Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA

A thesis submitted by

Christopher O. Boyson[1]

In partial fulfillment of the requirement for the degree of

Master of Science

In

Psychology

TUFTS UNIVERSITY

August 2011

Committee Members:

Klaus A. Miczek[1], Ph.D.; Joseph F. DeBold[1],Ph.D.; Stephen C. Heinrichs[2], Ph.D.

[1]Dept. of Psychology, Tufts University, Medford, MA, USA, [2]Boston VA Medical Center, Boston, MA, USA

Abstract

Intermittent exposure to social defeat stress can induce long-term neural plasticity that may influence escalated cocaine-taking behavior. Stressful encounters can lead to activation of dopamine neurons in the ventral tegmental area (VTA), which are modulated by corticotropin releasing factor (CRF) neurons. The study aims to prevent the effects of intermittently scheduled, brief social defeat stress on subsequent intravenous (IV) cocaine self-administration by pretreatment with a CRF receptor subtype 1 (CRF-R1) antagonist. Long-Evans rats were submitted to four intermittent social defeat experiences separated by 72 h over 10 days. Two experiments examined systemic or intra-VTA antagonism of CRF-R1 subtype during stress on the later expression of locomotor sensitization and cocaine self-administration during fixed (0.75 mg/kg/infusion) and progressive ratio schedules of reinforcement (0.3 mg/kg/infusion), including a continuous 24-h "binge" (0.3 mg/kg/infusion). Pretreatment with a CRF-R1 antagonist, CP 154,526, (20 mg/kg i.p.) prior to each social defeat episode prevented the development of stress-induced locomotor sensitization to a cocaine challenge and prevented escalated cocaine self-administration during a 24-h "binge". In addition, pretreatment with a CRF-R1 antagonist (0.3 μg/0.5 μl/side) into the VTA prior to each social defeat episode prevented stress-induced locomotor sensitization to a cocaine challenge and prevented escalated cocaine self-administration during a 24-h "binge". The current results suggest that CRF-R1 subtype in the VTA is critically involved in the development of stress-induced locomotor sensitization which may contribute to escalated cocaine self-administration during continuous access in a 24-h "binge".

Keywords Stress: Locomotor sensitization, Escalated cocaine self-administration, CRF-R1, CP 154,526, VTA

Acknowledgments

I would first like to thank my committee, Klaus, Joe and Steve. Everything you all have taught me over the past couple years is invaluable and I will use these skills for the rest of my career. Secondly, I would like to thank our highly skilled undergraduates for their assistance to accomplish our research, they have been tremendous. Lastly, I would like to thank our staff within the lab, especially Tom Sopko and Akiko Shimamoto for their wonderful assistance, but most importantly I am very happy to know that I am supported by everyone in the lab. I hope to continue producing excellent research and I am excited to see what my future holds. Also thanks for the funding by National Institute on Drug Abuse grant DA-002632 awarded to KAM.

Table of contents

Introduction

The Diagnostic and Statistical Manual for Mental Disorders (DSM-IV-TR; American Psychiatric Association, 2000) defines substance dependence as (1) compulsion to seek and take the drug (i.e. cocaine), (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented. Drug addiction has been conceptualized as a transition from impulsivity to compulsivity composed of three stages: 'binge/intoxication', 'withdrawal/negative affect', and 'preoccupation/anticipation' (craving) (Koob and Volkow 2010).

Psychostimulant abuse, including cocaine or amphetamine use continues to be a worldwide problem. According to the United Nations Office on Drugs and Crime, amphetamine-like stimulants are one of the most widely abused classes of drugs, with an estimated 30 million people abusing amphetamines. In addition, over 13 million people have been reported to abuse cocaine and the number continues to grow (UNODC, 2004). Reports from emergency rooms treating victims of violence and statistics from the criminal justice system on violent crimes committed by drug users as well as epidemiological evidence and neurobiological data all link social stress and drug use (Sinha et al. 2006;Sinha 2009;Substance Abuse and Mental Health Services Administration 2010). Several studies have indicated that one of the major factors contributing to relapse and continuous drug abuse is stress. Adverse life events, chronic distress and environmental stressors have been reported to be positively correlated with increased drug abuse in humans (Brady and Sinha 2005;Kosten et al. 1986;Sinha 2001;Sinha et al. 2006). The experience of stress and substance abuse seem to share common neurobiological substrates that include the release of neuropeptide corticotropin releasing factor (CRF) and the neurotransmitter

dopamine (DA). More specifically, the mesolimbic dopamine pathway, originating in the ventral tegmental area (VTA) seems to be a critical brain site that may mediate various behaviors including the transition from recreational drug use to addiction.

Mesolimbic Dopamine System

The mesolimbic dopamine system is the primary source of dopamine released throughout the brain and mediates behaviors of substance dependence and natural rewards including those of palatable foods, liquids and sex. These thoughts and behaviors can be attributed to an increased level in dopamine, primarily in the nucleus accumbens (NAC) (DiChiara and Imperato 1988;Pontieri et al. 1995;Wise and Rompre 1989). The major brain structures that constitute the mesolimbic pathway are the prefrontal cortex (PFC), amygdala, subiculum, the nucleus accumbens/striatal complex and the ventral tegmental area (Kalivas 1993). The primary source of dopamine release is by the neurons whose cell bodies lie within the ventromedial mesencephalon, primarily in the VTA. Within the VTA there are two subdivisions, the anterior portion and the posterior portion (Rodd et al. 2005). Major synaptic inputs into the VTA, containing CRF, originate from the paraventricular nucleus, bed nucleus of the stria terminalis (BNST) and the central amygdala (Rodaros et al. 2007). In addition, other afferent inputs to the VTA arise from many brain regions that include: the PFC, NAC, the diagonal band of Broca, substantia innominata, lateral preoptic area, and lateral hypothalamus. Some lower brainstem afferent connections to the VTA include the superior colliculus, substantia nigra, dorsal raphe, parabrachial nucleus, and dentate nucleus of the cerebellum.

