epstein DH, Rice KC, Shaham Y (2006) The anxiogenic drug yohimbine reinstates palatable food seeking in a rat relapse model: a role of CRF1 receptors. Neuropsychopharmacology 31:2188-2196.

Ghosh A, Ginty DD, Bading H, Greenberg ME (1994) Calcium regulation of gene expression in neuronal cells. J Neurobiol 25:294-303.

Giorgetti M, Hotsenpiller G, Froestl W, Wolf ME (2002) In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. Neuroscience 109:585-595.

Giorgetti M, Hotsenpiller G, Ward P, Teppen T, Wolf ME (2001) Amphetamine-induced plasticity of AMPA receptors in the ventral tegmental area: effects on extracellular levels of dopamine and glutamate in freely moving rats. J Neurosci 21:6362-6369.

Giorgetti M, Javaid JI, Davis JM, Costa E, Guidotti A, Appel SB, Brodie MS (1998) Imidazenil, a positive allosteric GABAA receptor modulator, inhibits the effects of cocaine on locomotor activity and extracellular dopamine in the nucleus accumbens shell without tolerance liability. J Pharmacol Exp Ther 287:58-66.

Goddard GV (1965) Immediate behavioral effects of minute lesions in the limbic system. Amer Psychol 20:149-150.

Goeders NE (2002) Stress and cocaine addiction. J Pharmacol Exp Ther 301:785-789.

Goeders NE (ed.) (2007) The Hypothalamic-Pituiatry-Adrenal Axis and Addiction. Burlington MA: Elsevier Academic Press.

Goeders NE, Bienvenu OJ, De Souza EB (1990) Chronic cocaine administration alters corticotropin-releasing factor receptors in the rat brain. Brain Res 531:322-328.

Goeders NE, Guerin GF (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology (Berl) 114:63-70.

Goeders NE, Guerin GF (1996a) Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. Brain Res 722:145-152.

Goeders NE, Guerin GF (1996b) Role of corticosterone in intravenous cocaine self-administration in rats. Neuroendocrinology 64:337-348.

Goeders NE, Peltier RL, Guerin GF (1998) Ketoconazole reduces low dose cocaine self-administration in rats. Drug Alcohol Depend 53:67-77.

Goldman-Rakic PS, Muly EC, 3rd, Williams GV (2000) D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 31:295-301.

Gonon F (1997) Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. J Neurosci 17:5972-5978.

Gonon FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 24:19-28.

Gonzalez GA, Montminy MR (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680.

Gosnell BA, Morley JE, Levine AS (1983) A comparison of the effects of corticotropin releasing factor and sauvagine on food intake. Pharmacol Biochem Behav 19:771-775.

Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. Nat Neurosci 8:805-812.

Goudie AJ, and Emmett-Oglesby, M.W. (ed.) (1989) Psychoactive Drug Tolerance and Sensitization. Clifton NJ: Humana Press.

Grace AA (1995) The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. Drug Alcohol Depend 37:111-129.

Grace AA, Bunney BS (1979) Paradoxical GABA excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons. Eur J Pharmacol 59:211-218.

Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons--1. Identification and characterization. Neuroscience 10:301-315.

Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877-2890.

Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4:2866-2876.

Grace AA, Bunney BS (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333:271-284.

Grace AA, Floresco SB, Goto Y, Lodge DJ (2007a) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220-227.

Grace AA, Onn SP (1989) Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded in vitro. J Neurosci 9:3463-3481.

Grace CR, Perrin MH, DiGruccio MR, Miller CL, Rivier JE, Vale WW, Riek R (2004) NMR structure and peptide hormone binding site of the first extracellular domain of a type B1 G protein-coupled receptor. Proc Natl Acad Sci U S A 101:12836-12841.

Grace CR, Perrin MH, Gulyas J, Digruccio MR, Cantle JP, Rivier JE, Vale WW, Riek R (2007b) Structure of the N-terminal domain of a type B1 G protein-coupled receptor in complex with a peptide ligand. Proc Natl Acad Sci U S A 104:4858-4863.

Graf EN, Hoks MA, Baumgardner J, Sierra J, Vranjkovic O, Bohr C, Baker DA, Mantsch JR (2011) Adrenal Activity during Repeated Long-Access Cocaine Self-Administration is Required for Later CRF-Induced and CRF-Dependent Stressor-Induced Reinstatement in Rats. Neuropsychopharmacology.

Grammatopoulos DK (2012) Insights into mechanisms of corticotropin-releasing hormone receptor signal transduction. Br J Pharmacol 166:85-97.

Grammatopoulos DK, Dai Y, Randeva HS, Levine MA, Karteris E, Easton AJ, Hillhouse EW (1999) A novel spliced variant of the type 1 corticotropin-releasing hormone receptor with a deletion in the seventh transmembrane domain present in the human pregnant term myometrium and fetal membranes. Mol Endocrinol 13:2189-2202.

Grammatopoulos DK, Randeva HS, Levine MA, Kanellopoulou KA, Hillhouse EW (2001) Rat cerebral cortex corticotropin-releasing hormone receptors: evidence for receptor coupling to multiple G-proteins. J Neurochem 76:509-519.

Grammatopoulos DK, Randeva HS, Levine MA, Katsanou ES, Hillhouse EW (2000) Urocortin, but not corticotropin-releasing hormone (CRH), activates the mitogen-activated protein kinase signal transduction pathway in human pregnant myometrium: an effect mediated via R1alpha and R2beta

CRH receptor subtypes and stimulation of Gq-proteins. Mol Endocrinol 14:2076-2091.

Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci U S A 87:6912-6916.

Graziane NM, Polter AM, Briand LA, Pierce RC, Kauer JA (2013) Kappa opioid receptors regulate stress-induced cocaine seeking and synaptic plasticity. Neuron 77:942-954.

Greenberg ME, Ziff EB (1984) Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature 311:433-438.

Greenberg ME, Ziff EB, Greene LA (1986) Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science 234:80-83.

Grillner P, Mercuri NB (2002) Intrinsic membrane properties and synaptic inputs regulating the firing activity of the dopamine neurons. Behav Brain Res 130:149-169.

Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Incubation of cocaine craving after withdrawal. Nature 412:141-142.

Gronier B, Rasmussen K (1998) Activation of midbrain presumed dopaminergic neurones by muscarinic cholinergic receptors: an in vivo electrophysiological study in the rat. Br J Pharmacol 124:455-464.

Grossman SP, Stumpf WE (1969) Intracranial drug implants: an autoradiographic analysis of diffusion. Science 166:1410-1412.

Grubb MC, Welch JR, Finn DA, Mark GP (2002) Cocaine self-administration alters the locomotor response to microinjection of bicuculline into the ventral tegmental area of rats. Brain Res 952:44-51.

Gu H, Salmeron BJ, Ross TJ, Geng X, Zhan W, Stein EA, Yang Y (2010) Mesocorticolimbic circuits are impaired in chronic cocaine users as demonstrated by resting-state functional connectivity. Neuroimage 53:593-601.

Guan X, Zhang R, Xu Y, Li S (2009) Cocaine withdrawal enhances long-term potentiation in rat hippocampus via changing the activity of corticotropin-releasing factor receptor subtype 2. Neuroscience 161:665-670.

Guarraci FA, Kapp BS (1999) An electrophysiological characterization of ventral tegmental area dopaminergic neurons during differential pavlovian fear conditioning in the awake rabbit. Behav Brain Res 99:169-179.

Gulledge AT, Jaffe DB (1998) Dopamine decreases the excitability of layer V pyramidal cells in the rat prefrontal cortex. J Neurosci 18:9139-9151.

Gulledge AT, Jaffe DB (2001) Multiple effects of dopamine on layer V pyramidal cell excitability in rat prefrontal cortex. J Neurophysiol 86:586-595.

Guo Z, Tellew JE, Gross RS, Dyck B, Grey J, Haddach M, Kiankarimi M, Lanier M, Li BF, Luo Z, McCarthy JR, Moorjani M, Saunders J, Sullivan R, Zhang X, Zamani-Kord S, Grigoriadis DE, Crowe PD, Chen TK, Williams JP (2005) Design and synthesis of tricyclic imidazo[4,5-b]pyridin-2-ones as corticotropin-releasing factor-1 antagonists. J Med Chem 48:5104-5107.

Haber SN, Ryoo H, Cox C, Lu W (1995) Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: comparison with the mRNA for the D2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. J Comp Neurol 362:400-410.

Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, Webster EL, Atkinson AJ, Schulkin J, Contoreggi C, Chrousos GP, McCann SM, Suomi SJ, Higley JD, Gold PW (2000) Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. Proc Natl Acad Sci U S A 97:6079-6084.

Hahn J, Hopf FW, Bonci A (2009) Chronic cocaine enhances corticotropin-releasing factor-dependent potentiation of excitatory transmission in ventral tegmental area dopamine neurons. J Neurosci 29:6535-6544.

Hairston JE, Ball GF, Nelson RJ (2003) Photoperiodic and temporal influences on chemosensory induction of brain fos expression in female prairie voles. J Neuroendocrinol 15:161-172.

Hall JN, Uchman, R.S., and Dominguez, R. (1988) Trends and Patterns of Methamphetamine Use in the United States. Report prepared for the Department of Epidemiology and Statistical Analysis. NIDA Order No. 88MO3105481D. Miami: Up Front Drug Information Center.

Hall SM, Havassy BE, Wasserman DA (1991) Effects of commitment to abstinence, positive moods, stress, and coping on relapse to cocaine use. J Consult Clin Psychol 59:526-532.

Halliday GM, Tork I (1986) Comparative anatomy of the ventromedial mesencephalic tegmentum in the rat, cat, monkey and human. J Comp Neurol 252:423-445.

Hamm HE, Gilchrist A (1996) Heterotrimeric G proteins. Curr Opin Cell Biol 8:189-196.

Harbuz MS, Chalmers J, De Souza L, Lightman SL (1993) Stress-induced activation of CRF and c-fos mRNAs in the paraventricular nucleus are not affected by serotonin depletion. Brain Res 609:167-173.

Harden DG, King D, Finlay JM, Grace AA (1998) Depletion of dopamine in the prefrontal cortex decreases the basal electrophysiological activity of mesolimbic dopamine neurons. Brain Res 794:96-102.

Harmar AJ (2001) Family-B G-protein-coupled receptors. Genome Biol 2:REVIEWS3013.

Harris GC, Aston-Jones G (2003a) Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. Neuropsychopharmacology 28:73-76.

Harris GC, Aston-Jones G (2003b) Enhanced morphine preference following prolonged abstinence: association with increased Fos expression in the extended amygdala. Neuropsychopharmacology 28:292-299.

Harris GC, 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 129:841-847.

Hartz RA, Nanda KK, Ingalls CL, Ahuja VT, Molski TF, Zhang G, Wong H, Peng Y, Kelley M, Lodge NJ, Zaczek R, Gilligan PJ, Trainor GL (2004) Design, synthesis, and biological evaluation of 1,2,3,7-tetrahydro-6h-purin-6-one and 3,7-dihydro-1h-purine-2,6-dione derivatives as corticotropin-releasing factor(1) receptor antagonists. J Med Chem 47:4741-4754.

Hasin DS, O'Brien CP, Auriacombe M, Borges G, Bucholz K, Budney A, Compton WM, Crowley T, Ling W, Petry NM, Schuckit M, Grant BF (2013) DSM-5 criteria for substance use disorders: recommendations and rationale. Am J Psychiatry 170:834-851.

Hauger RL, Olivares-Reyes JA, Braun S, Hernandez-Aranda J, Hudson CC, Gutknecht E, Dautzenberg FM, Oakley RH (2013) Desensitization of human CRF2(a) receptor signaling governed by agonist potency and betaarrestin2 recruitment. Regul Pept 186:62-76.

Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27:555-579.

Heilig M, Egli M, Crabbe JC, Becker HC (2010) Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? Addict Biol 15:169-184.

Heimer L, Alheid GF (1991) Piecing together the puzzle of basal forebrain anatomy. Adv Exp Med Biol 295:1-42.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C (1991) Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89-125.

Heinrichs SC, Cole BJ, Pich EM, Menzaghi F, Koob GF, Hauger RL (1992) Endogenous corticotropin-releasing factor modulates feeding induced by neuropeptide Y or a tail-pinch stressor. Peptides 13:879-884.

Heinrichs SC, Menzaghi F, Pich EM, Baldwin HA, Rassnick S, Britton KT, Koob GF (1994) Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. Neuropsychopharmacology 11:179-186.

Heinrichs SC, Menzaghi F, Schulteis G, Koob GF, Stinus L (1995) Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. Behav Pharmacol 6:74-80.

Heinz A, Beck A, Grusser SM, Grace AA, Wrase J (2009) Identifying the neural circuitry of alcohol craving and relapse vulnerability. Addict Biol 14:108-118.

Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI (1997) Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology (Berl) 133:7-16.

Henry B, Vale W, Markou A (2006) The effect of lateral septum corticotropin-releasing factor receptor 2 activation on anxiety is modulated by stress. J Neurosci 26:9142-9152.

Herb A, Burnashev N, Werner P, Sakmann B, Wisden W, Seeburg PH (1992) The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. Neuron 8:775-785.

Herkenham M (1987) Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. Neuroscience 23:1-38.

Herman JP (1993) Regulation of adrenocorticosteroid receptor mRNA expression in the central nervous system. Cell Mol Neurobiol 13:349-372.

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78-84.

Herman JP, Cullinan WE, Watson SJ (1994) Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. J Neuroendocrinol 6:433-442.

Herman JP, Guillonneau D, Dantzer R, Scatton B, Semerdjian-Rouquier L, Le Moal M (1982) Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci 30:2207-2214.

Hernandez G, Shizgal P (2009) Dynamic changes in dopamine tone during self-stimulation of the ventral tegmental area in rats. Behav Brain Res 198:91-97.

Herrera DG, Robertson HA (1996) Activation of c-fos in the brain. Prog Neurobiol 50:83-107.

Herringa RJ, Mackenrodt DB, Barlow JD, Roseboom PH, Nanda SA, Kalin NH (2006) Corticotropin-releasing factor (CRF), but not corticosterone, increases basolateral amygdala CRF-binding protein. Brain Res 1083:21-28.

Herringa RJ, Nanda SA, Hsu DT, Roseboom PH, Kalin NH (2004) The effects of acute stress on the regulation of central and basolateral amygdala CRF-binding protein gene expression. Brain Res Mol Brain Res 131:17-25.

Hester R, Garavan H (2004) Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 24:11017-11022.

Hill CS, Treisman R (1995) Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell 80:199-211.

Hillhouse EW, Grammatopoulos DK (2006) The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr Rev 27:260-286.

Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX (2001) The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 21:7463-7473.

Hoare SR, Sullivan SK, Ling N, Crowe PD, Grigoriadis DE (2003) Mechanism of corticotropin-releasing factor type I receptor regulation by nonpeptide antagonists. Mol Pharmacol 63:751-765.

Hoare SR, Sullivan SK, Schwarz DA, Ling N, Vale WW, Crowe PD, Grigoriadis DE (2004) Ligand affinity for amino-terminal and juxtamembrane domains of the corticotropin releasing factor type I receptor: regulation by G-protein and nonpeptide antagonists. Biochemistry 43:3996-4011.

Hoffman GE, Le WW, Abbud R, Lee WS, Smith MS (1994) Use of Fos-related antigens (FRAs) as markers of neuronal activity: FRA changes in dopamine neurons during proestrus, pregnancy and lactation. Brain Res 654:207-215.

Hoffman GE, Le WW, Sita LV (2008) The importance of titrating antibodies for immunocytochemical methods. Curr Protoc Neurosci Chapter 2:Unit 2 12.

Hofmann BA, Sydow S, Jahn O, van Werven L, Liepold T, Eckart K, Spiess J (2001) Functional and protein chemical characterization of the N-terminal domain of the rat corticotropin-releasing factor receptor 1. Protein science : a publication of the Protein Society 10:2050-2062.

Hokfelt T, Everitt BJ, Theodorsson-Norheim E, Goldstein M (1984a) Occurrence of neurotensinlike immunoreactivity in subpopulations of hypothalamic, mesencephalic, and medullary catecholamine neurons. J Comp Neurol 222:543-559.

Hokfelt T, Johansson O, Goldstein M (1984b) Chemical anatomy of the brain. Science 225:1326-1334.

Hokfelt T, Martensson, R., Bjorklund, A., Kleinau, S., and Goldstein, M. (ed.) (1984) Distributional maps of tyrosine-hydroxylase-immunoreactive neurons in the brain brain. Amsterdam: Elsevier.