Major synaptic outputs from the VTA go to the nucleus accumbens which is divided into two major subdivisions: the shell and the core. The shell has a very intricate efferent connection

to many brain regions including the ventromedial ventral pallidum, bed nucleus of the stria terminalis, central amygdala (Heimer et al. 1991), lateral preoptic area, lateral hypothalamus, mediodorsal substantia nigra pars compacta, mesopontine reticular formation, periaqueductal gray (PAG) and the VTA (Ikemoto and Panksepp 1999). The core sends major efferent projections to the dorsolateral ventral pallidum, endopeduncular nucleus, lateral part of the VTA and the substantia nigra (Oades and Halliday 1987).

In addition to dopamine as a primary neurotransmitter, CRF is emerging as a likely neuropeptide that modulates cellular release of dopamine in the VTA. Exposure to acute stressors can increase CRF release in the VTA (Wang et al. 2005). This stress activation of the CRF system within the mesolimbic pathway can presumably be attributed to efferent's from the PVN, BNST, or CeA, to the VTA which appear to be facilitated by glutamatergic input, increasing dopamine release (Rodaros et al. 2007;Ungless et al. 2003). Cocaine addiction has been hypothesized to be due to drug-induced neuroplastic changes in the mesolimbic DA pathway that may be modulated by CRF neurons. Repeated exposure to psychostimulants has been associated with increases in locomotor behaviors along with increases in c-Fos expression (Covington, III et al. 2005;Nikulina et al. 2004). This suggests that the VTA within the mesolimbic DA pathway is a primary brain site that involves many neurotransmitters and with repeated drug exposure, the VTA may play a critical role in the induction and expression of sensitization.

Repeated Psychostimulant Use and Behavioral Sensitization

Repeated administration of drugs (e.g. cocaine, amphetamine, morphine, phencyclidine (PCP), and nicotine) results in a progressive increase in the subsequent locomotor response to the

drug. This progressive change in locomotor behavior, such as, walking, running, and stereotyped behaviors (e.g. turning, sniffing, and climbing) can be defined as behavioral sensitization (Robinson and Berridge 1993). This development of sensitization including those behaviors of locomotor stimulation has been associated with an increased release of dopamine in the nucleus accumbens. In addition, stereotyped behaviors have been associated with changes in the striatum (Fibiger et al. 1973;Kelly et al. 1975;Sharp et al. 1987). Usually behavioral sensitization is measured by psychomotor-activating effects caused by the psychostimulant. Most frequently, locomotor tests (e.g. beam breaks, crossovers, distance traveled, walking frequency) are performed to quantify the psychomotor effects on the individual and external stimuli can either increase or decrease these psychomotor effects. For example, animals exposed to intermittent social defeat stress demonstrate a significantly higher walking behavior when injected with cocaine compared to contemporary controls (10 mg/kg i.p.) (Covington, III and Miczek 2005). The primary behavior displayed is an increase in walking, but other complex patterns may be demonstrated by repetitive motor actions (stereotyped behaviors), rearing, head movements, limb movements, sniffing, oral movements (licking and biting).

Sensitization is part of a two step process that first involves both induction and expression. Neural adaptations in the ventral tegmental area and prefrontal cortex most likely are due to the induction process (Vanderschuren and Kalivas 2000). Some drugs of abuse have been thought to exert their action on dopamine cell bodies in the midbrain to induce sensitization or changes in the neurochemical makeup. The induction process can occur during one exposure to drugs of abuse and it can be amplified after repeated administration. Once this induction process is produced it is known that these adapted cells will persist for very long periods (Miczek et al. 2004). After inducing neurobiological changes within the brain, the expression of sensitization or

augmented behavioral responses to a psychostimulant challenge can persist for more than a year, and this has been correlated with an increased release of dopamine in the nucleus accumbens (Paulson et al. 1991). However, the persistence of sensitization is dependent on many factors, including the type of drug, the dose, the number of exposures, and the pattern of exposure. One of the major factors contributing to the expression of sensitization is the context to which the animal or human is exposed. Contextual cues such as lights, housing, or odor can be a major influence in how an animal may react to an injection of a psychostimulant (Badiani et al. 1995b). For example, when an animal has developed a psychomotor sensitization to several drug exposures in a specific context over a specific time period and then is re-exposed to the drug in a different contextual arrangement, the expression of the psychomotor behavior may be lost (Badiani et al. 1995a). Contextual factors are not well understood and deserve further investigations that may prove useful in developing treatments for addiction (Robinson and Berridge 2008;Stewart and Badiani 1993). Overall the mesolimbic dopamine pathway is involved in the induction and expression of sensitization, and is a key component involved in the transition from recreational drug use to compulsive drug addiction (Robinson and Berridge 1993).