Hokfelt T, Millhorn D, Seroogy K, Tsuruo Y, Ceccatelli S, Lindh B, Meister B, Melander T, Schalling M, Bartfai T, et al. (1987) Coexistence of peptides with classical neurotransmitters. Experientia 43:768-780.

Holmes KD, Babwah AV, Dale LB, Poulter MO, Ferguson SS (2006) Differential regulation of corticotropin releasing factor 1alpha receptor endocytosis

and trafficking by beta-arrestins and Rab GTPases. J Neurochem 96:934-949.

Horvitz JC (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 96:651-656.

Hsu SY, Hsueh AJ (2001) Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat Med 7:605-611.

Hughes P, Lawlor P, Dragunow M (1992) Basal expression of Fos, Fos-related, Jun, and Krox 24 proteins in rat hippocampus. Brain Res Mol Brain Res 13:355-357.

Huising MO, Vaughan JM, Shah SH, Grillot KL, Donaldson CJ, Rivier J, Flik G, Vale WW (2008) Residues of corticotropin releasing factor-binding protein (CRF-BP) that selectively abrogate binding to CRF but not to urocortin 1. J Biol Chem 283:8902-8912.

Hunt SP, Pini A, Evan G (1987) Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature 328:632-634.

Hurd YL, Weiss F, Koob GF, And NE, Ungerstedt U (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an in vivo microdialysis study. Brain Res 498:199-203.

Hurley KM, Herbert H, Moga MM, Saper CB (1991) Efferent projections of the infralimbic cortex of the rat. J Comp Neurol 308:249-276.

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565-598.

Ikeda J, Nakajima T, Osborne OC, Mies G, Nowak TS, Jr. (1994) Coexpression of c-fos and hsp70 mRNAs in gerbil brain after ischemia: induction threshold, distribution and time course evaluated by in situ hybridization. Brain Res Mol Brain Res 26:249-258.

Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27-78.

Ikemoto S, Kohl RR, McBride WJ (1997a) GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. J Neurochem 69:137-143.

Ikemoto S, Murphy JM, McBride WJ (1997b) Self-infusion of GABA(A) antagonists directly into the ventral tegmental area and adjacent regions. Behav Neurosci 111:369-380.

Ikemoto S, Murphy JM, McBride WJ (1998) Regional differences within the rat ventral tegmental area for muscimol self-infusions. Pharmacol Biochem Behav 61:87-92.

Ikemoto S, Qin M, Liu ZH (2006) Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. J Neurosci 26:723-730.

Ikemoto S, Wise RA (2002) Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral tegmental area. J Neurosci 22:9895-9904.

Ikemoto S, Witkin BM, Morales M (2003) Rewarding injections of the cholinergic agonist carbachol into the ventral tegmental area induce locomotion and c-Fos expression in the retrosplenial area and supramammillary nucleus. Brain Res 969:78-87.

Imaki T, Shibasaki T, Hotta M, Demura H (1993) Intracerebroventricular administration of corticotropin-releasing factor induces c-fos mRNA expression in brain regions related to stress responses: comparison with pattern of c-fos mRNA induction after stress. Brain Res 616:114-125.

Imaki T, Xiao-Quan W, Shibasaki T, Yamada K, Harada S, Chikada N, Naruse M, Demura H (1995) Stress-induced activation of neuronal activity and corticotropin-releasing factor gene expression in the paraventricular nucleus is modulated by glucocorticoids in rats. J Clin Invest 96:231-238.

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 20:7489-7495.

Ito R, Robbins TW, Everitt BJ (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci 7:389-397.

Jacobs N, Dubois, L.C. (ed.) (2012) Drug Addiction: Science and Treatment. New York: Nova Science Publishers, Inc.

Jacobson L, Akana SF, Cascio CS, Shinsako J, Dallman MF (1988) Circadian variations in plasma corticosterone permit normal termination of adrenocorticotropin responses to stress. Endocrinology 122:1343-1348.

Jacobson L, Sapolsky R (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr Rev 12:118-134.

Jaffe EH, Marty A, Schulte A, Chow RH (1998) Extrasynaptic vesicular transmitter release from the somata of substantia nigra neurons in rat midbrain slices. J Neurosci 18:3548-3553.

Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology (Berl) 97:59-64.

Jahn O, Tezval H, van Werven L, Eckart K, Spiess J (2004) Three-amino acid motifs of urocortin II and III determine their CRF receptor subtype selectivity. Neuropharmacology 47:233-242.

Jalabert M, Bourdy R, Courtin J, Veinante P, Manzoni OJ, Barrot M, Georges F (2011) Neuronal circuits underlying acute morphine action on dopamine neurons. Proc Natl Acad Sci U S A 108:16446-16450.

Jan YN, Jan LY (1983) Coexistence and corelease of cholinergic and peptidergic transmitters in frog sympathetic ganglia. Fed Proc 42:2929-2933.

Janssen D, Kozicz T (2013) Is it really a matter of simple dualism? Corticotropin-releasing factor receptors in body and mental health. Front Endocrinol (Lausanne) 4:28.

Jedema HP, Moghaddam B (1994) Glutamatergic control of dopamine release during stress in the rat prefrontal cortex. J Neurochem 63:785-788.

Jedema HP, Moghddam B (1996) Characterization of excitatory amino acid modulation of dopamine release in the prefrontal cortex of conscious rats. J Neurochem 66:1448-1453.

Jekel JF, Allen DF, Podlewski H, Clarke N, Dean-Patterson S, Cartwright P (1986) Epidemic free-base cocaine abuse. Case study from the Bahamas. Lancet 1:459-462.

Jennings JH, Sparta DR, Stamatakis AM, Ung RL, Pleil KE, Kash TL, Stuber GD (2013) Distinct extended amygdala circuits for divergent motivational states. Nature 496:224-228.

Jhou T (2005) Neural mechanisms of freezing and passive aversive behaviors. J Comp Neurol 493:111-114.

Jhou TC, Fields HL, Baxter MG, Saper CB, Holland PC (2009) The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine

neurons, encodes aversive stimuli and inhibits motor responses. Neuron 61:786-800.

Ji G, Neugebauer V (2012) Modulation of medial prefrontal cortical activity using in vivo recordings and optogenetics. Molecular brain 5:36.

Jobes ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ, Preston KL (2011) Clonidine blocks stress-induced craving in cocaine users. Psychopharmacology (Berl) 218:83-88.

Joels M, de Kloet ER (1994) Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. Prog Neurobiol 43:1-36.

Johanson CE, Fischman MW (1989) The pharmacology of cocaine related to its abuse. Pharmacol Rev 41:3-52.

Johnson SW, North RA (1992a) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12:483-488.

Johnson SW, North RA (1992b) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol 450:455-468.

Johnson SW, Seutin V, North RA (1992) Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. Science 258:665-667.

Jones IW, Wonnacott S (2004) Precise localization of alpha7 nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. J Neurosci 24:11244-11252.

Joseph MH, Datla K, Young AM (2003) The interpretation of the measurement of nucleus accumbens dopamine by in vivo dialysis: the kick, the craving or the cognition? Neurosci Biobehav Rev 27:527-541.

Justice NJ, Yuan ZF, Sawchenko PE, Vale W (2008) Type 1 corticotropin-releasing factor receptor expression reported in BAC transgenic mice: implications for reconciling ligand-receptor mismatch in the central corticotropin-releasing factor system. J Comp Neurol 511:479-496.

Kaczmarek L, Chaudhuri A (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. Brain Res Brain Res Rev 23:237-256.

Kageyama K, Li C, Vale WW (2003) Corticotropin-releasing factor receptor type 2 messenger ribonucleic acid in rat pituitary: localization and regulation by

immune challenge, restraint stress, and glucocorticoids. Endocrinology 144:1524-1532.

Kalin NH, Sherman, J.E., Takahashi, L.K. (1998) Antagonism of endogenous CRH systems attenuates stress-induced freezing behavior in rats. Brain Res 457:130-135.

Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res Brain Res Rev 18:75-113.

Kalivas PW, Churchill L, Romanides A (1999) Involvement of the pallidal-thalamocortical circuit in adaptive behavior. Ann N Y Acad Sci 877:64-70.

Kalivas PW, Duffy P (1989) Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biol Psychiatry 25:913-928.

Kalivas PW, Duffy P (1991) A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. J Neurochem 56:961-967.

Kalivas PW, Duffy P (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Res 675:325-328.

Kalivas PW, Duffy P (1998) Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. J Neurochem 70:1497-1502.

Kalivas PW, Duffy P, Barrow J (1989) Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J Pharmacol Exp Ther 251:378-387.

Kalivas PW, Duffy P, Eberhardt H (1990) Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J Pharmacol Exp Ther 253:858-866.

Kalivas PW, Duffy P, Latimer LG (1987) Neurochemical and behavioral effects of corticotropin-releasing factor in the ventral tegmental area of the rat. J Pharmacol Exp Ther 242:757-763.

Kalivas PW, O'Brien C (2008) Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacology 33:166-180.

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223-244.

Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45:647-650.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Kane JK, Hwang Y, Konu O, Loughlin SE, Leslie FM, Li MD (2005) Regulation of Homer and group I metabotropic glutamate receptors by nicotine. Eur J Neurosci 21:1145-1154.

Karhunen T, Vilim FS, Alexeeva V, Weiss KR, Church PJ (2001) Targeting of peptidergic vesicles in cotransmitting terminals. J Neurosci 21:RC127.

Karlsgodt KH, Lukas SE, Elman I (2003) Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. Am J Drug Alcohol Abuse 29:539-551.

Karolyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, Seong E, Camper SA, Seasholtz AF (1999) Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. Proc Natl Acad Sci U S A 96:11595-11600.

Karreman M, Moghaddam B (1996) Effect of a pharmacological stressor on glutamate efflux in the prefrontal cortex. Brain Res 716:180-182.

Karreman M, Westerink BH, Moghaddam B (1996) Excitatory amino acid receptors in the ventral tegmental area regulate dopamine release in the ventral striatum. J Neurochem 67:601-607.

Kasagi Y, Horiba N, Sakai K, Fukuda Y, Suda T (2002) Involvement of cAMP-response element binding protein in corticotropin-releasing factor (CRF)-induced down-regulation of CRF receptor 1 gene expression in rat anterior pituitary cells. J Neuroendocrinol 14:587-592.

Kaufling J, Veinante P, Pawlowski SA, Freund-Mercier MJ, Barrot M (2009) Afferents to the GABAergic tail of the ventral tegmental area in the rat. J Comp Neurol 513:597-621.

Kaufling J, Veinante P, Pawlowski SA, Freund-Mercier MJ, Barrot M (2010) gamma-Aminobutyric acid cells with cocaine-induced DeltaFosB in the ventral tegmental area innervate mesolimbic neurons. Biol Psychiatry 67:88-92.

Kaufman JN, Ross TJ, Stein EA, Garavan H (2003) Cingulate hypoactivity in cocaine users during a GO-NOGO task as revealed by event-related functional magnetic resonance imaging. J Neurosci 23:7839-7843.

Kehne J, De Lombaert S (2002) Non-peptidic CRF1 receptor antagonists for the treatment of anxiety, depression and stress disorders. Curr Drug Targets CNS Neurol Disord 1:467-493.

Kehne JH, Cain CK (2010) Therapeutic utility of non-peptidic CRF1 receptor antagonists in anxiety, depression, and stress-related disorders: evidence from animal models. Pharmacol Ther 128:460-487.

Kemp CF, Woods RJ, Lowry PJ (1998) The corticotrophin-releasing factor-binding protein: an act of several parts. Peptides 19:1119-1128.

Kenakin T (2002) Efficacy at G-protein-coupled receptors. Nat Rev Drug Discov 1:103-110.

Kessler M, Terramani T, Lynch G, Baudry M (1989) A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. J Neurochem 52:1319-1328.

Kim Y, Wood J, Moghaddam B (2012) Coordinated activity of ventral tegmental neurons adapts to appetitive and aversive learning. PLoS One 7:e29766.

King D, Zigmond MJ, Finlay JM (1997) Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increase in extracellular dopamine in the nucleus accumbens core and shell. Neuroscience 77:141-153.

Kippin TE, Fuchs RA, See RE (2006) Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 187:60-67.

Kishimoto T, Pearse RV, 2nd, Lin CR, Rosenfeld MG (1995) A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc Natl Acad Sci U S A 92:1108-1112.

Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J (2000) Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nat Genet 24:415-419.

Klitenick MA, DeWitte P, Kalivas PW (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. J Neurosci 12:2623-2632.

Kobayashi T, Washiyama K, Ikeda K (2004) Modulators of G protein-activated inwardly rectifying K+ channels: potentially therapeutic agents for addictive drug users. Ann N Y Acad Sci 1025:590-594.

Kodangattil JN, Dacher M, Authement ME, Nugent FS (2013) Spike timing-dependent plasticity at GABAergic synapses in the Ventral tegmental area. J Physiol.

Konkoy CS, Davis TP (1996) Ectoenzymes as sites of peptide regulation. Trends Pharmacol Sci 17:288-294.

Koob G, Kreek MJ (2007) Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry 164:1149-1159.

Koob GF (1999a) The role of the striatopallidal and extended amygdala systems in drug addiction. Ann N Y Acad Sci 877:445-460.

Koob GF (1999b) Stress, corticotropin-releasing factor, and drug addiction. Ann N Y Acad Sci 897:27-45.

Koob GF (2008) A role for brain stress systems in addiction. Neuron 59:11-34.

Koob GF (2009) Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology 56 Suppl 1:18-31.

Koob GF (2010) The role of CRF and CRF-related peptides in the dark side of addiction. Brain Res 1314:3-14.

Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, O'Dell LE, Parsons LH, Sanna PP (2004) Neurobiological mechanisms in the transition from drug use to drug dependence. Neurosci Biobehav Rev 27:739-749.

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715-723.

Koob GF, Heinrichs SC (1999) A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 848:141-152.

Koob GF, Heinrichs, S.C., Menzaghi, F., Pich, E.M., and Britton, K.T. (1994)

Corticotropin releasing factor, stress and behavior. Seminars in Neuroscience 6:221-229.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52-58.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129.

Koob GF, Le Moal M (2008) Review. Neurobiological mechanisms for opponent motivational processes in addiction. Philos Trans R Soc Lond B Biol Sci 363:3113-3123.

Koob GF, Thatcher-Britton K (1985) Stimulant and anxiogenic effects of corticotropin releasing factor. Prog Clin Biol Res 192:499-506.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217-238.

Koob GF, Zorrilla EP (2010) Neurobiological mechanisms of addiction: focus on corticotropin-releasing factor. Curr Opin Investig Drugs 11:63-71.

Korf J, Aghajanian GK, Roth RH (1973) Increased turnover of norepinephrine in the rat cerebral cortex during stress: role of the locus coeruleus. Neuropharmacology 12:933-938.

Korosi A, Kozicz T, Richter J, Veening JG, Olivier B, Roubos EW (2007) Corticotropin-releasing factor, urocortin 1, and their receptors in the mouse spinal cord. J Comp Neurol 502:973-989.

Korosi A, Veening JG, Kozicz T, Henckens M, Dederen J, Groenink L, van der Gugten J, Olivier B, Roubos EW (2006) Distribution and expression of CRF receptor 1 and 2 mRNAs in the CRF over-expressing mouse brain. Brain Res 1072:46-54.

Korotkova TM, Brown RE, Sergeeva OA, Ponomarenko AA, Haas HL (2006) Effects of arousal- and feeding-related neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area of the rat. Eur J Neurosci 23:2677-2685.

Korte SM, Bouws GA, Bohus B (1993) Central actions of corticotropin-releasing hormone (CRH) on behavioral, neuroendocrine, and cardiovascular regulation: brain corticoid receptor involvement. Horm Behav 27:167-183.

Kostich WA, Chen A, Sperle K, Largent BL (1998) Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2gamma receptor. Mol Endocrinol 12:1077-1085.

Kovacs KJ (1998) c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int 33:287-297.

Kovacs KJ (2008) Measurement of immediate-early gene activation- c-fos and beyond. J Neuroendocrinol 20:665-672.

Kovacs KJ, Sawchenko PE (1996a) Regulation of stress-induced transcriptional changes in the hypothalamic neurosecretory neurons. Journal of molecular neuroscience : MN 7:125-133.

Kovacs KJ, Sawchenko PE (1996b) Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. J Neurosci 16:262-273.

Koyrakh L, Lujan R, Colon J, Karschin C, Kurachi Y, Karschin A, Wickman K (2005) Molecular and cellular diversity of neuronal G-protein-gated potassium channels. J Neurosci 25:11468-11478.

Kozicz T (2007) On the role of urocortin 1 in the non-preganglionic Edinger-Westphal nucleus in stress adaptation. Gen Comp Endocrinol 153:235-240.

Kozicz T (2009) Neurobiology of corticotrophin-releasing factor system components in stress. In: Neuropeptides and Peptide analogs: Adaptation to Maladaptation (Kovacs, M., and Merchenthaler, I., ed), pp 59-90: Kerala: Research Signpost.

Kozicz T, Li M, Arimura A (2001) The activation of urocortin immunoreactive neurons in the Einger-Westphal nucleus following stress in rats. Stress 4:85-90.

Kozicz T, Sterrenburg L, Xu L (2011) Does midbrain urocortin 1 matter? A 15-year journey from stress (mal)adaptation to energy metabolism. Stress 14:376-383.

Kreek MJ (ed.) (1987) Psychopharmacology: The Third Generation of Progress. New York: Raven Press.

Kreek MJ, Koob GF (1998) Drug dependence: stress and dysregulation of brain reward pathways. Drug Alcohol Depend 51:23-47.

Kretschmer BD (1999) Modulation of the mesolimbic dopamine system by glutamate: role of NMDA receptors. J Neurochem 73:839-848.

Kudo T, Uchigashima M, Miyazaki T, Konno K, Yamasaki M, Yanagawa Y, Minami M, Watanabe M (2012) Three types of neurochemical projection

from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. J Neurosci 32:18035-18046.

Kufahl PR, Zavala AR, Singh A, Thiel KJ, Dickey ED, Joyce JN, Neisewander JL (2009) c-Fos expression associated with reinstatement of cocaine-seeking behavior by response-contingent conditioned cues. Synapse 63:823-835.

Kuhar MJ, Pilotte NS (1996) Neurochemical changes in cocaine withdrawal. Trends Pharmacol Sci 17:260-264.

Kushner SA, and Unterwald, E.M. (2001) Chronic cocaine administration decreases the functional coupling of GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Life Sci 69.

Labouebe G, Lomazzi M, Cruz HG, Creton C, Lujan R, Li M, Yanagawa Y, Obata K, Watanabe M, Wickman K, Boyer SB, Slesinger PA, Luscher C (2007) RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Nat Neurosci 10:1559-1568.

Lacey MG, Mercuri NB, North RA (1987) Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. J Physiol 392:397-416.

Lacey MG, Mercuri NB, North RA (1988) On the potassium conductance increase activated by GABAB and dopamine D2 receptors in rat substantia nigra neurones. J Physiol 401:437-453.

LaLumiere RT, Kalivas PW (2008) Glutamate release in the nucleus accumbens core is necessary for heroin seeking. J Neurosci 28:3170-3177.

LaLumiere RT, Niehoff KE, Kalivas PW (2010) The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. Learn Mem 17:168-175.

LaLumiere RT, Smith KC, Kalivas PW (2012) Neural circuit competition in cocaine-seeking: roles of the infralimbic cortex and nucleus accumbens shell. Eur J Neurosci 35:614-622.

Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J (2008) Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. Neuron 57:760-773.

Lammel S, Ion DI, Roeper J, Malenka RC (2011) Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. Neuron 70:855-862.

Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC (2012) Input-specific control of reward and aversion in the ventral tegmental area. Nature 491:212-217.

Land BB, Bruchas MR, Lemos JC, Xu M, Melief EJ, Chavkin C (2008) The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. J Neurosci 28:407-414.

Lane DA, Reed B, Kreek MJ, Pickel VM (2011) Differential glutamate AMPA-receptor plasticity in subpopulations of VTA neurons in the presence or absence of residual cocaine: implications for the development of addiction. Neuropharmacology 61:1129-1140.

Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, Baldwin RM, Charney DS, Hoffer PB, Kung HF, et al. (1995) SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 36:1182-1190.

Laskowitz DT, Lei B, Dawson HN, Wang H, Bellows ST, Christensen DJ, Vitek MP, James ML (2012) The apoE-mimetic peptide, COG1410, improves functional recovery in a murine model of intracerebral hemorrhage. Neurocritical care 16:316-326.

Lavicky J, Dunn AJ (1993) Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. J Neurochem 60:602-612.

Lavielle S, Tassin, J.P., Thierry, A.M., Blanc, G., Herve, D., Bathelemy, C., and Glowinski, J. (1978) Blockade by benzodiazepines of the selective high increase in dopamine turnover induced by stress in mesocortical dopaminergic neurons of the rat. Brain Res 168:585-594.

Laviolette SR, Gallegos RA, Henriksen SJ, van der Kooy D (2004) Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area. Nat Neurosci 7:160-169.

Laviolette SR, van der Kooy D (2001) GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. Eur J Neurosci 13:1009-1015.

Laviolette SR, van der Kooy D (2004) GABAA receptors signal bidirectional reward transmission from the ventral tegmental area to the tegmental pedunculopontine nucleus as a function of opiate state. Eur J Neurosci 20:2179-2187.

Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y (2000) The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. Psychopharmacology (Berl) 150:317-324.

Lee Y, Davis M (1997) Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. J Neurosci 17:6434-6446.

Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko PE, Vale WW (2001) Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci U S A 98:7570-7575.

Li C, Vaughan J, Sawchenko PE, Vale WW (2002) Urocortin III-immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. J Neurosci 22:991-1001.

Li CS, Huang C, Yan P, Bhagwagar Z, Milivojevic V, Sinha R (2008) Neural correlates of impulse control during stop signal inhibition in cocaine-dependent men. Neuropsychopharmacology 33:1798-1806.

Liaw CW, Lovenberg TW, Barry G, Oltersdorf T, Grigoriadis DE, de Souza EB (1996) Cloning and characterization of the human corticotropin-releasing factor-2 receptor complementary deoxyribonucleic acid. Endocrinology 137:72-77.

Lima MS, Reisser AA, Soares BG, Farrell M (2003) Antidepressants for cocaine dependence. Cochrane Database Syst Rev CD002950.

Linthorst AC, Flachskamm C, Hopkins SJ, Hoadley ME, Labeur MS, Holsboer F, Reul JM (1997) Long-term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral, and cytokine responses to a systemic inflammatory challenge. J Neurosci 17:4448-4460.

Littman GK, Eiser, J.R., and Rawson, N.S. (1977) Towards a typology of relapse: a preliminary report. Drug Alcohol Depend 2:157-162.

Liu J, Yu B, Orozco-Cabal L, Grigoriadis DE, Rivier J, Vale WW, Shinnick-Gallagher P, Gallagher JP (2005) Chronic cocaine administration switches corticotropin-releasing factor2 receptor-mediated depression to facilitation of glutamatergic transmission in the lateral septum. J Neurosci 25:577-583.

Lodge DJ, Grace AA (2005) Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity. J Pharmacol Exp Ther 314:201-206.

Lomax P (1966) The distribution of morphine following intracerebral microinjection. Experientia 22:249-250.

Lombardo KA, Herringa RJ, Balachandran JS, Hsu DT, Bakshi VP, Roseboom PH, Kalin NH (2001) Effects of acute and repeated restraint stress on corticotropin-releasing hormone binding protein mRNA in rat amygdala and dorsal hippocampus. Neurosci Lett 302:81-84.

Loughlin SE, Fallon JH (1984) Substantia nigra and ventral tegmental area projections to cortex: topography and collateralization. Neuroscience 11:425-435.

Lovenberg TW, Chalmers DT, Liu C, De Souza EB (1995a) CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136:4139-4142.

Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T (1995b) Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A 92:836-840.

Loweth JA, Tseng KY, Wolf ME (2013) Adaptations in AMPA receptor transmission in the nucleus accumbens contributing to incubation of cocaine craving. Neuropharmacology.

Lu L, Grimm JW, Dempsey J, Shaham Y (2004) Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. Psychopharmacology (Berl) 176:101-108.

Lu L, Liu D, Ceng X (2001) Corticotropin-releasing factor receptor type 1 mediates stress-induced relapse to cocaine-conditioned place preference in rats. Eur J Pharmacol 415:203-208.

Lu L, Liu Z, Huang M, Zhang Z (2003a) Dopamine-dependent responses to cocaine depend on corticotropin-releasing factor receptor subtypes. J Neurochem 84:1378-1386.

Lu L, Shepard JD, Hall FS, Shaham Y (2003b) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev 27:457-491.

Lu XY, Churchill L, Kalivas PW (1997) Expression of D1 receptor mRNA in projections from the forebrain to the ventral tegmental area. Synapse 25:205-214.

Luckman SM, Dyball RE, Leng G (1994) Induction of c-fos expression in hypothalamic magnocellular neurons requires synaptic activation and not simply increased spike activity. J Neurosci 14:4825-4830.

Ludwig AM, Wikler A (1974) "Craving" and relapse to drink. Q J Stud Alcohol 35:108-130.

Lukkes JL, Staub DR, Dietrich A, Truitt W, Neufeld-Cohen A, Chen A, Johnson PL, Shekhar A, Lowry CA (2011) Topographical distribution of corticotropin-releasing factor type 2 receptor-like immunoreactivity in the rat dorsal raphe nucleus: co-localization with tryptophan hydroxylase. Neuroscience 183:47-63.

Luo X, Kiss A, Rabadan-Diehl C, Aguilera G (1995) Regulation of hypothalamic and pituitary corticotropin-releasing hormone receptor messenger ribonucleic acid by adrenalectomy and glucocorticoids. Endocrinology 136:3877-3883.

Luscher C, Huber KM (2010) Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 65:445-459.

Luscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650-663.

Lymangrover JR, Brodish A (1973) Tissue CRF: an extra-hypothalamic corticotrophin releasing factor (CRF) in the peripheral blood of stressed rats. Neuroendocrinology 12:225-235.

Lysakowski A, Figueras H, Price SD, Peng YY (1999) Dense-cored vesicles, smooth endoplasmic reticulum, and mitochondria are closely associated with non-specialized parts of plasma membrane of nerve terminals: implications for exocytosis and calcium buffering by intraterminal organelles. J Comp Neurol 403:378-390.

Ma QP, Zhou Y, Han JS (1993a) Electroacupuncture accelerated the expression of c-Fos protooncogene in dopaminergic neurons in the ventral tegmental area of the rat. Int J Neurosci 70:217-222.

Ma QP, Zhou Y, Han JS (1993b) Noxious stimulation accelerated the expression of c-fos protooncogene in cholecystokininergic and dopaminergic neurons in the ventral tegmental area. Peptides 14:561-566.

Maclean PD (1957) Chemical and electrical stimulation of hippocampus in unrestrained animals. II. Behavioral findings. AMA archives of neurology and psychiatry 78:128-142.

Madayag A, Kau KS, Lobner D, Mantsch JR, Wisniewski S, Baker DA (2011) Drug-induced plasticity contributing to heightened relapse susceptibility: neurochemical changes and augmented reinstatement in high-intake rats. J Neurosci 30:210-217.

Madhavan A, Bonci A, Whistler JL (2010) Opioid-Induced GABA potentiation after chronic morphine attenuates the rewarding effects of opioids in the ventral tegmental area. J Neurosci 30:14029-14035.

Magreta-Mitrovic M, Mitrovic, I., Riley, R.C., Jan, L.Y., and Basbaum, A.I. (1999) Immunohistochemical localization of GABAB receptors in the rat central nervous system. J Comp Neurol 405:85-114.

Mahler SV, Aston-Jones GS (2012) Fos activation of selective afferents to ventral tegmental area during cue-induced reinstatement of cocaine seeking in rats. J Neurosci 32:13309-13326.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.

Malonek D, Grinvald A (1996) Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. Science 272:551-554.

Mameli M, Balland B, Lujan R, Luscher C (2007) Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. Science 317:530-533.

Mameli M, Bellone C, Brown MT, Luscher C (2011) Cocaine inverts rules for synaptic plasticity of glutamate transmission in the ventral tegmental area. Nat Neurosci 14:414-416.

Mansi JA, Rivest S, Drolet G (1996) Regulation of corticotropin-releasing factor type 1 (CRF1) receptor messenger ribonucleic acid in the paraventricular nucleus of rat hypothalamus by exogenous CRF. Endocrinology 137:4619-4629.

Mansvelder HD, Keath JR, McGehee DS (2002) Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. Neuron 33:905-919.

Mansvelder HD, McGehee DS (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron 27:349-357.

Mantsch JR, Baker DA, Francis DM, Katz ES, Hoks MA, Serge JP (2008a) Stressor- and corticotropin releasing factor-induced reinstatement and active stress-related behavioral responses are augmented following long-access cocaine self-administration by rats. Psychopharmacology (Berl) 195:591-603.

Mantsch JR, Baker DA, Serge JP, Hoks MA, Francis DM, Katz ES (2008b) Surgical adrenalectomy with diurnal corticosterone replacement slows escalation and prevents the augmentation of cocaine-induced reinstatement in rats self-administering cocaine under long-access conditions. Neuropsychopharmacology 33:814-826.

Mantsch JR, Cullinan WE, Tang LC, Baker DA, Katz ES, Hoks MA, Ziegler DR (2007) Daily cocaine self-administration under long-access conditions augments restraint-induced increases in plasma corticosterone and impairs glucocorticoid receptor-mediated negative feedback in rats. Brain Res 1167:101-111.

Mantsch JR, Goeders NE (1999) Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: relationship to the discriminative stimulus effects of cocaine. Psychopharmacology (Berl) 142:399-407.

Mantsch JR, Goeders NE (2000) Effects of cocaine self-administration on plasma corticosterone in rats: relationship to hippocampal type II glucocorticoid receptors. Prog Neuropsychopharmacol Biol Psychiatry 24:633-646.

Mantsch JR, Katz ES (2007) Elevation of glucocorticoids is necessary but not sufficient for the escalation of cocaine self-administration by chronic electric footshock stress in rats. Neuropsychopharmacology 32:367-376.

Mantsch JR, Saphier D, Goeders NE (1998) Corticosterone facilitates the acquisition of cocaine self-administration in rats: opposite effects of the type II glucocorticoid receptor agonist dexamethasone. J Pharmacol Exp Ther 287:72-80.

Mantsch JR, Schlussman SD, Ho A, Kreek MJ (2000) Effects of cocaine self-administration on plasma corticosterone and prolactin in rats. J Pharmacol Exp Ther 294:239-247.

Mantsch JR, Vranjkovic O, Twining RC, Gasser PJ, McReynolds JR, Blacktop JM (2014) Neurobiological mechanisms that contribute to stress-related cocaine use. Neuropharmacology 76 Pt B:383-394.

Mantsch JR, Weyer A, Vranjkovic O, Beyer CE, Baker DA, Caretta H (2010) Involvement of noradrenergic neurotransmission in the stress- but not cocaine-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: role for beta-2 adrenergic receptors. Neuropsychopharmacology 35:2165-2178.

Mantsch JR, Yuferov V, Mathieu-Kia AM, Ho A, Kreek MJ (2003) Neuroendocrine alterations in a high-dose, extended-access rat self-administration model of escalating cocaine use. Psychoneuroendocrinology 28:836-862.

Mantsch JR, Yuferov V, Mathieu-Kia AM, Ho A, Kreek MJ (2004) Effects of extended access to high versus low cocaine doses on self-administration, cocaine-induced reinstatement and brain mRNA levels in rats. Psychopharmacology (Berl) 175:26-36.

Mantz J, Thierry AM, Glowinski J (1989) Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons: selective activation of the mesocortical system. Brain Res 476:377-381.

Manzoni OJ, Williams JT (1999) Presynaptic regulation of glutamate release in the ventral tegmental area during morphine withdrawal. J Neurosci 19:6629-6636.

Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY, Basbaum AI (1999) Immunohistochemical localization of GABA(B) receptors in the rat central nervous system. J Comp Neurol 405:299-321.

Margolis EB, Lock H, Hjelmstad GO, Fields HL (2006) The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? J Physiol 577:907-924.

Margolis EB, Mitchell JM, Ishikawa J, Hjelmstad GO, Fields HL (2008) Midbrain dopamine neurons: projection target determines action potential duration and dopamine D(2) receptor inhibition. J Neurosci 28:8908-8913.

Margolis EB, Toy B, Himmels P, Morales M, Fields HL (2012) Identification of rat ventral tegmental area GABAergic neurons. PLoS One 7:e42365.

Marinelli M, Cooper DC, Baker LK, White FJ (2003) Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior. Psychopharmacology (Berl) 168:84-98.