Behavioral sensitization has been hypothesized to be associated with many neuroadaptive changes in the brain which may be important to compulsive drug use and psychotic disorders that may be contributing to addiction (Robinson and Berridge 1993;Stam et al. 2000;Stewart and Badiani 1993;Vanderschuren and Kalivas 2000). In humans, behavioral sensitization is harder to describe, but some people, after repeated amphetamine use may begin to resemble an individual with schizophrenia or paranoia (Ellinwood 1967;Griffith et al. 1972). Repeated amphetamine exposure has been shown to induce behavioral sensitization (e.g. increased eye-blink responses,

vigor, and energy ratings) and neural sensitization in humans (e.g., an increase in evoked dopamine "release" as indicated by raclopride displacement) (Leyton 2007). However, it is very difficult to investigate behavioral sensitization in humans, but it can be useful to identify the neural changes that occur during repeated administration of psychostimulants. Indeed, exposure to many drugs of abuse can lead to a very complex form of neurobehavioral plasticity that can induce increases and decreases of cellular release in different neural systems that may cause long-term deficits to ones cognitive ability (Kalivas and Volkow 2005). As mentioned above, context specificity is a key component to sensitization and can be a trigger for an individual to relapse. Among other contextual cues, stress also can induce sensitization that could persist for years.

Social Stress

Some specific types of social stress can promote drug abuse and trigger relapse, whereas others do not, each stressor activating discrete neurobiological mechanisms (Miczek et al. 2008;Miczek et al. 2011). For example, continuous subordinate social stress, where the animal is under constant threat can cause neurobiological changes in the raphe and prefrontal cortex, creating anhedonic-like effects. One example of anhedonic-like behavior is observed when an animal once saw sucrose as a reward and tasty fluid and preferred it over water, but once exposed to chronic stress the preference for sucrose diminishes, meaning the animals may have lost interest in the rewarding features of sucrose. In contrast, maternal separation can cause long-lasting changes in regards to, γ-aminobutyric acid (GABA), glutamate, serotonin and opiate receptors that may cause anxiolytic-like responses (Pickering et al. 2006). Maternal separation can cause an animal to be less likely to go out in the open arms of an elevated plus-maze, which

is a sign of anxiety-like behavior (Miczek et al. 2008). Social stress, depending on intensity, duration and controllability, may increase the susceptibility to drug dependence, anxiety, post traumatic stress syndrome, panic disorders or depression. In this study, we have chosen brief, intermittent social stress because this allows us to investigate the neural circuit comprising the VTA-accumbens-prefrontal cortex-amygdala, which is a circuit critical for the dopamine mediated behavioral sensitization and escalated stimulant consumption (Covington, III et al. 2008;Miczek et al. 2011). The brain continually adapts by redefining and reorganizing synaptic connections to reinforce positive or negative behavioral responses (Gould et al. 1999). An important feature of stress in regulating the behavioral outcome of an individual's response is not only the duration of the exposure, but also whether the stress is experienced continuously or intermittently. Continuous, or chronic subordination stress in rats, is considered highly debilitating and could result in eventual death. Chronic stress is defined by animals being exposed to constant threat for more than 10 days, each day being exposed to a confrontation with an aggressive counterpart (Miczek et al. 2004). Chronic stress can result in many physiological consequences involving hyperactivation in various endocrine, immune, and cardiovascular parameters. For example, long-term visual exposure to a dominant male treeshew (Tupaia belangerii) is sufficient to cause severe physiological consequences in an intruding male leading to major endocrine dysfunction, weight loss and eventually death (Von Holst 1998). A third of longhaired rats that were introduced into a small colony of *Rattus villosissimus* eventually died due the aggressive dominant male's presence (Barnett 1975). In addition, chronic subordination can lead to a severely constrained behavioral profile; they exhibit lower reproductive success, disturbed circadian rhythms, and persistent endocrine activation leading to atrophy of the gonads

and suppressed immune function (Bronson and Marsden 1973;Meerlo et al. 1999;Miczek et al. 1991;Raab et al. 1986;Tornatzky and Miczek 1993).

In contrast to chronic stress, where the animal is under constant threat by residing in the aggressive animal's homecage, intermittent social stress is characterized by defeat response to attacks and threats during brief, aggressive confrontations. A brief intermittent episode of social defeat can consist of a 5 minute "fight" between an aggressive resident and an intruder. A "fight" usually consists of 4-5 bites and eventual display of a submissive posture where the intruder has demonstrated a sign of defeat. Brief, intermittent social defeats are usually repeated 4 times over the course of 10 days, separated by 72 hours. Therefore, intermittent brief episodes of social defeat stress give us the opportunity to investigate immediate responses to attacks and threats, the recovery period after defeat, and discrete neurobiological adaptations that persist long after the defeat. The stress of being defeated in an aggressive encounter leads to increases in adrenocorticotropic hormone (ACTH) and glucocorticoids, while decreasing androgens (Brain 1972;Bronson and Marsden 1973;Raab et al. 1986). Tachycardic, hypertensive, and hyperthermic responses are observed in the initial phase of an aggressive encounter in both the resident attacker and the defeated intruder (Schuurman 1980;Tornatzky and Miczek 1993). These large deviations from their normal homeostatic set points are much longer lasting in defeated intruders compared to aggressive residents. For example, after a 10 minute confrontation the normal heart rate for the defeated intruder only returns 3-4 hours post defeat (Tornatzky and Miczek 1993). In addition, plasma corticosterone levels in repeated episodes of defeated rats takes nearly 24 hours to rebound back to baseline levels, whereas in aggressive residents the rebound for normal baseline corticosterone levels occurs within a few hours (Covington, III and Miczek 2005;Schuurman 1980). Immunologically, intermittently defeated rats show reduced

antibody production, indicating immunosuppression (Fleshner et al. 1989;Stefanski 2001). Intermittent social defeat stress can also lead a rat to an escalated response to cocaine self-administration during a 24-hour "binge" (Covington, III and Miczek 2005). By using intermittent social defeat stress it allows us to investigate consequences from stressful experiences. Overall, intermittent social defeat stress allows us to better understand how the body and brain may adapt to cope with stressful stimuli, such as an aggressive resident. Most importantly it has been well established that intruders being intermittently stressed are more vulnerable to escalated drug taking behaviors and this characteristic is very beneficial to understand the neurobiological mechanisms involved in stress and substance abuse.