Marinelli M, Rudick CN, Hu XT, White FJ (2006) Excitability of dopamine neurons: modulation and physiological consequences. CNS Neurol Disord Drug Targets 5:79-97.

Markou A, Arroyo M, Everitt BJ (1999) Effects of contingent and non-contingent cocaine on drug-seeking behavior measured using a second-order schedule of cocaine reinforcement in rats. Neuropsychopharmacology 20:542-555.

Markou A, Koob GF (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology 4:17-26.

Markou A, Koob GF (1992) Bromocriptine reverses the elevation in intracranial self-stimulation thresholds observed in a rat model of cocaine withdrawal. Neuropsychopharmacology 7:213-224.

Marlatt AG (1996) Models of relapse and relapse prevention: a commentary. Exp Clin Psychopharmacol 4:55-60.

Marlatt G (ed.) (1978) Behavioral assessments of social drinking and alcoholism. New Brunswick, New Jersey: Rutgers Center for Alcohol Studies Publications

Marlatt G, Gordon, J. (ed.) (1980) Determinants of relapse: implications for the maintenance of behavioral change. New York: Brunner/Mazel.

Marlatt GA (1990) Cue exposure and relapse prevention in the treatment of addictive behaviors. Addict Behav 15:395-399.

Marlatt GA, Baer JS, Donovan DM, Kivlahan DR (1988) Addictive behaviors: etiology and treatment. Annu Rev Psychol 39:223-252.

Marlatt GA, Gordon, J.R. (1985) Relapse prevention: maintenance strategies in the treatment of addictive behavior. New York: Guilford.

Martin WR, Jasinski DR (1969) Physiological parameters of morphine dependence in man--tolerance, early abstinence, protracted abstinence. J Psychiatr Res 7:9-17.

Mathe JM, Nomikos GG, Schilstrom B, Svensson TH (1998) Non-NMDA excitatory amino acid receptors in the ventral tegmental area mediate systemic dizocilpine (MK-801) induced hyperlocomotion and dopamine release in the nucleus accumbens. Journal of neuroscience research 51:583-592.

Matochik JA, London ED, Eldreth DA, Cadet JL, Bolla KI (2003) Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. Neuroimage 19:1095-1102.

Matsumoto M, Hikosaka O (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447:1111-1115.

Matsumoto M, Hikosaka O (2009a) Representation of negative motivational value in the primate lateral habenula. Nat Neurosci 12:77-84.

Matsumoto M, Hikosaka O (2009b) Two types of dopamine neuron distinctly convey positive and negative motivational signals. Nature 459:837-841.

Matsuzaki I, Takamatsu Y, Moroji T (1989) The effects of intracerebroventricularly injected corticotropin-releasing factor (CRF) on the central nervous system: behavioural and biochemical studies. Neuropeptides 13:147-155.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 309:261-263.

Maywood ES, Bittman EL, Ebling FJ, Barrett P, Morgan P, Hastings MH (1995) Regional distribution of iodomelatonin binding sites within the suprachiasmatic nucleus of the Syrian hamster and the Siberian hamster. J Neuroendocrinol 7:215-223.

McClure SM, Daw ND, Montague PR (2003) A computational substrate for incentive salience. Trends Neurosci 26:423-428.

McEwen BS, Stellar E (1993) Stress and the individual. Mechanisms leading to disease. Arch Intern Med 153:2093-2101.

McFarland K, Davidge SB, Lapish CC, Kalivas PW (2004) Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J Neurosci 24:1551-1560.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655-8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531-3537.

McKay JR, Alterman AI, McLellan AT, Boardman CR, Mulvaney FD, O'Brien CP (1998) Random versus nonrandom assignment in the evaluation of treatment for cocaine abusers. J Consult Clin Psychol 66:697-701.

McKay JR, Alterman AI, Rutherford MJ, Cacciola JS, McLellan AT (1999) The relationship of alcohol use to cocaine relapse in cocaine dependent patients in an aftercare study. J Stud Alcohol 60:176-180.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl) 168:57-65.

McNally GP, Johansen JP, Blair HT (2011) Placing prediction into the fear circuit. Trends Neurosci 34:283-292.

Mead AN, Stephens DN (1999) CNQX but not NBQX prevents expression of amphetamine-induced place preference conditioning: a role for the glycine site of the NMDA receptor, but not AMPA receptors. J Pharmacol Exp Ther 290:9-15.

Mendelson JH, Mello NK (1996) Management of cocaine abuse and dependence. The New England journal of medicine 334:965-972.

Menzaghi F, Howard RL, Heinrichs SC, Vale W, Rivier J, Koob GF (1994) Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J Pharmacol Exp Ther 269:564-572.

Merchenthaler I, Vigh S, Petrusz P, Schally AV (1982) Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am J Anat 165:385-396.

Mereu G, Costa E, Armstrong DM, Vicini S (1991) Glutamate receptor subtypes mediate excitatory synaptic currents of dopamine neurons in midbrain slices. J Neurosci 11:1359-1366.

Merighi A (2002) Costorage and coexistence of neuropeptides in the mammalian CNS. Prog Neurobiol 66:161-190.

Meyer RE, Mirin, S.M. (ed.) (1979) The Heroin Stimulus: Implications for a Theory of Addiction. New York: Plenum Medical Book Company.

Michaeli A, Yaka R (2010) Dopamine inhibits GABA(A) currents in ventral tegmental area dopamine neurons via activation of presynaptic G-protein coupled inwardly-rectifying potassium channels. Neuroscience 165:1159-1169.

Miczek KA, Nikulina EM, Takahashi A, Covington HE, 3rd, Yap JJ, Boyson CO, Shimamoto A, de Almeida RM (2011) Gene expression in aminergic and

peptidergic cells during aggression and defeat: relevance to violence, depression and drug abuse. Behav Genet 41:787-802.

Miliaressis E, Rompre PP, Laviolette P, Philippe L, Coulombe D (1986) The curve-shift paradigm in self-stimulation. Physiol Behav 37:85-91.

Millan EZ, Marchant NJ, McNally GP (2011) Extinction of drug seeking. Behav Brain Res 217:454-462.

Millan EZ, McNally GP (2011) Accumbens shell AMPA receptors mediate expression of extinguished reward seeking through interactions with basolateral amygdala. Learn Mem 18:414-421.

Miller NE, Gottesman KS, Emery N (1964) Dose Response to Carbachol and Norepinephrine in Rat Hypothalamus. Am J Physiol 206:1384-1388.

Miller NS, Goldsmith RJ (2001) Craving for alcohol and drugs in animals and humans: biology and behavior. J Addict Dis 20:87-104.

Miller NS, Summers GL, Gold MS (1993) Cocaine dependence: alcohol and other drug dependence and withdrawal characteristics. J Addict Dis 12:25-35.

Miner P, Borkuhova Y, Shimonova L, Khaimov A, Bodnar RJ (2010) GABA-A and GABA-B receptors mediate feeding elicited by the GABA-B agonist baclofen in the ventral tegmental area and nucleus accumbens shell in rats: reciprocal and regional interactions. Brain Res 1355:86-96.

Mirenowicz J, Schultz W (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379:449-451.

Misgeld U, Bijak M, Jarolimek W (1995) A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system. Prog Neurobiol 46:423-462.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69-97.

Moghaddam B (1993) Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. J Neurochem 60:1650-1657.

Moghaddam B (2002) Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biol Psychiatry 51:775-787.

Moghaddam B, Bolinao ML, Stein-Behrens B, Sapolsky R (1994) Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. Brain Res 655:251-254.

Moisan J, Rompre PP (1998) Electrophysiological evidence that a subset of midbrain dopamine neurons integrate the reward signal induced by electrical stimulation of the posterior mesencephalon. Brain Res 786:143-152.

Moldow RL, Fischman AJ (1987) Cocaine induced secretion of ACTH, beta-endorphin, and corticosterone. Peptides 8:819-822.

Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29:365-402.

Montague PR, Hyman SE, Cohen JD (2004) Computational roles for dopamine in behavioural control. Nature 431:760-767.

Moore RY, Bloom FE (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. Annu Rev Neurosci 1:129-169.

Moore RY, Bloom FE (1979) Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annu Rev Neurosci 2:113-168.

Moreau J-L, Kilpatrick, G., Jenck, F., and Hoffman, F. (1997) Corticotropin-releasing factor and anxiety: Animal studies. European Neuropsychopharmacology 7:S88-89.

Morgan JI, Cohen DR, Hempstead JL, Curran T (1987) Mapping patterns of c-fos expression in the central nervous system after seizure. Science 237:192-197.

Morgan JI, Curran T (1986) Role of ion flux in the control of c-fos expression. Nature 322:552-555.

Morgan JI, Curran T (1989) Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci 12:459-462.

Morita M, Yano S, Yamaguchi T, Yamauchi M, Sugimoto T Phenylacetic acid stimulates reactive oxygen species generation and tumor necrosis factor-alpha secretion in vascular endothelial cells. Ther Apher Dial 15:147-150.

Morrow BA, Elsworth JD, Roth RH (2001) Prenatal exposure to cocaine reduces the number and enhances reactivity of A10 dopaminergic neurons to environmental stress. Synapse 41:337-344.

Mueller D, Stewart J (2000) Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav Brain Res 115:39-47.

Murase S, Grenhoff J, Chouvet G, Gonon FG, Svensson TH (1993a) Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. Neurosci Lett 157:53-56.

Murase S, Mathe JM, Grenhoff J, Svensson TH (1993b) Effects of dizocilpine (MK-801) on rat midbrain dopamine cell activity: differential actions on firing pattern related to anatomical localization. J Neural Transm Gen Sect 91:13-25.

Myers RD (1966) Injection of solutions into cerebral tissue: Relation between volume and diffusion. Physiol Behav 1:171-174.

Nassel DR (2009) Neuropeptide signaling near and far: how localized and timed is the action of neuropeptides in brain circuits? Invert Neurosci 9:57-75.

NDIC (2011) National Drug Threat Assessment. National Drug Intelligence Center: US Department of Justice National Drug Intelligence Center Prdouct No. 2011-Q0317-001.

Nemeroff CB (1997) Overview of CRF in psychiatric diseases European Neuropsychopharmacology 7:S86.

Nemoto T, Yamauchi N, Shibasaki T (2009) Novel action of pituitary urocortin 2 in the regulation of expression and secretion of gonadotropins. J Endocrinol 201:105-114.

Nestler EJ, Terwilliger RZ, Walker JR, Sevarino KA, Duman RS (1990) Chronic cocaine treatment decreases levels of the G protein subunits Gi alpha and Go alpha in discrete regions of rat brain. J Neurochem 55:1079-1082.

Neufeld-Cohen A, Evans AK, Getselter D, Spyroglou A, Hill A, Gil S, Tsoory M, Beuschlein F, Lowry CA, Vale W, Chen A (2010a) Urocortin-1 and -2 double-deficient mice show robust anxiolytic phenotype and modified serotonergic activity in anxiety circuits. Mol Psychiatry 15:426-441, 339.

Neufeld-Cohen A, Kelly PA, Paul ED, Carter RN, Skinner E, Olverman HJ, Vaughan JM, Issler O, Kuperman Y, Lowry CA, Vale WW, Seckl JR, Chen A, Jamieson PM (2012) Chronic activation of corticotropin-releasing factor

type 2 receptors reveals a key role for 5-HT1A receptor responsiveness in mediating behavioral and serotonergic responses to stressful challenge. Biol Psychiatry 72:437-447.

Neufeld-Cohen A, Tsoory MM, Evans AK, Getselter D, Gil S, Lowry CA, Vale WW, Chen A (2010b) A triple urocortin knockout mouse model reveals an essential role for urocortins in stress recovery. Proc Natl Acad Sci U S A 107:19020-19025.

Newton I (1686) Principia Mathematica Philosophiae Naturalis: Three Laws of Motion.

Nie Z, Zorrilla EP, Madamba SG, Rice KC, Roberto M, Siggins GR (2009) Presynaptic CRF1 receptors mediate the ethanol enhancement of GABAergic transmission in the mouse central amygdala. ScientificWorldJournal 9:68-85.

Niehaus JL, Murali M, Kauer JA (2010) Drugs of abuse and stress impair LTP at inhibitory synapses in the ventral tegmental area. Eur J Neurosci 32:108-117.

Nielsen SM, Nielsen LZ, Hjorth SA, Perrin MH, Vale WW (2000) Constitutive activation of tethered-peptide/corticotropin-releasing factor receptor chimeras. Proc Natl Acad Sci U S A 97:10277-10281.

Nikulina EM, Covington HE, 3rd, Ganschow L, Hammer RP, Jr., Miczek KA (2004) Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. Neuroscience 123:857-865.

Nishino H, Ono T, Muramoto K, Fukuda M, Sasaki K (1987) Neuronal activity in the ventral tegmental area (VTA) during motivated bar press feeding in the monkey. Brain Res 413:302-313.

Nissbrandt H, Elverfors A, Engberg G (1994) Pharmacologically induced cessation of burst activity in nigral dopamine neurons: significance for the terminal dopamine efflux. Synapse 17:217-224.

Nolan BC, Saliba M, Tanchez C, Ranaldi R (2010) Behavioral activating effects of selective AMPA receptor antagonism in the ventral tegmental area. Pharmacology 86:336-343.

Novak CM, Smale L, Nunez AA (2000) Rhythms in Fos expression in brain areas related to the sleep-wake cycle in the diurnal Arvicanthis niloticus. American journal of physiology Regulatory, integrative and comparative physiology 278:R1267-1274.

Nugent FS, Niehaus JL, Kauer JA (2009) PKG and PKA signaling in LTP at GABAergic synapses. Neuropsychopharmacology 34:1829-1842.

Nugent FS, Penick EC, Kauer JA (2007) Opioids block long-term potentiation of inhibitory synapses. Nature 446:1086-1090.

Nusbaum MP (2002) Regulating peptidergic modulation of rhythmically active neural circuits. Brain Behav Evol 60:378-387.

O'Brien CP (1997) A range of research-based pharmacotherapies for addiction. Science 278:66-70.

O'Brien CP (2005) Anticraving medications for relapse prevention: a possible new class of psychoactive medications. Am J Psychiatry 162:1423-1431.

O'Brien CP (ed.) (2011) Drug Addiction. In Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York: McGraw-Hill.

O'Brien CP, Childress AR, Ehrman R, Robbins SJ (1998) Conditioning factors in drug abuse: can they explain compulsion? J Psychopharmacol 12:15-22.

O'Brien CP, Ehrman, R., and Ternes, J.W. (ed.) (1986) Behavioral Analysis of Drug Dependence. Orlando, FL: Academic Press.

O'Brien CP, McLellan AT (1996) Myths about the treatment of addiction. Lancet 347:237-240.

Oades RD, Halliday GM (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 434:117-165.

Oakley NR, Hayes AG, Sheehan MJ (1991) Effect of typical and atypical neuroleptics on the behavioural consequences of activation by muscimol of mesolimbic and nigro-striatal dopaminergic pathways in the rat. Psychopharmacology (Berl) 105:204-208.

Oakley RH, Olivares-Reyes JA, Hudson CC, Flores-Vega F, Dautzenberg FM, Hauger RL (2007) Carboxyl-terminal and intracellular loop sites for CRF1 receptor phosphorylation and beta-arrestin-2 recruitment: a mechanism regulating stress and anxiety responses. American journal of physiology Regulatory, integrative and comparative physiology 293:R209-222.

Okuyama S, Chaki S, Kawashima N, Suzuki Y, Ogawa S, Nakazato A, Kumagai T, Okubo T, Tomisawa K (1999) Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor

subtype 1 receptor antagonists CRA1000 and CRA1001. J Pharmacol Exp Ther 289:926-935.

Olds J, Yuwiler A, Olds ME, Yun C (1964) Neurohumors in Hypothalamic Substrates of Reward. Am J Physiol 207:242-254.

Oleson EB, Gentry RN, Chioma VC, Cheer JF (2012) Subsecond dopamine release in the nucleus accumbens predicts conditioned punishment and its successful avoidance. J Neurosci 32:14804-14808.

Olpe HR, Koella WP, Wolf P, Haas HL (1977) The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. Brain Res 134:577-580.

Olschowka JA, O'Donohue TL, Mueller GP, Jacobowitz DM (1982) Hypothalamic and extrahypothalamic distribution of CRF-like immunoreactive neurons in the rat brain. Neuroendocrinology 35:305-308.

Olson VG, Nestler EJ (2007) Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. Synapse 61:87-95.

Olson VG, Zabetian CP, Bolanos CA, Edwards S, Barrot M, Eisch AJ, Hughes T, Self DW, Neve RL, Nestler EJ (2005) Regulation of drug reward by cAMP response element-binding protein: evidence for two functionally distinct subregions of the ventral tegmental area. J Neurosci 25:5553-5562.

Omelchenko N, Bell R, Sesack SR (2009) Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. Eur J Neurosci 30:1239-1250.

Omelchenko N, Sesack SR (2009) Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. Synapse 63:895-906.

Orozco-Cabal L, Liu J, Pollandt S, Schmidt K, Shinnick-Gallagher P, Gallagher JP (2008) Dopamine and corticotropin-releasing factor synergistically alter basolateral amygdala-to-medial prefrontal cortex synaptic transmission: functional switch after chronic cocaine administration. J Neurosci 28:529-542.

Orth DN, Mount CD (1987) Specific high-affinity binding protein for human corticotropin-releasing hormone in normal human plasma. Biochemical and biophysical research communications 143:411-417.

Otis TS, Mody I (1992) Differential activation of GABAA and GABAB receptors by spontaneously released transmitter. J Neurophysiol 67:227-235.

Overton P, Clark D (1992) Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse 10:131-140.

Overton PG, Clark D (1997) Burst firing in midbrain dopaminergic neurons. Brain Res Brain Res Rev 25:312-334.

Pacak K, Palkovits M (2001) Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocr Rev 22:502-548.

Padgett CL, Lalive AL, Tan KR, Terunuma M, Munoz MB, Pangalos MN, Martinez-Hernandez J, Watanabe M, Moss SJ, Lujan R, Luscher C, Slesinger PA (2012) Methamphetamine-Evoked Depression of GABA(B) Receptor Signaling in GABA Neurons of the VTA. Neuron 73:978-989.

Paladini CA, Celada P, Tepper JM (1999a) Striatal, pallidal, and pars reticulata evoked inhibition of nigrostriatal dopaminergic neurons is mediated by GABA(A) receptors in vivo. Neuroscience 89:799-812.

Paladini CA, Iribe Y, Tepper JM (1999b) GABAA receptor stimulation blocks NMDA-induced bursting of dopaminergic neurons in vitro by decreasing input resistance. Brain Res 832:145-151.

Paladini CA, Tepper JM (1999) GABA(A) and GABA(B) antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. Synapse 32:165-176.

Palchaudhuri MR, Hauger RL, Wille S, Fuchs E, Dautzenberg FM (1999) Isolation and pharmacological characterization of two functional splice variants of corticotropin-releasing factor type 2 receptor from Tupaia belangeri. J Neuroendocrinol 11:419-428.

Pan B, Hillard CJ, Liu QS (2008) Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. J Neurosci 28:1385-1397.

Papadopoulou N, Chen J, Randeva HS, Levine MA, Hillhouse EW, Grammatopoulos DK (2004) Protein kinase A-induced negative regulation of the corticotropin-releasing hormone R1alpha receptor-extracellularly regulated kinase signal transduction pathway: the critical role of Ser301 for signaling switch and selectivity. Mol Endocrinol 18:624-639.

Pape JR, Ciofi P, Tramu G (1996) Suckling-induced Fos-immunoreactivity in subgroups of hypothalamic POMC neurons of the lactating rat: investigation of a role for prolactin. J Neuroendocrinol 8:375-386.

Parham KL, Zervou S, Karteris E, Catalano RD, Old RW, Hillhouse EW (2004) Promoter analysis of human corticotropin-releasing factor (CRF) type 1 receptor and regulation by CRF and urocortin. Endocrinology 145:3971-3983.

Park J, Wheeler RA, Fontillas K, Keithley RB, Carelli RM, Wightman RM (2012) Catecholamines in the bed nucleus of the stria terminalis reciprocally respond to reward and aversion. Biol Psychiatry 71:327-334.

Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC (2002) Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. J Neurosci 22:2916-2925.

Pastor R, Reed C, Burkhart-Kasch S, Li N, Sharpe AL, Coste SC, Stenzel-Poore MP, Phillips TJ (2011) Ethanol concentration-dependent effects and the role of stress on ethanol drinking in corticotropin-releasing factor type 1 and double type 1 and 2 receptor knockout mice. Psychopharmacology (Berl) 218:169-177.

Patton MH, Bizup BT, Grace AA (2013) The infralimbic cortex bidirectionally modulates mesolimbic dopamine neuron activity via distinct neural pathways. J Neurosci 33:16865-16873.

Paulus MP, Tapert SF, Schuckit MA (2005) Neural activation patterns of methamphetamine-dependent subjects during decision making predict relapse. Arch Gen Psychiatry 62:761-768.

Paxinos G, and Franklin, K. (ed.) (2000) The Mouse Brain in Stereotaxic Corrdinates. Sand Diego, CA: Academic Press.

Paxinos G, and Watson, C. (ed.) (2004) The Rat Brain In Stereotaxic Coordinates - The New Coronal Set: Elsevier Academic Press.

Paxinos G, Watson, C. (ed.) (2007) The Rat Brain in Stereotaxic Coordinates: Academic Press.

Pelleymounter MA, Joppa M, Carmouche M, Cullen MJ, Brown B, Murphy B, Grigoriadis DE, Ling N, Foster AC (2000) Role of corticotropin-releasing factor (CRF) receptors in the anorexic syndrome induced by CRF. J Pharmacol Exp Ther 293:799-806.

Penit-Soria J, Audinat E, Crepel F (1987) Excitation of rat prefrontal cortical neurons by dopamine: an in vitro electrophysiological study. Brain Res 425:263-274.

Perez-Garci E, Gassmann M, Bettler B, Larkum ME (2006) The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca2+ spikes in layer 5 somatosensory pyramidal neurons. Neuron 50:603-616.

Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P, Vale W (1995) Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc Natl Acad Sci U S A 92:2969-2973.

Perrin MH, DiGruccio MR, Koerber SC, Rivier JE, Kunitake KS, Bain DL, Fischer WH, Vale WW (2003) A soluble form of the first extracellular domain of mouse type 2beta corticotropin-releasing factor receptor reveals differential ligand specificity. J Biol Chem 278:15595-15600.

Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW (1993) Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology 133:3058-3061.

Perrin MH, Fischer WH, Kunitake KS, Craig AG, Koerber SC, Cervini LA, Rivier JE, Groppe JC, Greenwald J, Moller Nielsen S, Vale WW (2001) Expression, purification, and characterization of a soluble form of the first extracellular domain of the human type 1 corticotropin releasing factor receptor. J Biol Chem 276:31528-31534.

Perrin MH, Grace CR, Riek R, Vale WW (2006) The three-dimensional structure of the N-terminal domain of corticotropin-releasing factor receptors: sushi domains and the B1 family of G protein-coupled receptors. Ann N Y Acad Sci 1070:105-119.

Perrin MH, Vale WW (1999) Corticotropin releasing factor receptors and their ligand family. Ann N Y Acad Sci 885:312-328.

Perrotti LI, Bolanos CA, Choi KH, Russo SJ, Edwards S, Ulery PG, Wallace DL, Self DW, Nestler EJ, Barrot M (2005) DeltaFosB accumulates in a GABAergic cell population in the posterior tail of the ventral tegmental area after psychostimulant treatment. Eur J Neurosci 21:2817-2824.

Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279-288.

Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neurosci 28:6046-6053.

Petrusz P, and Merchenthaler, I. (ed.) (1992) The corticotropin-releasing factor system. Boca Raton FL: CRC Press.

Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84:167-173.

Pettit HO, Justice JB, Jr. (1989) Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. Pharmacol Biochem Behav 34:899-904.

Pettit HO, Justice JB, Jr. (1991) Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res 539:94-102.

Pezzone MA, Lee WS, Hoffman GE, Pezzone KM, Rabin BS (1993) Activation of brainstem catecholaminergic neurons by conditioned and unconditioned aversive stimuli as revealed by c-Fos immunoreactivity. Brain Res 608:310-318.

Pezzone MA, Lee WS, Hoffman GE, Rabin BS (1992) Induction of c-Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. Brain Res 597:41-50.

Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. Nature 422:614-618.

Phillipson OT (1979a) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. J Comp Neurol 187:117-143.

Phillipson OT (1979b) The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. J Comp Neurol 187:85-98.

Piazza PV, Deroche V, Deminiere JM, Maccari S, Le Moal M, Simon H (1993) Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviors. Proc Natl Acad Sci U S A 90:11738-11742.

Piazza PV, Le Moal ML (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 36:359-378.

Piazza PV, Marinelli M, Jodogne C, Deroche V, Rouge-Pont F, Maccari S, Le Moal M, Simon H (1994) Inhibition of corticosterone synthesis by Metyrapone decreases cocaine-induced locomotion and relapse of cocaine self-administration. Brain Res 658:259-264.

Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y (2011) Neurobiology of the incubation of drug craving. Trends Neurosci 34:411-420.

Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401-404.

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30:215-238.

Pinnock RD (1984) Hyperpolarizing action of baclofen on neurons in the rat substantia nigra slice. Brain Res 322:337-340.

Pirot S, Godbout R, Mantz J, Tassin JP, Glowinski J, Thierry AM (1992) Inhibitory effects of ventral tegmental area stimulation on the activity of prefrontal cortical neurons: evidence for the involvement of both dopaminergic and GABAergic components. Neuroscience 49:857-865.

Pisarchik A, Slominski AT (2001) Alternative splicing of CRH-R1 receptors in human and mouse skin: identification of new variants and their differential expression. FASEB J 15:2754-2756.

Pollandt S, Liu J, Orozco-Cabal L, Grigoriadis DE, Vale WW, Gallagher JP, Shinnick-Gallagher P (2006) Cocaine withdrawal enhances long-term potentiation induced by corticotropin-releasing factor at central amygdala glutamatergic synapses via CRF, NMDA receptors and PKA. Eur J Neurosci 24:1733-1743.

Potter E, Behan DP, Fischer WH, Linton EA, Lowry PJ, Vale WW (1991) Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. Nature 349:423-426.

Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW (1992) The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. Proc Natl Acad Sci U S A 89:4192-4196.

Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W (1994) Distribution of corticotropin-releasing factor receptor mRNA

expression in the rat brain and pituitary. Proc Natl Acad Sci U S A 91:8777-8781.

Preil J, Muller MB, Gesing A, Reul JM, Sillaber I, van Gaalen MM, Landgrebe J, Holsboer F, Stenzel-Poore M, Wurst W (2001) Regulation of the hypothalamic-pituitary-adrenocortical system in mice deficient for CRH receptors 1 and 2. Endocrinology 142:4946-4955.

Prescott C, Weeks AM, Staley KJ, Partin KM (2006) Kynurenic acid has a dual action on AMPA receptor responses. Neurosci Lett 402:108-112.

Preston KL, Sullivan JT, Strain EC, Bigelow GE (1992) Effects of cocaine alone and in combination with bromocriptine in human cocaine abusers. J Pharmacol Exp Ther 262:279-291.

Primus RJ, Yevich E, Baltazar C, Gallager DW (1997) Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain. Neuropsychopharmacology 17:308-316.

Pucak ML, Grace AA (1994) Evidence that systemically administered dopamine antagonists activate dopamine neuron firing primarily by blockade of somatodendritic autoreceptors. J Pharmacol Exp Ther 271:1181-1192.

Radulovic J, Ruhmann A, Liepold T, Spiess J (1999) Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. J Neurosci 19:5016-5025.

Ranaldi R, Kest K, Zellner MR, Lubelski D, Muller J, Cruz Y, Saliba M (2011) The effects of VTA NMDA receptor antagonism on reward-related learning and associated c-fos expression in forebrain. Behav Brain Res 216:424-432.

Rassnick S, Heinrichs SC, Britton KT, Koob GF (1993) Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. Brain Res 605:25-32.

Raveh A, Riven I, Reuveny E (2009) Elucidation of the gating of the GIRK channel using a spectroscopic approach. J Physiol 587:5331-5335.

Recio J, Pevet P, Masson-Pevet M (1996) Serotonergic modulation of photically induced increase in melatonin receptor density and Fos immunoreactivity in the suprachiasmatic nuclei of the rat. J Neuroendocrinol 8:839-845.

Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM, Dedic N, Schumacher M, von Wolff G, Avrabos C, Touma C, Engblom D, Schutz G, Nave KA, Eder M, Wotjak CT, Sillaber I, Holsboer F, Wurst W,

Deussing JM (2011) Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. Science 333:1903-1907.

Regev L, Neufeld-Cohen A, Tsoory M, Kuperman Y, Getselter D, Gil S, Chen A (2011) Prolonged and site-specific over-expression of corticotropin-releasing factor reveals differential roles for extended amygdala nuclei in emotional regulation. Mol Psychiatry 16:714-728.

Reinhard JF, Jr., Bannon MJ, Roth RH (1982) Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam. Naunyn-Schmiedeberg's archives of pharmacology 318:374-377.

Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117:2505-2511.

Reyes BA, Carvalho AF, Vakharia K, Van Bockstaele EJ (2011) Amygdalar peptidergic circuits regulating noradrenergic locus coeruleus neurons: linking limbic and arousal centers. Exp Neurol 230:96-105.

Reyes BA, Fox K, Valentino RJ, Van Bockstaele EJ (2006) Agonist-induced internalization of corticotropin-releasing factor receptors in noradrenergic neurons of the rat locus coeruleus. Eur J Neurosci 23:2991-2998.

Reyes BA, Valentino RJ, Van Bockstaele EJ (2008) Stress-induced intracellular trafficking of corticotropin-releasing factor receptors in rat locus coeruleus neurons. Endocrinology 149:122-130.

Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, Hogenesch JB, Gulyas J, Rivier J, Vale WW, Sawchenko PE (2001) Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A 98:2843-2848.

Reynolds SM, Zahm DS (2005) Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala. J Neurosci 25:11757-11767.

Rice ME, Cragg SJ, Greenfield SA (1997) Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. J Neurophysiol 77:853-862.

Richter RM, Weiss F (1999) In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. Synapse 32:254-261.

Riegel AC, Williams JT (2008) CRF facilitates calcium release from intracellular stores in midbrain dopamine neurons. Neuron 57:559-570.

Rijkers DT, Kruijtzer JA, van Oostenbrugge M, Ronken E, den Hartog JA, Liskamp RM (2004) Structure-activity studies on the corticotropin releasing factor antagonist astressin, leading to a minimal sequence necessary for antagonistic activity. Chembiochem 5:340-348.

Risbrough VB, Hauger RL, Roberts AL, Vale WW, Geyer MA (2004) Corticotropin-releasing factor receptors CRF1 and CRF2 exert both additive and opposing influences on defensive startle behavior. J Neurosci 24:6545-6552.

Ritz MC, Cone EJ, Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. Life Sci 46:635-645.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219-1223.

Rivest S, Deshaies Y, Richard D (1989) Effects of corticotropin-releasing factor on energy balance in rats are sex dependent. Am J Physiol 257:R1417-1422.

Rivier C, Rivier J, Mormede P, Vale W (1984) Studies of the nature of the interaction between vasopressin and corticotropin-releasing factor on adrenocorticotropin release in the rat. Endocrinology 115:882-886.

Rivier C, Vale W (1987) Cocaine stimulates adrenocorticotropin (ACTH) secretion through a corticotropin-releasing factor (CRF)-mediated mechanism. Brain Res 422:403-406.

Rivier J, Gulyas J, Kirby D, Low W, Perrin MH, Kunitake K, DiGruccio M, Vaughan J, Reubi JC, Waser B, Koerber SC, Martinez V, Wang L, Tache Y, Vale W (2002) Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. J Med Chem 45:4737-4747.

Rivier J, Spiess J, Vale W (1983) Characterization of rat hypothalamic corticotropin-releasing factor. Proc Natl Acad Sci U S A 80:4851-4855.

Robbins TW, Cador M, Taylor JR, Everitt BJ (1989) Limbic-striatal interactions in reward-related processes. Neurosci Biobehav Rev 13:155-162.

Roberts AJ, Koob GF (1997) The neurobiology of addiction: an overview. Alcohol Health Res World 21:101-106.

Roberts DC, Andrews MM, Vickers GJ (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. Neuropsychopharmacology 15:417-423.

Roberts DC, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615-620.

Robinson TE, Becker JB, Moore CJ, Castaneda E, Mittleman G (1985) Enduring enhancement in frontal cortex dopamine utilization in an animal model of amphetamine psychosis. Brain Res 343:374-377.