Stress and Behavioral Cross-Sensitization

Stressful situations can induce behavioral sensitization to psychostimulants, which has been described as cross-sensitization. One study reported that repeated tail-pinch stress in rats can induce behavioral sensitization to a later injection of amphetamine (Antelman et al. 1980). Also, repeated foot-shock (Kalivas and Duffy 1989;Sorg and Kalivas 1991), restraint (Hahn et al. 1986), food restriction (Cabib et al. 2000), and maternal separation (Meaney et al. 2002) induced behavioral sensitization to injections of psychostimulants. In addition, neural changes in the nucleus accumbens have been linked to the mesolimbic dopamine system in regards to psychostimulant self-administration after stress manipulations (Kalivas and Duffy 1989;Piazza et al. 1989). Dopamine is the major neurotransmitter in the development of behavioral sensitization to a psychomotor stimulant. In addition to dopamine, other neurotransmitters such as glutamate and corticotropin releasing factor have been associated with increased neuronal activation that may cause negative changes regarding the composition of a particular neuron (Tagliaferro and

Morales 2008;Wang et al. 2005). Many types of stressors, including handling stress, swim stress, foot-shock stress and social defeat stress increase the dopaminergic activity in the nucleus accumbens and in the prefrontal cortex (Abercrombie et al. 1989;Bannon and Roth 1983;Covington, III et al. 2008;Dazzi et al. 1995;Imperato et al. 1991;Tidey and Miczek 1996). Therefore, stressful stimuli seem to activate many areas in the brain that are also activated by drug of abuse. This may be critical when a person may relapse due to a stressful encounter and these activational effects may be detrimental to an individual's overall health.

Stress and Escalated Drug Taking

Intermittent social defeat stress is extremely valuable due to its persistent, long-lasting nature. For example, studies have demonstrated neural adaptations in immediate early gene expression in the brain (Covington, III and Miczek 2005), induction of behavioral cross-sensitization to psychostimulants, and increases in Fos-like protein in the ventral tegmental area (Nikulina et al. 2004). Even a single social defeat can cause profound changes in defensive behaviors and daily temperature rhythms that could last for weeks (Meerlo et al. 1996;Tornatzky and Miczek 1993). In contrast to continuous subordination where animals face severe pathophysiological consequences and often require the rescue of the stressed animal (Barnett 1975;Blanchard et al. 1985), intermittent stress can show enduring functional activation in mesolimbic structures that may be implicated in escalated drug-taking behaviors (Covington, III and Miczek 2005;Louilot et al. 1986;Mos and Van Valkenburg 1979;Tidey and Miczek 1996).

Social defeat stress in rodents is a paradigm that attempts to mimic real life social events and for this reason its translational value to that of human social stress is of major importance (Björkqvist 2001). Episodic activation of neural and hormonal stress mechanisms can produce

behavioral and neuro chemical changes that may lead to a sensitized individual who may be more prone to drug-seeking and drug-taking behaviors ("behavioral and neurochemical sensitization" (Goeders 2002b;Marinelli and Piazza 2002)). Activation of ascending projections from the mesolimbic pathway is critical for the induction of behavioral sensitization to psychomotor stimulants (Wolf et al. 1994). Considerable evidence points to different kinds of social stress as risk factors for initiating, escalating, and resuming drug abuse (Brady et al. 1998;Sinha et al. 2006).

Intermittent social defeat stress promotes and intensifies cocaine self-administration assessed by performance under the control of a progressive ratio schedule and by conditions of extended binge-like access (Covington, III and Miczek 2001). A progressive ratio schedule has been implemented as a useful protocol to assess the maximal number of infusions prior to the 'break point' value (Hodos 1961). The 'break point' is defined by the last completed ratio achieved before a session is terminated. A session is terminated if the rat does not complete a ratio within 60 minutes of their last infusion. Each ratio is defined as the amount of lever presses needed to obtain an infusion of drug and is as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178...(Richardson and Roberts 1996). Previous studies have demonstrated that intermittent social defeat stress can increase the total number of cocaine infusions taken under a progressive ratio schedule (Covington, III and Miczek 2001;Covington, III and Miczek 2005). In addition, another cocaine self-administration protocol involves a continuous access to cocaine for 24-hours. This protocol allows us to capture dysregulated features of cocaine taking behavior that may be due to previous exposure to intermittent social defeat stress. Many studies have demonstrated that brief, intermittent social defeat stress can significantly increase the total number of cocaine infusions during a continuous 24-hour "binge" which may capture certain

variables of compulsive drug-taking behaviors (Covington, III et al. 2008;Covington, III and Miczek 2005;Miczek et al. 2008). Since we know CRF is the primary neuropeptide involved in the response to a stressor it is of interest to consider manipulations of the CRF receptor in midbrain targets to further investigate the role of social stress, cocaine taking, and CRF.