Robinson TE, Becker JB, Young EA, Akil H, Castaneda E (1987) The effects of footshock stress on regional brain dopamine metabolism and pituitary beta-endorphin release in rats previously sensitized to amphetamine. Neuropharmacology 26:679-691.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Browman KE, Crombag HS, Badiani A (1998) Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. Neurosci Biobehav Rev 22:347-354.

Rodaros D, Caruana DA, Amir S, Stewart J (2007) Corticotropin-releasing factor projections from limbic forebrain and paraventricular nucleus of the hypothalamus to the region of the ventral tegmental area. Neuroscience 150:8-13.

Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ (2000) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology (Berl) 149:217-224.

Rodd ZA, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ (2005a) Intracranial self-administration of cocaine within the posterior ventral tegmental area of Wistar rats: evidence for involvement of serotonin-3 receptors and dopamine neurons. J Pharmacol Exp Ther 313:134-145.

Rodd ZA, Bell RL, Melendez RI, Kuc KA, Lumeng L, Li TK, Murphy JM, McBride WJ (2004) Comparison of intracranial self-administration of ethanol within the posterior ventral tegmental area between alcohol-preferring and Wistar rats. Alcohol Clin Exp Res 28:1212-1219.

Rodd ZA, Bell RL, Zhang Y, Murphy JM, Goldstein A, Zaffaroni A, Li TK, McBride WJ (2005b) Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. Neuropsychopharmacology 30:330-338.

Rodriguez de Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F (1997) Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 276:2050-2054.

Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GF, Navarro M (1996) Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. J Pharmacol Exp Ther 276:56-64.

Roitman MF, Wheeler RA, Wightman RM, Carelli RM (2008) Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci 11:1376-1377.

Roseboom PH, Nanda SA, Bakshi VP, Trentani A, Newman SM, Kalin NH (2007) Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression. Psychoneuroendocrinology 32:44-55.

Ross PC, Kostas CM, Ramabhadran TV (1994) A variant of the human corticotropin-releasing factor (CRF) receptor: cloning, expression and pharmacology. Biochemical and biophysical research communications 205:1836-1842.

Rossant CJ, Pinnock RD, Hughes J, Hall MD, McNulty S (1999) Corticotropin-releasing factor type 1 and type 2alpha receptors regulate phosphorylation of calcium/cyclic adenosine 3',5'-monophosphate response element-binding protein and activation of p42/p44 mitogen-activated protein kinase. Endocrinology 140:1525-1536.

Roth RH (1984) CNS dopamine autoreceptors: distribution, pharmacology, and function. Ann N Y Acad Sci 430:27-53.

Roth RH, Tam SY, Ida Y, Yang JX, Deutch AY (1988) Stress and the mesocorticolimbic dopamine systems. Ann N Y Acad Sci 537:138-147.

Rotllant D, Marquez C, Nadal R, Armario A (2010) The brain pattern of c-fos induction by two doses of amphetamine suggests different brain

processing pathways and minor contribution of behavioural traits. Neuroscience 168:691-705.

Routtenberg A (1972) Intracranial chemical injection and behavior: a critical review. Behavioral biology 7:601-641.

Routtenberg A, and Bondareff, W. (1971) Protein synthesis and memory consolidation: Radioautographic study of intrahippocampal microinjections of 3H-Leucine in awake, freely moving animals. Fed Proc 30:215.

Routtenberg A, Bondareff, W.B., and Pysh, J. (1968) Carbachol stimulation of caudate nucleus: A preliminary behavioral, physiological, and electron microscopic study. Eastern Psychological Association Washington DC.

Routtenberg A, Olds J (1966) Stimulation of dorsal midbrain during septal and hypothalamic self-stimulation. Journal of comparative and physiological psychology 62:250-255.

Routtenberg A, Simpson JB (1971) Carbachol-induced drinking at ventricular and subfornical organ sites of application. Life sciences Pt 1: Physiology and pharmacology 10:481-490.

Rozsa E, Robotka H, Vecsei L, Toldi J (2008) The Janus-face kynurenic acid. J Neural Transm 115:1087-1091.

Ruckebusch Y, Malbert CH (1986) Stimulation and inhibition of food intake in sheep by centrally-administered hypothalamic releasing factors. Life Sci 38:929-934.

Rudolph U, Crestani F, Mohler H (2001) GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 22:188-194.

Rudoy CA, Van Bockstaele EJ (2007) Betaxolol, a selective beta(1)-adrenergic receptor antagonist, diminishes anxiety-like behavior during early withdrawal from chronic cocaine administration in rats. Prog Neuropsychopharmacol Biol Psychiatry 31:1119-1129.

Ruhmann A, Bonk I, Lin CR, Rosenfeld MG, Spiess J (1998) Structural requirements for peptidic antagonists of the corticotropin-releasing factor receptor (CRFR): development of CRFR2beta-selective antisauvagine-30. Proc Natl Acad Sci U S A 95:15264-15269.

Ruhmann A, Chapman J, Higelin J, Butscha B, Dautzenberg FM (2002) Design, synthesis and pharmacological characterization of new highly selective CRF(2) antagonists: development of 123I-K31440 as a potential SPECT ligand. Peptides 23:453-460.

Ryabinin AE, Bachtell RK, Heinrichs SC, Lee S, Rivier C, Olive MF, Mehmert KK, Camarini R, Kim JA, Koenig HN, Nannini MA, Hodge CW, Roberts AJ, Koob GF (2002) The corticotropin-releasing factor/urocortin system and alcohol. Alcohol Clin Exp Res 26:714-722.

Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577-582.

Sadja R, Smadja K, Alagem N, Reuveny E (2001) Coupling Gbetagamma-dependent activation to channel opening via pore elements in inwardly rectifying potassium channels. Neuron 29:669-680.

Sagar SM, Sharp FR, Curran T (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240:1328-1331.

Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A (1999) Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. Behav Brain Res 100:207-215.

Sakanaka M, Shibasaki T, Lederis K (1986) Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. Brain Res 382:213-238.

Salio C, Lossi L, Ferrini F, Merighi A (2006) Neuropeptides as synaptic transmitters. Cell Tissue Res 326:583-598.

SAMHSA (ed.) (2009) Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2012 National Survery on Drug Use and Health. Rockville, MD: Office of Applied Studies.

SAMHSA (ed.) (2012) Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2010 National Survey on Drug Use and Health. Rockville, MD: Office of Applied Studies.

Sanchez CJ, Bailie TM, Wu WR, Li N, Sorg BA (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 119:497-505.

Sandner G, Bielajew C, Fouriezos G (1996) Bicuculline microinjections into the ventral tegmental area of the rat: alteration of self-stimulation thresholds and of cytochrome oxidase activity in the brain. Behav Brain Res 79:145-151.

Saphier D, Welch JE, Farrar GE, Goeders NE (1993) Effects of intracerebroventricular and intrahypothalamic cocaine administration on adrenocortical secretion. Neuroendocrinology 57:54-62.

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1993) Alterations of corticotropin-releasing factor-like immunoreactivity in different brain regions after acute cocaine administration in rats. Brain Res 616:315-319.

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1995) Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. Brain Res 675:89-97.

Sarnyai Z, Hohn J, Szabo G, Penke B (1992) Critical role of endogenous corticotropin-releasing factor (CRF) in the mediation of the behavioral action of cocaine in rats. Life Sci 51:2019-2024.

Sarnyai Z, Shaham Y, Heinrichs SC (2001) The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 53:209-243.

Sartorius A, Kiening KL, Kirsch P, von Gall CC, Haberkorn U, Unterberg AW, Henn FA, Meyer-Lindenberg A (2010) Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. Biol Psychiatry 67:e9-e11.

Sassone-Corsi P, Visvader J, Ferland L, Mellon PL, Verma IM (1988) Induction of proto-oncogene fos transcription through the adenylate cyclase pathway: characterization of a cAMP-responsive element. Genes & development 2:1529-1538.

Sauvage M, Steckler T (2001) Detection of corticotropin-releasing hormone receptor 1 immunoreactivity in cholinergic, dopaminergic and noradrenergic neurons of the murine basal forebrain and brainstem nuclei--potential implication for arousal and attention. Neuroscience 104:643-652.

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251:947-950.

Schank JR, Ryabinin AE, Giardino WJ, Ciccocioppo R, Heilig M (2012) Stress-related neuropeptides and addictive behaviors: beyond the usual suspects. Neuron 76:192-208.

Schilstrom B, Fagerquist MV, Zhang X, Hertel P, Panagis G, Nomikos GG, Svensson TH (2000) Putative role of presynaptic alpha7* nicotinic receptors in nicotine stimulated increases of extracellular levels of

glutamate and aspartate in the ventral tegmental area. Synapse 38:375-383.

Schilstrom B, Nomikos GG, Nisell M, Hertel P, Svensson TH (1998) N-methyl-D-aspartate receptor antagonism in the ventral tegmental area diminishes the systemic nicotine-induced dopamine release in the nucleus accumbens. Neuroscience 82:781-789.

Schilstrom B, Nomikos, G.G., Nisell, M., Hertel, P., Svensson, T.H. (1998) NMDA receptor antagonism in the ventral tegmental area diminishes the systemic nicotine-induced dopamine release in the nucleus accumbens. Neuroscience 82:781-789.

Schilstrom B, Rawal N, Mameli-Engvall M, Nomikos GG, Svensson TH (2003) Dual effects of nicotine on dopamine neurons mediated by different nicotinic receptor subtypes. Int J Neuropsychopharmacol 6:1-11.

Schilstrom B, Yaka R, Argilli E, Suvarna N, Schumann J, Chen BT, Carman M, Singh V, Mailliard WS, Ron D, Bonci A (2006) Cocaine enhances NMDA receptor-mediated currents in ventral tegmental area cells via dopamine D5 receptor-dependent redistribution of NMDA receptors. J Neurosci 26:8549-8558.

Schmidt HD, Famous KR, Pierce RC (2009) The limbic circuitry underlying cocaine seeking encompasses the PPTg/LDT. Eur J Neurosci 30:1358-1369.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1-27.

Schultz W (2001) Reward signaling by dopamine neurons. Neuroscientist 7:293-302.

Schultz W (2007a) Behavioral dopamine signals. Trends Neurosci 30:203-210.

Schultz W (2007b) Multiple dopamine functions at different time courses. Annu Rev Neurosci 30:259-288.

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593-1599.

Schultz W, Romo R (1987) Responses of nigrostriatal dopamine neurons to high-intensity somatosensory stimulation in the anesthetized monkey. J Neurophysiol 57:201-217.

Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD, 3rd, Winston EN, Chen YL, Heym J (1996) CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. Proc Natl Acad Sci U S A 93:10477-10482.

Schuster CR, Thompson T (1969) Self administration of and behavioral dependence on drugs. Annu Rev Pharmacol 9:483-502.

Seabrook GR, Howson W, Lacey MG (1990) Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic GABAB receptors on neurones in rat brain slices. Br J Pharmacol 101:949-957.

Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ (2001) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proc Natl Acad Sci U S A 98:301-306.

Seamans JK, Floresco SB, Phillips AG (1995) Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. Behav Neurosci 109:1063-1073.

Seamans JK, Floresco SB, Phillips AG (1998) D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. J Neurosci 18:1613-1621.

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 74:1-58.

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 32.

Selye H (1937) Studies on adaptation. Endocrinology 21:169-188.

Selye H (1951) The general-adaptation-syndrome and the diseases of adaptation. South Med Surg 113:315-323.

Selye H (ed.) (1983) The stress concept: Past, present, and future. New York: McGraw Hill.

Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213-242.

Sesack SR, Grace AA (2010) Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology 35:27-47.

Sesack SR, Pickel VM (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. J Comp Neurol 320:145-160.

Seutin V, Johnson SW, North RA (1994) Effect of dopamine and baclofen on N-methyl-D-aspartate-induced burst firing in rat ventral tegmental neurons. Neuroscience 58:201-206.

Seutin V, Verbanck P, Massotte L, Dresse A (1990) Evidence for the presence of N-methyl-D-aspartate receptors in the ventral tegmental area of the rat: an electrophysiological in vitro study. Brain Res 514:147-150.

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. J Neurosci 28:8406-8416.

Shaham Y, Erb S, Leung S, Buczek Y, Stewart J (1998) CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. Psychopharmacology (Berl) 137:184-190.

Shaham Y, Erb S, Stewart J (2000) Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev 33:13-33.

Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J (1997) Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 17:2605-2614.

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3-20.

Shaham Y, Stewart J (1995) Effects of restraint stress and intra-ventral tegmental area injections of morphine and methyl naltrexone on the discriminative stimulus effects of heroin in the rat. Pharmacol Biochem Behav 51:491-498.

Shalev U, Erb S, Shaham Y (2010) Role of CRF and other neuropeptides in stress-induced reinstatement of drug seeking. Brain Res 1314:15-28.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54:1-42.

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 (Berl) 150:337-346.

Shalev U, Morales M, Hope B, Yap J, Shaham Y (2001) Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. Psychopharmacology (Berl) 156:98-107.

Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821-861.

Sheardown MJ, Nielsen EO, Hansen AJ, Jacobsen P, Honore T (1990) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. Science 247:571-574.

Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Brain Res Rev 46:71-117.

Shelton KL, and Beardsley, P.M. (2005) Interaction of extinguished cocaine-conditoned stimuli and footshock on reinstatement in rats. Int J Comp Psych 18:154-166.

Shen KZ, Johnson SW (1997) Presynaptic GABAB and adenosine A1 receptors regulate synaptic transmission to rat substantia nigra reticulata neurones. J Physiol 505 (Pt 1):153-163.

Sheng M, Greenberg ME (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron 4:477-485.

Shepard JD, Bossert JM, Liu SY, Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biol Psychiatry 55:1082-1089.

Sherman JE, Kalin NH (1986) ICV-CRH potently affects behavior without altering antinociceptive responding. Life Sci 39:433-441.

Sherman JE, Kalin NH (1987) The effects of ICV-CRH on novelty-induced behavior. Pharmacol Biochem Behav 26:699-703.

Sherman JE, Kalin NH (1988) ICV-CRH alters stress-induced freezing behavior without affecting pain sensitivity. Pharmacol Biochem Behav 30:801-807.

Shibahara S, Morimoto Y, Furutani Y, Notake M, Takahashi H, Shimizu S, Horikawa S, Numa S (1983) Isolation and sequence analysis of the human corticotropin-releasing factor precursor gene. EMBO J 2:775-779.

Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl) 158:343-359.

Sinha R (2008) Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci 1141:105-130.

Sinha R (2009) Modeling stress and drug craving in the laboratory: implications for addiction treatment development. Addict Biol 14:84-98.

Sinha R (2013) The clinical neurobiology of drug craving. Curr Opin Neurobiol 23:649-654.

Sinha R, Catapano D, O'Malley S (1999) Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology (Berl) 142:343-351.

Sinha R, Fuse T, Aubin LR, O'Malley SS (2000) Psychological stress, drug-related cues and cocaine craving. Psychopharmacology (Berl) 152:140-148.

Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ (2006) Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Arch Gen Psychiatry 63:324-331.

Sinha R, Lacadie C, Skudlarski P, Fulbright RK, Rounsaville BJ, Kosten TR, Wexler BE (2005) Neural activity associated with stress-induced cocaine craving: a functional magnetic resonance imaging study. Psychopharmacology (Berl) 183:171-180.

Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ (2003) Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. Psychopharmacology (Berl) 170:62-72.

Skelton KH, Owens MJ, Nemeroff CB (2000) The neurobiology of urocortin. Regul Pept 93:85-92.

Smith KS, Tindell AJ, Aldridge JW, Berridge KC (2009) Ventral pallidum roles in reward and motivation. Behav Brain Res 196:155-167.

Smith MA, Banerjee S, Gold PW, Glowa J (1992) Induction of c-fos mRNA in rat brain by conditioned and unconditioned stressors. Brain Res 578:135-141.

Smith MA, Bissette G, Slotkin TA, Knight DL, Nemeroff CB (1986) Release of corticotropin-releasing factor from rat brain regions in vitro. Endocrinology 118:1997-2001.

Smith RJ, Aston-Jones G (2008) Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. Brain Struct Funct 213:43-61.

Snyder S (ed.) (1986) Drugs and the Brain. New York: Scientific American Library.

Solomon RL, Corbit JD (1974) An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol Rev 81:119-145.

Sonino N (1987) The use of ketoconazole as an inhibitor of steroid production. The New England journal of medicine 317:812-818.

Sonnenberg JL, Macgregor-Leon PF, Curran T, Morgan JI (1989) Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. Neuron 3:359-365.