Corticotropin Releasing Factor (CRF)

The 41-amino acid peptide CRF has been implicated as a key component in the mechanisms by which stress promotes cocaine self-administration (Shaham et al. 1998). In particular, the action on CRF receptor subtype 1 (CRF-R1) rather than CRF-R2 may be critical for escalated cocaine, alcohol and nicotine self-administration in dependent individuals (Goeders 2002a). In addition, many types of stressors potently activate the CRF system both in the CNS and in the hypothalamic-pituitary-adrenal (HPA) axis (Bhatnagar and Vining 2003;Dunn and Berridge 1990;Iwasaki-Sekino et al. 2009).

CRF-immunoreactive fibers densely innervate many intra-hypothalamic and extra-hypothalamic brain areas, including the VTA. Afferent projections of neurons containing high concentrations of CRF are primarily located within the paraventricular nucleus of the hypothalamus (PVN) (Sawchenko et al. 1993), an intra-hypothalamic structure that is part of the HPA axis and is a major contributor in regulating the behavioral and physiological responses to stress. In addition, extra-hypothalamic CRF-containing neurons primarily exist in the bed nucleus of the stria terminalis, the central nucleus of the amygdala (Erb and Stewart 1999;Weiss et al. 2001), and the prefrontal cortex (Millan et al. 1986;Rodaros et al. 2007;Swanson et al. 1983).

CRF acts on two G-protein linked receptors, CRF-R1 and CRF-R2, and can interact with the CRF-binding protein (CRF-BP) (Higelin et al. 2001). More specifically, in the VTA these receptor subtypes are expressed in a topographically intricate manner and are proposed to be involved in brain reward and stress circuits (Sauvage and Steckler 2001;Van Pett et al. 2000). Efferent projections of CRF activation in the VTA is part of an interconnected network involving structures such as, the prefrontal cortex, the shell of the nucleus accumbens, and the basolateral amygdala (Abercrombie et al. 1989;Kalivas and Duffy 1995;Moghaddam 2002)

CRF and the first synthesized CRF receptor antagonists were of a peptide nature and are unable to cross the blood-brain-barrier. Therefore the studies that used these compounds had to administer them via a guide cannula directly into the brain. Intra-cerebro-ventricular (i.c.v.) injections of CRF induced reinstatement of heroin, alcohol, and cocaine seeking behavior, suggesting a role for CRF in stress-induced reinstatement (Brown et al. 2009;Le et al. 2000;Shaham et al. 1997). An initial study reported that i.c.v. injections of alpha-helical CRF_{9-41} (a nonselective peptide CRF receptor antagonist/partial agonist) decreased footshock-induced reinstatement of heroin seeking (Shaham et al. 1997). Subsequent studies reported that i.c.v. injections of a newer nonselective CRF receptor antagonist, D-Phe CRF_{12-41} also decreased footshock-induced reinstatement of nicotine, alcohol and cocaine seeking (Erb et al. 1998;Liu and Weiss 2003;Zislis et al. 2007). Most recently, the VTA has been reported to be a critical site for footshock-induced reinstatement of cocaine seeking (Wang et al. 2005). In addition, local VTA perfusions (via a microdialysis probe) of alpha-helical CRF_{9-41} decreased footshock-induced reinstatement of cocaine seeking (Wang et al. 2005). Therefore, hyperactivation of CRF receptors in the VTA could potentially be a target for neural sensitization that may induce escalated cocaine intake as a result of prior experiences with intermittent social stress. Most

studies link CRF-R1 to the regulation of emotional behaviors, including anxiety, stress, and drug-taking (Heinrichs et al. 1992).

Cellular Distributions in the VTA

The cellular distribution in the VTA has recently revealed CRF and glutamate (GLU) axon terminals establishing asymmetric synaptic connections with VTA DA neurons which are part of the mesolimbic pathways mediating brain reward and stress (Tagliaferro and Morales 2008). CRF in the VTA is predominantly found in axons and axon terminals but rarely found in dendrites. Neurons in the VTA coexpress vesicular glutamate transporters (vGluT2) and CRF, or coexpress tyrosine hydroylase (TH) and CRF, or coexpress glutamic acid decarboxylase (GAD) and CRF. One major difference between the neurons containing either VGluT2 or TH with the coexpression of CRF is that the axon terminals establish asymmetric synaptic connections, whereas neurons containing the coexpression of GAD and CRF establish mostly symmetric synaptic connections. Morphological analysis concluded that about 60% of all CRF synapses in the VTA are asymmetric, being characterized by a thick postsynaptic density. The remaining 40% of synapses were characterized as having a thin postsynaptic density and were identified as symmetric synapses. One major finding is that most CRF axon terminals that contact dopaminergic neurons, as expressed by a TH marker, form asymmetric synapses that may suggest glutamateric activation. Based on these morphological analyses, one proposed mechanism is that stress causes activation of CRF glutamatergic neurons innervating the VTA. Electrophysiological studies have demonstrated that glutamatergic regulation of DA neurons in the VTA is potentiated by NMDA receptor mediated neurotransmission which is also facilitated by CRF (Ungless et al. 2003). In accordance with Ungless et al. (2003), Korotkova et al. (2006)

saw a similar increase in the firing rate of dopaminergic VTA cells after the application of CRF. Brain slices that received CRF not only increased VTA dopamine neuron firing, but did so in a concentration-dependent manner. The results from experiments using CRF receptor agonists, CRF receptor antagonists and CRF receptor-deficient mice all lead to the same conclusion that CRF increased VTA dopamine neuron firing through activation of the CRF-R1 subtype (Wanat et al. 2008). These activational effects within the VTA most likely affect downstream elevation of DA and activation of DA receptors in the mPFC and NAC (Capriles et al. 2003;Kalivas et al. 1987;McFarland et al. 2004). Upon an external stimulus, such as stress, innervations in the VTA can cause a local release of CRF, glutamate or both that may produce activation of VTA dopamine neurons (Tagliaferro and Morales 2008).

The current study focuses on the CRF-R1 subtype in the ventral tegmental area for several reasons. Immunohistochemical investigations have demonstrated that CRF-R1 are localized throughout the VTA on a proportion of neurons that synthesize dopamine and project to forebrain regions such as the nucleus accumbens and medial prefrontal cortex (mPFC) (Sauvage and Steckler 2001). The VTA receives many synaptic inputs from limbic regions and from the CRF-rich paraventricular nucleus of the hypothalamus (Rodaros et al. 2007). CRF is co-localized in a number of excitatory or inhibitory afferents in the VTA that synapse on dopaminergic and nondopaminergic neurons. Synaptic connections between CRF terminals and dopamine neurons are predominately (83%) asymmetric, glutamatergic, and presumably excitatory (Tagliaferro and Morales 2008). Injections of CRF dose-dependently increased the firing rate of dopamine neurons in the VTA, and this effect was prevented by CRF-R1 antagonism (Wanat et al. 2008). In addition, many neurochemical and behavioral effects have been observed after injections of a CRF-R1 antagonist into the VTA (Kalivas et al. 1987;Specio

et al. 2008;Wang et al. 2005). Our rationale for the current experiments was to study whether it is possible to prevent the effects of intermittently scheduled, brief social defeat stress on subsequent intravenous cocaine self-administration by pretreatment with a CRF-R1 antagonist into the VTA.

Hypothesis and Aims

We aimed to test the hypothesis that brief, intermittently scheduled social defeat activates CRF-R1 in the VTA, and that this activation is necessary for the development of increased behavioral responses to cocaine. Our study used a selective non-peptide CRF-R1 antagonist (CP 154,526), to specifically block the stimulation of dopamine neurons by CRF-R1activation (Schulz et al. 1996). The choice of CP 154,526 is based on its ability to penetrate the blood-brain barrier (Schulz et al. 1996). If we are able to block the downstream release of dopamine during social stress we may be able to prevent the induction of behavioral cross-sensitization to cocaine and we may be able to prevent escalated cocaine intake during a 24-hour "binge". The first experiment attempts to characterize whether pretreatment with a CRF-R1antagonist, CP 154,526, (via systemic administration 20 mg/kg) can prevent the stress-induced locomotor sensitization, and subsequently prevent escalated cocaine self-administration. The second experiment specifically targets the VTA as a potential site of intervention to examine if microinjections of a CRF-R1 antagonist at this site prior to each social defeat episode can attenuate or prevent an escalated response to cocaine.

Materials and Methods

Subjects. Male Long Evans rats (Charles River Laboratories, Wilmington, MA) weighing 225-250g were individually housed in custom-built acrylic chambers (30 x 30.5 x 24.5 cm) on arrival (n=105). The floor of each cage was lined with Cellu-Dri^{TM} pellet bedding (Shepherd Specialty Papers, Kalamazoo, MI). All rats were on a reversed light/dark cycle (lights on 2000-0800 hours), with controlled temperature (21^{o} C ±10). Rats were given unlimited access to food (Purina laboratory rodent chow) and tap water. The housing conditions were maintained consistently throughout the experiment (See below, social defeat phase, cocaine challenge and cocaine intravenous (IV) self-administration). All experimental procedures were approved by the Tufts Institutional Animal Care and Use Committee, following the principles of Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Social Defeat Stress. Upon arrival rats were randomly assigned to two groups, defeat stressed or non-stressed controls. Subsequently separate groups of rats were assigned to pharmacological treatment conditions. Experimentally stressed rats were socially defeated as described previously (Covington, III et al. 2008), on days 1, 4, 7, and 10. Each social defeat episode took place in a room adjacent to the vivarium where the rats were normally housed.

Residents. A separate group of 20 male Long Evans rats (Charles River Laboratories, Wilmington, MA) was housed with females in large stainless steel cages (45.7 x 71.1 x 45.7 cm) and served as aggressive stimulus rats (residents). Each resident was chosen on the basis of displaying consistent aggressive behavior during confrontations with an intruder rat (Miczek 1979).

Systemic Experiment

Before each defeat, rats in the defeat stress and control groups were weighed and then injected with the appropriate agent, either vehicle (saline), or the CRF-R1 antagonist CP 154,526

dissolved in 0.1% methylcellulose with an injection volume of 1.0 ml/kg. Contemporary controls were administered either saline or CP 154,526 and then were returned to their homecages. Twenty minutes later, experimentally stressed rats were placed in a steel mesh protective cage inside the home cage of a larger aggressive "resident" rat. After 10 minutes of social threat in the protective cage, experimentally stressed rats were then removed from the protective unit, and exposed to attacks by the resident until they showed behavioral evidence of defeat, defined as the display of 4 seconds of supine position. Typically the defeat episode included 4 attack flurries, within a maximum of 5 minutes. Immediately after the defeat the experimentally stressed rats were then returned to the protective cage, which was placed back in the aggressive rat's home cage for an additional 10 minutes. Rats were then returned to their home cages in the vivarium until their next defeat, 72 hours later. Each experimentally defeated rat was exposed to 4 different resident aggressors over the course of the 10 days.