Sorg BA (2012) Reconsolidation of drug memories. Neurosci Biobehav Rev 36:1400-1417.

Sorg BA, Kalivas PW (1993) Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53:695-703.

Sorge RE, Rajabi H, Stewart J (2005) Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement. Neuropsychopharmacology 30:1681-1692.

Sorge RE, Stewart J (2005) The contribution of drug history and time since termination of drug taking to footshock stress-induced cocaine seeking in rats. Psychopharmacology (Berl) 183:210-217.

Spealman RD, Lee B, Tiefenbacher S, Platt DM, Rowlett JK, Khroyan TV (2004) Triggers of relapse: nonhuman primate models of reinstated cocaine seeking. Nebr Symp Motiv 50:57-84.

Specio SE, Wee S, O'Dell LE, Boutrel B, Zorrilla EP, Koob GF (2008) CRF(1) receptor antagonists attenuate escalated cocaine self-administration in rats. Psychopharmacology (Berl) 196:473-482.

Spina MG, Basso AM, Zorrilla EP, Heyser CJ, Rivier J, Vale W, Merlo-Pich E, Koob GF (2000) Behavioral effects of central administration of the novel CRF antagonist astressin in rats. Neuropsychopharmacology 22:230-239.

Spina MG, Merlo-Pich E, Akwa Y, Balducci C, Basso AM, Zorrilla EP, Britton KT, Rivier J, Vale WW, Koob GF (2002) Time-dependent induction of anxiogenic-like effects after central infusion of urocortin or corticotropin-releasing factor in the rat. Psychopharmacology (Berl) 160:113-121.

Stanford IM, Lacey MG (1996) Differential actions of serotonin, mediated by 5-HT1B and 5-HT2C receptors, on GABA-mediated synaptic input to rat substantia nigra pars reticulata neurons in vitro. J Neurosci 16:7566-7573.

Stavraky GW (1961) "Supersensitivity Following Lesions of the Nervous System.". Toronto: University of Toronto Press.

Steckler T, Holsboer F (1999) Corticotropin-releasing hormone receptor subtypes and emotion. Biol Psychiatry 46:1480-1508.

Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ (1998) Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. J Neurosci 18:8003-8015.

Steffensen SC, Taylor SR, Horton ML, Barber EN, Lyle LT, Stobbs SH, Allison DW (2008) Cocaine disinhibits dopamine neurons in the ventral tegmental area via use-dependent blockade of GABA neuron voltage-sensitive sodium channels. Eur J Neurosci 28:2028-2040.

Stein GW, Levitt RA (1971) Lesion effects on cholinergically elicited drinking in the rat. Physiol Behav 7:517-522.

Sterling P, and Eyer, J. (ed.) (1988) Handbook of Life Stress Cognition and Health. New York: Wiley.

Stewart J (1992) Neurobiology of conditioning to drugs of abuse. Ann N Y Acad Sci 654:335-346.

Stewart J (2003) Stress and relapse to drug seeking: studies in laboratory animals shed light on mechanisms and sources of long-term vulnerability. Am J Addict 12:1-17.

Stewart J, de Wit, H. (1987a) Reinstatement of drug-taking behaivor as a method of assessing incentive motivational properties of drugs. In: Methods of assessing the reinforcing properties of abused drugs (Bozarth, M. A., ed), pp 211-227 New York: Springer.

Stewart J, Eikelboom, R., (ed.) (1987b) Conditioned Drug Effects. Plenum, NY.

Stine SM, Southwick SM, Petrakis IL, Kosten TR, Charney DS, Krystal JH (2002) Yohimbine-induced withdrawal and anxiety symptoms in opioid-dependent patients. Biol Psychiatry 51:642-651.

Stone TW (1993) Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 45:309-379.

Stone TW (2007) Kynurenic acid blocks nicotinic synaptic transmission to hippocampal interneurons in young rats. Eur J Neurosci 25:2656-2665.

Stretch R, Gerber GJ, Wood SM (1971) Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. Can J Physiol Pharmacol 49:581-589.

Striplin CD, Kalivas PW (1992) Correlation between behavioral sensitization to cocaine and G protein ADP-ribosylation in the ventral tegmental area. Brain Res 579:181-186.

Striplin CD, Kalivas PW (1993) Robustness of G protein changes in cocaine sensitization shown with immunoblotting. Synapse 14:10-15.

Stuber GD, Hnasko TS, Britt JP, Edwards RH, Bonci A (2010) Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. J Neurosci 30:8229-8233.

Stuber GD, Klanker M, de Ridder B, Bowers MS, Joosten RN, Feenstra MG, Bonci A (2008) Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. Science 321:1690-1692.

Suaud-Chagny MF, 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 49:63-72.

Suda T, Iwashita M, Tozawa F, Ushiyama T, Tomori N, Sumitomo T, Nakagami Y, Demura H, Shizume K (1988) Characterization of corticotropin-releasing hormone binding protein in human plasma by chemical cross-linking and its binding during pregnancy. J Clin Endocrinol Metab 67:1278-1283.

Sugita S, Johnson SW, North RA (1992) Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. Neurosci Lett 134:207-211.

Sun W (2011) Dopamine neurons in the ventral tegmental area: drug-induced synaptic plasticity and its role in relapse to drug-seeking behavior. Curr Drug Abuse Rev 4:270-285.

Sun W, Akins, C.K., Mattingly, A.E., and Rebec, G.V. (2005) Ionotropic Glutamate Receptors in the Ventral Tegmental Area Regulate Cocaine-Seeking Behavior in Rats. Neuropsychopharmacology 30.

Sutton MA, Karanian DA, Self DW (2000) Factors that determine a propensity for cocaine-seeking behavior during abstinence in rats. Neuropsychopharmacology 22:626-641.

Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W (1982) Corticotropin releasing factor produces behavioural activation in rats. Nature 297:331-333.

Svensson TH, Tung CS (1989) Local cooling of pre-frontal cortex induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area in vivo. Acta Physiol Scand 136:135-136.

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165-186.

Swerdlow NR, Britton KT, Koob GF (1989) Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by alpha-helical CRF (9-41). Neuropsychopharmacology 2:285-292.

Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15:2057-2061.

Szalardy L, Zadori D, Toldi J, Fulop F, Klivenyi P, Vecsei L (2012) Manipulating kynurenic acid levels in the brain - on the edge between neuroprotection and cognitive dysfunction. Curr Top Med Chem 12:1797-1806.

Szekely AM, Barbaccia ML, Alho H, Costa E (1989) In primary cultures of cerebellar granule cells the activation of N-methyl-D-aspartate-sensitive glutamate receptors induces c-fos mRNA expression. Mol Pharmacol 35:401-408.

Szekely AM, Barbaccia ML, Costa E (1987) Activation of specific glutamate receptor subtypes increases C-fos proto-oncogene expression in primary

cultures of neonatal rat cerebellar granule cells. Neuropharmacology 26:1779-1782.

Sziraki I, Sershen H, Hashim A, Lajtha A (2002) Receptors in the ventral tegmental area mediating nicotine-induced dopamine release in the nucleus accumbens. Neurochem Res 27:253-261.

Taber MT, Das S, Fibiger HC (1995) Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. J Neurochem 65:1407-1410.

Tagliaferro P, Morales M (2008) Synapses between corticotropin-releasing factor-containing axon terminals and dopaminergic neurons in the ventral tegmental area are predominantly glutamatergic. J Comp Neurol 506:616-626.

Takahashi LK, Ho SP, Livanov V, Graciani N, Arneric SP (2001) Antagonism of CRF(2) receptors produces anxiolytic behavior in animal models of anxiety. Brain Res 902:135-142.

Takahata R, Moghaddam B (1998) Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. J Neurochem 71:1443-1449.

Takahata R, Moghaddam B (2000) Target-specific glutamatergic regulation of dopamine neurons in the ventral tegmental area. J Neurochem 75:1775-1778.

Tan KR, Brown M, Labouebe G, Yvon C, Creton C, Fritschy JM, Rudolph U, Luscher C (2010) Neural bases for addictive properties of benzodiazepines. Nature 463:769-774.

Tan KR, Yvon C, Turiault M, Mirzabekov JJ, Doehner J, Labouebe G, Deisseroth K, Tye KM, Luscher C (2012) GABA Neurons of the VTA Drive Conditioned Place Aversion. Neuron 73:1173-1183.

Tanaka M, Telegdy G (2008) Antidepressant-like effects of the CRF family peptides, urocortin 1, urocortin 2 and urocortin 3 in a modified forced swimming test in mice. Brain Res Bull 75:509-512.

Tanimoto H, Heisenberg M, Gerber B (2004) Experimental psychology: event timing turns punishment to reward. Nature 430:983.

Tasken K, Aandahl EM (2004) Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol Rev 84:137-167.

Tassin JP, Herve D, Blanc G, Glowinski J (1980) Differential effects of a two-minute open-field session on dopamine utilization in the frontal cortices of BALB/C and C57 BL/6 mice. Neurosci Lett 17:67-71.

Tazi A, Dantzer R, Le Moal M, Rivier J, Vale W, Koob GF (1987) Corticotropin-releasing factor antagonist blocks stress-induced fighting in rats. Regul Pept 18:37-42.

Tecuapetla F, Patel JC, Xenias H, English D, Tadros I, Shah F, Berlin J, Deisseroth K, Rice ME, Tepper JM, Koos T (2010) Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. J Neurosci 30:7105-7110.

Tetel MJ, Getzinger MJ, Blaustein JD (1993) Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. J Neuroendocrinol 5:397-404.

Tezval H, Jahn O, Todorovic C, Sasse A, Eckart K, Spiess J (2004) Cortagine, a specific agonist of corticotropin-releasing factor receptor subtype 1, is anxiogenic and antidepressive in the mouse model. Proc Natl Acad Sci U S A 101:9468-9473.

Thierry AM, Tassin JP, Blanc G, Glowinski J (1976) Selective activation of mesocortical DA system by stress. Nature 263:242-244.

Tidey JW, Miczek KA (1996) Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res 721:140-149.

Tissari AH, Argiolas A, Fadda F, Serra G, Gessa GL (1979) Foot-shock stress accelerates non-striatal dopamine synthesis without activating tyrosine hydroxylase. Naunyn-Schmiedeberg's archives of pharmacology 308:155-157.

Todorovic C, Radulovic J, Jahn O, Radulovic M, Sherrin T, Hippel C, Spiess J (2007) Differential activation of CRF receptor subtypes removes stress-induced memory deficit and anxiety. Eur J Neurosci 25:3385-3397.

Tong ZY, Overton PG, Clark D (1996) Antagonism of NMDA receptors but not AMPA/kainate receptors blocks bursting in dopaminergic neurons induced by electrical stimulation of the prefrontal cortex. J Neural Transm 103:889-904.

Tran-Nguyen LT, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL (1998) Time-dependent changes in cocaine-seeking behavior and

extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19:48-59.

Treisman R (1992) The serum response element. Trends in biochemical sciences 17:423-426.

Trojniar W, Klejbor I (1999) Facilitatory effect of unilateral lesion of the ventral tegmental area on locomotor response to stimulation of the contralateral ventral tegmental area: involvement of GABAergic transmission. Brain Res 842:419-430.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324:1080-1084.

Turnbull AV, Rivier C (1997) Corticotropin-releasing factor (CRF) and endocrine responses to stress: CRF receptors, binding protein, and related peptides. Proc Soc Exp Biol Med 215:1-10.

Tye SJ, Miller AD, Blaha CD (2013) Ventral tegmental ionotropic glutamate receptor stimulation of nucleus accumbens tonic dopamine efflux blunts hindbrain-evoked phasic neurotransmission: implications for dopamine dysregulation disorders. Neuroscience 252:337-345.

Tzschentke TM (2000) The medial prefrontal cortex as a part of the brain reward system. Amino acids 19:211-219.

Tzschentke TM (2001) Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog Neurobiol 63:241-320.

Tzschentke TM, Schmidt WJ (2003) Glutamatergic mechanisms in addiction. Mol Psychiatry 8:373-382.

Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 10:397-409.

Ulrich CD, 2nd, Holtmann M, Miller LJ (1998) Secretin and vasoactive intestinal peptide receptors: members of a unique family of G protein-coupled receptors. Gastroenterology 114:382-397.

Ungless MA, Argilli E, Bonci A (2010) Effects of stress and aversion on dopamine neurons: implications for addiction. Neurosci Biobehav Rev 35:151-156.

Ungless MA, Magill PJ, Bolam JP (2004) Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. Science 303:2040-2042.

Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A (2003) Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. Neuron 39:401-407.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411:583-587.

UNODC (2010) World drug report. In: United Nations Publications, vol. Sales No. E.10.XI.13 New York.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Res 797:73-93.

Vaccarino FM, Hayward MD, Nestler EJ, Duman RS, Tallman JF (1992) Differential induction of immediate early genes by excitatory amino acid receptor types in primary cultures of cortical and striatal neurons. Brain Res Mol Brain Res 12:233-241.

Valdez GR, Inoue K, Koob GF, Rivier J, Vale W, Zorrilla EP (2002) Human urocortin II: mild locomotor suppressive and delayed anxiolytic-like effects of a novel corticotropin-releasing factor related peptide. Brain Res 943:142-150.

Valdez GR, Zorrilla EP, Rivier J, Vale WW, Koob GF (2003) Locomotor suppressive and anxiolytic-like effects of urocortin 3, a highly selective type 2 corticotropin-releasing factor agonist. Brain Res 980:206-212.

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394-1397.

Valentino RJ, Foote SL, Page ME (1993) The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. Ann N Y Acad Sci 697:173-188.

Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res 682:215-221.

Van den Oever MC, Spijker S, Smit AB, De Vries TJ (2010) Prefrontal cortex plasticity mechanisms in drug seeking and relapse. Neurosci Biobehav Rev 35:276-284.

Van Dyke C, Ungerer J, Jatlow P, Barash P, Byck R (1982) Intranasal cocaine: dose relationships of psychological effects and plasma levels. Int J Psychiatry Med 12:1-13.

Van Eden CG, Hoorneman EM, Buijs RM, Matthijssen MA, Geffard M, Uylings HB (1987) Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. Neuroscience 22:849-862.

Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 428:191-212.

van Zessen R, Phillips JL, Budygin EA, Stuber GD (2012) Activation of VTA GABA Neurons Disrupts Reward Consumption. Neuron 73:1184-1194.

Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, et al. (1995) Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378:287-292.

Veldhuis HD, De Wied D (1984) Differential behavioral actions of corticotropin-releasing factor (CRF). Pharmacol Biochem Behav 21:707-713.

Viau V, Sawchenko PE (2002) Hypophysiotropic neurons of the paraventricular nucleus respond in spatially, temporally, and phenotypically differentiated manners to acute vs. repeated restraint stress: rapid publication. J Comp Neurol 445:293-307.

Vigot R, Barbieri S, Brauner-Osborne H, Turecek R, Shigemoto R, Zhang YP, Lujan R, Jacobson LH, Biermann B, Fritschy JM, Vacher CM, Muller M, Sansig G, Guetg N, Cryan JF, Kaupmann K, Gassmann M, Oertner TG, Bettler B (2006) Differential compartmentalization and distinct functions of GABAB receptor variants. Neuron 50:589-601.

Vincent SL, Khan Y, Benes FM (1993) Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. J Neurosci 13:2551-2564.

Vita N, Laurent P, Lefort S, Chalon P, Lelias JM, Kaghad M, Le Fur G, Caput D, Ferrara P (1993) Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. FEBS Lett 335:1-5.

Vlachou S, Markou A (2010) GABAB receptors in reward processes. Advances in pharmacology 58:315-371.

Vocci FJ, Elkashef A (2005) Pharmacotherapy and other treatments for cocaine abuse and dependence. Curr Opin Psychiatry 18:265-270.

Vogt BA (2005) Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 6:533-544.

Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler M, Logan J, Franceschi D, Gatley J, Hitzemann R, Gifford A, Wong C, Pappas N (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry 158:2015-2021.

Volkow ND, Ding YS, Fowler JS, Wang GJ (1996) Cocaine addiction: hypothesis derived from imaging studies with PET. J Addict Dis 15:55-71.

Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex 10:318-325.

Volkow ND, Fowler JS, Wang GJ (1999a) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. J Psychopharmacol 13:337-345.

Volkow ND, Fowler JS, Wang GJ (2002) Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol 13:355-366.

Volkow ND, Fowler JS, Wang GJ (2004) The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. Neuropharmacology 47 Suppl 1:3-13.

Volkow ND, Fowler JS, Wolf AP, Hitzemann R, Dewey S, Bendriem B, Alpert R, Hoff A (1991) Changes in brain glucose metabolism in cocaine dependence and withdrawal. Am J Psychiatry 148:621-626.

Volkow ND, Hitzemann R, Wang GJ, Fowler JS, Wolf AP, Dewey SL, Handlesman L (1992) Long-term frontal brain metabolic changes in cocaine abusers. Synapse 11:184-190.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Wong C, Hitzemann R, Pappas NR (1999b) Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. J Pharmacol Exp Ther 291:409-415.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26:6583-6588.

Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci 27:468-474.

Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL (2001) Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 292:1175-1178.

Vranjkovic O, Hang S, Baker DA, Mantsch JR (2012) beta-adrenergic receptor mediation of stress-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: roles for beta1 and beta2 adrenergic receptors. J Pharmacol Exp Ther 342:541-551.

Vuillez P, Jacob N, Teclemariam-Mesbah R, Van Rossum A, Vivien-Roels B, Pevet P (1998) Effect of NMDA receptor antagonist MK-801 on light-induced Fos expression in the suprachiasmatic nuclei and on melatonin production in the Syrian hamster. J Neuroendocrinol 10:671-677.

Wagner FA, Anthony JC (2002) From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. Neuropsychopharmacology 26:479-488.

Walker BM, Koob GF (2007) The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. Alcohol Clin Exp Res 31:11-18.

Walker DL, Toufexis DJ, Davis M (2003) Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur J Pharmacol 463:199-216.

Wallace BC (1989) Psychological and environmental determinants of relapse in crack cocaine smokers. J Subst Abuse Treat 6:95-106.

Wallace DM, Magnuson DJ, Gray TS (1989) The amygdalo-brainstem pathway: selective innervation of dopaminergic, noradrenergic and adrenergic cells in the rat. Neurosci Lett 97:252-258.

Walsh SL, Stoops WW, Moody DE, Lin SN, Bigelow GE (2009) Repeated dosing with oral cocaine in humans: assessment of direct effects, withdrawal, and pharmacokinetics. Exp Clin Psychopharmacol 17:205-216.

Wan W, Janz L, Vriend CY, Sorensen CM, Greenberg AH, Nance DM (1993) Differential induction of c-Fos immunoreactivity in hypothalamus and brain stem nuclei following central and peripheral administration of endotoxin. Brain Res Bull 32:581-587.

Wan W, Wetmore L, Sorensen CM, Greenberg AH, Nance DM (1994) Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. Brain Res Bull 34:7-14.

Wanat MJ, Bonci A, Phillips PE (2013) CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors. Nat Neurosci.

Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A (2008) Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. J Physiol 586:2157-2170.

Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB (2005) Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci 25:5389-5396.

Wang B, You ZB, Rice KC, Wise RA (2007) Stress-induced relapse to cocaine seeking: roles for the CRF(2) receptor and CRF-binding protein in the ventral tegmental area of the rat. Psychopharmacology (Berl) 193:283-294.

Wang J, O'Donnell P (2001) D(1) dopamine receptors potentiate nmda-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. Cereb Cortex 11:452-462.

Wang T, French ED (1993a) Electrophysiological evidence for the existence of NMDA and non-NMDA receptors on rat ventral tegmental dopamine neurons. Synapse 13:270-277.

Wang T, French ED (1993b) L-glutamate excitation of A10 dopamine neurons is preferentially mediated by activation of NMDA receptors: extra- and intracellular electrophysiological studies in brain slices. Brain Res 627:299-306.

Wang T, French ED (1995) NMDA, kainate, and AMPA depolarize nondopamine neurons in the rat ventral tegmentum. Brain Res Bull 36:39-43.

Wang T, O'Connor WT, Ungerstedt U, French ED (1994) N-methyl-D-aspartic acid biphasically regulates the biochemical and electrophysiological

response of A10 dopamine neurons in the ventral tegmental area: in vivo microdialysis and in vitro electrophysiological studies. Brain Res 666:255-262.

Waselus M, Van Bockstaele EJ (2007) Co-localization of corticotropin-releasing factor and vesicular glutamate transporters within axon terminals of the rat dorsal raphe nucleus. Brain Res 1174:53-65.

Waszczak BL, Walters JR (1980) Intravenous GABA agonist administration stimulates firing of A10 dopaminergic neurons. Eur J Pharmacol 66:141-144.

Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N Whole-brain mapping of direct inputs to midbrain dopamine neurons. Neuron 74:858-873.

Waters AJ, Shiffman S, Sayette MA, Paty JA, Gwaltney CJ, Balabanis MH (2004) Cue-provoked craving and nicotine replacement therapy in smoking cessation. J Consult Clin Psychol 72:1136-1143.

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21:165-204.

Watts AE, Williams JT, Henderson G (1996) Baclofen inhibition of the hyperpolarization-activated cation current, Ih, in rat substantia nigra zona compacta neurons may be secondary to potassium current activation. J Neurophysiol 76:2262-2270.

Webb SM, Vollrath-Smith FR, Shin R, Jhou TC, Xu S, Ikemoto S (2012) Rewarding and incentive motivational effects of excitatory amino acid receptor antagonists into the median raphe and adjacent regions of the rat. Psychopharmacology (Berl) 224:401-412.

Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP (1996) In vivo and in vitro characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. Endocrinology 137:5747-5750.

Weddington WW, Brown BS, Cone EJ, Haertzen CA, Dax EM, Herning RI, Michaelson BS (1990) Changes in mood, craving and sleep during acute abstinence reported by male cocaine addicts. NIDA Res Monogr 105:453-454.

Weeks JR (1962) Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. Science 138:143-144.

Weiss B, Heller A (1969) Methodological problems in evaluating the role of cholinergic mechanisms in behavior. Fed Proc 28:135-146.

Weiss F (2005) Neurobiology of craving, conditioned reward and relapse. Curr Opin Pharmacol 5:9-19.

Weiss F, Hurd YL, Ungerstedt U, Markou A, Plotsky PM, Koob GF (1992a) Neurochemical correlates of cocaine and ethanol self-administration. Ann N Y Acad Sci 654:220-241.

Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci U S A 97:4321-4326.

Weiss F, Markou A, Lorang MT, Koob GF (1992b) Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Res 593:314-318.

Weninger SC, Peters LL, Majzoub JA (2000) Urocortin expression in the Edinger-Westphal nucleus is up-regulated by stress and corticotropin-releasing hormone deficiency. Endocrinology 141:256-263.

Werner P, Voigt M, Keinanen K, Wisden W, Seeburg PH (1991) Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. Nature 351:742-744.

Wersinger SR, Baum MJ, Erskine MS (1993) Mating-induced FOS-like immunoreactivity in the rat forebrain: a sex comparison and a dimorphic effect of pelvic nerve transection. J Neuroendocrinol 5:557-568.

West AR, Floresco SB, Charara A, Rosenkranz JA, Grace AA (2003) Electrophysiological interactions between striatal glutamatergic and dopaminergic systems. Ann N Y Acad Sci 1003:53-74.

Westerink BH, Enrico P, Feimann J, De Vries JB (1998) The pharmacology of mesocortical dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. J Pharmacol Exp Ther 285:143-154.

Westerink BH, Korf J (1976) Acidic dopamine metabolites in cortical areas of the rat brain: localization and effects of drug. Brain Res 113:429-434.

Westerink BH, Kwint HF, deVries JB (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J Neurosci 16:2605-2611.

White FJ (1996) Synaptic regulation of mesocorticolimbic dopamine neurons. Annu Rev Neurosci 19:405-436.

White NM (1989) Reward or reinforcement: what's the difference? Neurosci Biobehav Rev 13:181-186.

Whitnall MH (1993) Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. Prog Neurobiol 40:573-629.

Wietfeld D, Heinrich N, Furkert J, Fechner K, Beyermann M, Bienert M, Berger H (2004) Regulation of the coupling to different G proteins of rat corticotropin-releasing factor receptor type 1 in human embryonic kidney 293 cells. J Biol Chem 279:38386-38394.

Wightman RM, Zimmerman JB (1990) Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. Brain Res Brain Res Rev 15:135-144.

Wikler A (1973) Dynamics of drug dependence. Implications of a conditioning theory for research and treatment. Arch Gen Psychiatry 28:611-616.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572-575.

Winter C, Vollmayr B, Djodari-Irani A, Klein J, Sartorius A (2011) Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. Behav Brain Res 216:463-465.

Wirtshafter D, Klitenick MA (1989) Comparative studies of locomotor behavior following microinjections of muscimol into various sites in the paramedian tegmentum. Pharmacol Biochem Behav 32:625-628.

Wirtshafter D, Sheppard AC (2001) Localization of GABA(B) receptors in midbrain monoamine containing neurons in the rat. Brain Res Bull 56:1-5.

Wisden W, Seeburg PH (1993) A complex mosaic of high-affinity kainate receptors in rat brain. J Neurosci 13:3582-3598.

Wise RA (1996) Addictive drugs and brain stimulation reward. Annu Rev Neurosci 19:319-340.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483-494.

Wise RA (2009) Ventral tegmental glutamate: a role in stress-, cue-, and cocaine-induced reinstatement of cocaine-seeking. Neuropharmacology 56 Suppl 1:174-176.

Wise RA, Bozarth MA (1982) Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. Pharmacol Biochem Behav 17:239-243.

Wise RA, Hoffman DC (1992) Localization of drug reward mechanisms by intracranial injections. Synapse 10:247-263.

Wise RA, Morales M (2010) A ventral tegmental CRF-glutamate-dopamine interaction in addiction. Brain Res 1314:38-43.

Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB, Jr. (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. Psychopharmacology (Berl) 120:10-20.

Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191-225.

Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zukin S, Grace AA, Lee JS, Rohde C, Jasinski DR, Gjedde A, London ED (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacology 31:2716-2727.

Woods RJ, Grossman A, Saphier P, Kennedy K, Ur E, Behan D, Potter E, Vale W, Lowry PJ (1994) Association of human corticotropin-releasing hormone to its binding protein in blood may trigger clearance of the complex. J Clin Endocrinol Metab 78:73-76.

Wu YN, Shen KZ, Johnson SW (1999) Presynaptic inhibition preferentially reduces in NMDA receptor-mediated component of transmission in rat midbrain dopamine neurons. Br J Pharmacol 127:1422-1430.

Xi ZX, Stein EA (1998) Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. Brain Res 798:156-165.

Xi ZX, Stein EA (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. J Pharmacol Exp Ther 290:1369-1374.

Yamada Y, Mizutani K, Mizusawa Y, Hantani Y, Tanaka M, Tanaka Y, Tomimoto M, Sugawara M, Imai N, Yamada H, Okajima N, Haruta J (2004) New class of corticotropin-releasing factor (CRF) antagonists: small peptides having high binding affinity for CRF receptor. J Med Chem 47:1075-1078.

Yamauchi N, Otagiri A, Nemoto T, Sekino A, Oono H, Kato I, Yanaihara C, Shibasaki T (2005) Distribution of urocortin 2 in various tissues of the rat. J Neuroendocrinol 17:656-663.

Yap JJ, Miczek KA (2008) Stress and Rodent Models of Drug Addiction: Role of VTA-Accumbens-PFC-Amygdala Circuit. Drug Discov Today Dis Models 5:259-270.

Ye JH, Zalcman SS, Tao L (2005) Kainate-activated currents in the ventral tegmental area of neonatal rats are modulated by interleukin-2. Brain Res 1049:227-233.

Yim CY, Mogenson GJ (1980) Effect of picrotoxin and nipecotic acid on inhibitory response of dopaminergic neurons in the ventral tegmental area to stimulation of the nucleus accumbens. Brain Res 199:466-473.

You ZB, Wang B, Zitzman D, Azari S, Wise RA (2007) A role for conditioned ventral tegmental glutamate release in cocaine seeking. J Neurosci 27:10546-10555.

Young AB, Fagg GE (1990) Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. Trends Pharmacol Sci 11:126-133.

Young AM (2004) Increased extracellular dopamine in nucleus accumbens in response to unconditioned and conditioned aversive stimuli: studies using 1 min microdialysis in rats. J Neurosci Methods 138:57-63.

Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. Proc Natl Acad Sci U S A 88:1291-1295.

Yu W, Miller RF (1995) NBQX, an improved non-NMDA antagonist studied in retinal ganglion cells. Brain Res 692:190-194.

Zahm DS, Cheng AY, Lee TJ, Ghobadi CW, Schwartz ZM, Geisler S, Parsely KP, Gruber C, Veh RW (2011) Inputs to the midbrain dopaminergic

complex in the rat, with emphasis on extended amygdala-recipient sectors. J Comp Neurol 519:3159-3188.

Zahm DS, Heimer L (1990) Two transpallidal pathways originating in the rat nucleus accumbens. J Comp Neurol 302:437-446.

Zahm DS, Williams E, Wohltmann C (1996) Ventral striatopallidothalamic projection: IV. Relative involvements of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. J Comp Neurol 364:340-362.

Zahrt J, Taylor JR, Mathew RG, Arnsten AF (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J Neurosci 17:8528-8535.

Zangen A, Ikemoto S, Zadina JE, Wise RA (2002) Rewarding and psychomotor stimulant effects of endomorphin-1: anteroposterior differences within the ventral tegmental area and lack of effect in nucleus accumbens. J Neurosci 22:7225-7233.

Zavala AR, Osredkar T, Joyce JN, Neisewander JL (2008) Upregulation of Arc mRNA expression in the prefrontal cortex following cue-induced reinstatement of extinguished cocaine-seeking behavior. Synapse 62:421-431.

Zhang Z, Morse AC, Koob GF, Schulteis G (2007) Dose- and time-dependent expression of anxiety-like behavior in the elevated plus-maze during withdrawal from acute and repeated intermittent ethanol intoxication in rats. Alcohol Clin Exp Res 31:1811-1819.

Zhao-Shea R, Liu L, Soll LG, Improgo MR, Meyers EE, McIntosh JM, Grady SR, Marks MJ, Gardner PD, Tapper AR (2011) Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. Neuropsychopharmacology 36:1021-1032.

Zhao MG, Ko SW, Wu LJ, Toyoda H, Xu H, Quan J, Li J, Jia Y, Ren M, Xu ZC, Zhuo M (2006) Enhanced presynaptic neurotransmitter release in the anterior cingulate cortex of mice with chronic pain. J Neurosci 26:8923-8930.

Zheng F, Johnson SW (2002) Group I metabotropic glutamate receptor-mediated enhancement of dopamine cell burst firing in rat ventral tegmental area in vitro. Brain Res 948:171-174.

Zhou Y, Spangler R, LaForge KS, Maggos CE, Ho A, Kreek MJ (1996) Corticotropin-releasing factor and type 1 corticotropin-releasing factor

receptor messenger RNAs in rat brain and pituitary during "binge"-pattern cocaine administration and chronic withdrawal. J Pharmacol Exp Ther 279:351-358.

Zoli M, Agnati LF (1996) Wiring and volume transmission in the central nervous system: the concept of closed and open synapses. Prog Neurobiol 49:363-380.

Zoli M, Jansson A, Sykova E, Agnati LF, Fuxe K (1999) Volume transmission in the CNS and its relevance for neuropsychopharmacology. Trends Pharmacol Sci 20:142-150.

Zorrilla EP, Koob, G.F. (ed.) (2005) The roles of urocortins 1, 2, and 3 in the brain. New York: Elsevier Science.

Zorrilla EP, Tache Y, Koob GF (2003) Nibbling at CRF receptor control of feeding and gastrocolonic motility. Trends Pharmacol Sci 24:421-427.

Zorrilla EP, Valdez GR, Weiss F (2001) Changes in levels of regional CRF-like-immunoreactivity and plasma corticosterone during protracted drug withdrawal in dependent rats. Psychopharmacology (Berl) 158:374-381.

Zorrilla EP, Wee S, Zhao Y, Specio S, Boutrel B, Koob GF, Weiss F (2012) Extended access cocaine self-administration differentially activates dorsal raphe and amygdala corticotropin-releasing factor systems in rats. Addict Biol 17:300-308.

Zupanc GK (1996) Peptidergic transmission: from morphological correlates to functional implications. Micron 27:35-91.

Zweifel LS, Argilli E, Bonci A, Palmiter RD (2008) Role of NMDA receptors in dopamine neurons for plasticity and addictive behaviors. Neuron 59:486-496.

Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, Darvas M, Kim MJ, Mizumori SJ, Paladini CA, Phillips PE, Palmiter RD (2009) Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. Proc Natl Acad Sci U S A 106:7281-7288.