Intra-VTA Experiment

Intra-VTA cannula surgery. The CRF-R1antagonist CP 154,526 was directly infused into the VTA before each social defeat. Rats were surgically implanted with two guide cannulae for the delivery of CP 154,526 or artificial cerebrospinal fluid (aCSF) vehicle microinjections. While under ketamine (100 mg/kg) and xylazine (6 mg/kg) anesthesia, rats were surgically implanted with bilateral cannulae (23 gauge stainless steel, 11 mm) aimed 1 mm above the VTA (AP: -5.2, DV: -7.5, ML: +1.8 mm) at a 10° angle relative to bregma (Paxinos and Watson 1997). Cannulae were affixed with dental acrylic and three stainless steel screws, #0, anchored into the skull. Patency of cannulae was maintained by inserting obdurators (0.010/.25 mm fit 11 mm with 1 mm projection) except during microinjections. Each infusion of CP 154,526 (0.3µg/side) or vehicle (aCSF) was delivered over 3 minutes in a volume of 0.5 µl using a CMA/100

microinfusion pump (CMA Microdialysis, Sweden). After each infusion the injector was left in place for an additional 1 minute to allow for diffusion and to prevent backflow. Infusions were administered once every 72 hours, 20 minutes before each of the four stress episodes, or once every 72 hours in the absence of social stress (control). After the completion of the self-administration experiments, rats were sacrificed and cannula placement was verified by mounting 50-µm sections on gelatin coated slides and then staining them with cresyl violet (Figure 2).

IV Catheter Surgery. All rats were permanently implanted with indwelling catheter (Siltastic® silicon tubing, ID 0.63 mm, OD 1.17 mm) into the right jugular vein (Covington, III and Miczek 2001;Remie et al. 1990) under a combination of ketamine (100 mg/kg) and xylazine (6 mg/kg) anesthesia. The catheter was passed subcutaneously through the rat's back where it exited through a small incision and was affixed to a small plastic pedestal (Plastics One, Roanoke, VA) mounted inside a harness (Instech Laboratories Inc., Plymouth Meeting, PA). After catheter surgery rats were allowed 5 days to recover, and were handled and weighed daily.

Locomotor sensitization

Cocaine Challenge. Eleven days after the fourth social defeat episode (day 21), stressed and non-stressed rats were given a challenge injection of cocaine (10 mg/kg i.p.), to asses behavioral cross-sensitization (Covington, III and Miczek 2001). Each rat was moved to an adjacent room and was briefly removed from its cage to be weighed and injected with saline, after which it was immediately returned to the home cage. 5 min after the saline injection, rats were videotaped for 5 minutes. All rats were then injected with cocaine (10 mg/kg i.p.) and additional behavioral recordings took place 5-10 and 25-30 min later. A trained observer analyzed each video recording, using a custom keyboard and commercial software (The

Observer Video-Pro© version 8.0, Noldus Information Technology, Wageningen, The Netherlands) to record the frequency and duration of rearing, walking, grooming, and inactivity.

Cocaine self-administration

After 5 days of recovery, rats were moved from their home cage and housed in the IV self-administration test chambers (Miczek and Mutschler 1996). Catheters were flushed with 0.2 ml of saline and 0.2 ml of heparinized saline (20 IU/ml) each morning, and 0.17 ml pulses of saline were delivered every 30 minutes except during the daily cocaine self-administration sessions.

Acquisition and maintenance. Initially, rats were allowed to self-administer cocaine (0.75 mg/kg/infusion), without a priming injection, and each lever press resulted in an IV infusion (fixed ratio; FR 1 schedule of reinforcement) followed by a 30 second timeout. Each daily session terminated after the delivery of 15 infusions or after 5 hours of access. After reliable self-administration behavior was verified (two consecutive days of 15 infusions), the FR schedule was progressively increased over three to five additional days until every fifth lever press resulted in an IV infusion (FR 5). Rats were maintained on a limited access FR 5 schedule for at least 5 consecutive days. Rats that did not meet the criterion of two consecutive days of 15 infusions underwent behavioral shaping to facilitate lever pressing for IV cocaine self-administration.

Progressive ratio schedule. Once rats showed reliable, stable self-administration on FR5 for 5 days, they were tested using a progressive ratio (PR) schedule (Richardson and Roberts 1996). The progressive response requirement incremented as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178... Sessions terminated once no cocaine infusion was delivered within 60 minutes. The average number of completed infusions for each rat was the

dependent variable. Three daily PR sessions alternated with FR 5 sessions of 15 infusions, 0.75 mg/kg/infusion.

Twenty-four-hour binge. After their final PR session, rats were given one more day of limited access to cocaine (FR5, 0.75 mg/kg/infusion, 15 infusions). The following day rats were given the opportunity to self-administer cocaine (FR5, 0.3 mg/kg/infusion) for 24 hours. The total number of infusions of cocaine was the dependent variable for analysis. Upon completion of the 24-hour binge, catheter patency was verified by IV administration of methohexital sodium (Brevital™) (Figure 1).

Statistics. Two-way analyses of variance (ANOVA) were used to analyze walking frequency in response to a cocaine injection (10 mg/kg i.p.), average number of infusions received over 3 PR self-administration sessions, total number of infusions received during the 24-hour "binge", and in the cumulative cocaine infusions at hour 24 across the treatment groups (stress or control, CP 154,526 or vehicle). When the F-ratios were statistically significant Holm-Sidak post hoc analyses of all pair-wise comparisons were made.

Systemic experiment (n=58)

Cocaine challenge: Non-stressed vehicle n=17; Non-stressed CP 154-526 n=10; Episodic defeat vehicle n=20; Episodic defeat CP 154-526 n=11.

Cocaine self-administration: Non-stressed vehicle n=9; Non-stressed CP 154-526 n=8; Episodic defeat vehicle n=9; Episodic defeat CP 154-526 n=9.

Intra-VTA experiment (n=47)

Cocaine challenge: Non-stressed vehicle n=11; Non-stressed CP 154-526 n=9; Episodic defeat vehicle n=14; Episodic defeat CP 154-526 n=13.

Cocaine self-administration: Non-stressed vehicle n=8; Non-stressed CP 154-526 n=7; Episodic defeat vehicle n=7; Episodic defeat CP 154-526 n=8.

Animals dropped from analysis during the cocaine self-administration phase were due to illness, or failure of catheter patency. Seventeen microinjected animals were excluded from the cocaine challenge analysis due to missed placements.

Drug solutions. Cocaine hydrochloride was obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD) and was dissolved in sterile 0.9% saline. CP 154,526 was obtained from Pfizer as a gift or was purchased from Tocris Bioscience (St Louis, MO). The CP 154,526 doses were based on previous reports (Schulz et al. 1996;Shaham et al. 1998).

Results

Figure 1 is a line graph of the experimental design, timeline, and drugs used in phase I-III (Figure 1). Figure 2 is a schematic portrayal of accurately placed intra-VTA sites. Each figure corresponds to coronal sections of the rat brain from -5.2 to -6.04 mm from bregma. Filled circles represent the average location of each pair of bilateral cannulae. The injection sites from seventeen rats were inaccurate placements and are shown as open circles. On the right is photomicrograph of an intra-VTA injection site (Figure 2).

Locomotor sensitization to cocaine

Systemic Experiment

Cocaine Challenge. Eleven days after the last social defeat (i.e., day 21) no significant differences were found between groups in terms of the frequency of any behavior after an initial saline challenge. However, the cocaine (10 mg/kg i.p.) challenge produced a significant increase in the frequency of walking behavior in those rats that were episodically stressed and pretreated

with vehicle injections prior to each defeat (Fig. 3A). During the cocaine challenge, a significant main effect of stress was detected on the frequency of walking behavior in episodically stressed animals compared to non-stressed ($F_{1,54}$=11.502, p=0.001), and a significant interaction between stress and drug treatment was also observed ($F_{1,54}$=7.014, p=0.011) (Figure 3A).

Post hoc analysis revealed a significant increase in the walking frequency in those rats exposed to intermittent stress and pretreated with vehicle compared to the vehicle-treated non-stress condition (p<0.001). A significant reduction was detected in the walking frequency during the cocaine challenge in stressed rats pretreated with CP 154,526 (20 mg/kg i.p.), as compared to the vehicle-treated stress condition (p=0.001), showing that systemic injections of CP 154,526 before each exposure to stress prevented the induction of locomotor sensitization (Figure 3B).

Intra-VTA Experiment

Cocaine Challenge. Microinfusions of CP 154,526 (0.3µg/0.5µl/side) into the VTA prior to each episode of social defeat significantly reduced the walking frequency in response to a cocaine challenge as compared to the vehicle-treated stress condition (Fig. 3B). Overall, a significant interaction between stress and drug treatment was observed ($F_{1,43}$=6.317 p=0.016) (Figure 4A).

Walking frequency was significantly higher in rats that were stressed and microinfused with vehicle compared to non-stressed vehicle-treated controls (p=0.049), while walking frequency was significantly reduced in stressed rats pretreated with CP 154,526 compared to the vehicle-treated stress condition (p=0.006). Of the seventeen missed placements, four were part of the group that received both stress and microinjections of CP 154,526. The average walking frequency for rats with misplacements outside the VTA was 75 ±10.6, roughly 1.7 times higher than those rats receiving CP 154,526 directly into the VTA (Figure 4B).

Cocaine Self-Administration

Systemic Experiment

Acquisition and maintenance. The statistical power was not great enough to run accurate statistics for the first two daily sessions of cocaine self-administration, rather the percentage of rats that met the criterion within the first two daily sessions are reported. Maintenance (resp/min) of self-administering cocaine was significantly greater in those rats that received systemic injections of vehicle compared to those rats that received systemic injections of CP 154,526 ($F_{1,36}$=5.288 p=.027). In addition, rats that were stressed compared to non-stressed controls showed a significant increase in rate of self-administering cocaine ($F_{1,36}$=8.417 p=0.006). (see Table 1)

Progressive ratio of cocaine reinforcement. The "break point" for self-administering cocaine was significantly less in those rats that received systemic injections of CP 154,526 compared to those rats that received systemic injections of vehicle. Specifically, there was an overall drug effect between conditions ($F_{1,31}$=6.451, p=0.016). (see Table 1).

Twenty-four-hour cocaine binge. The total number of cocaine infusions during the 24-h "binge" was highest in the vehicle-treated stress sensitized rats, which contributed to a significant main effect of stress ($F_{1,31}$=8.0, p=0.008), a significant main effect of drug ($F_{1,31}$=5.330, p=0.028), and a significant interaction between stress and drug treatment ($F_{1,31}$=4.975, p=0.033) (Fig. 4A). Stressed, vehicle-treated rats achieved significantly more cocaine infusions during the 24-h "binge" than non-stressed vehicle-treated controls (p=0.001). Rats given systemic injections of CP 154,526 prior to each defeat showed a significant reduction in the total number of cocaine infusions during the 24-h "binge" compared to the stressed vehicle-treated group (p=0.003). In addition, cumulative cocaine infusions represent the same

statistics described above since we only did statistics at hour 24 during the cocaine binge. (Fig. 5A).

Intra-VTA Experiment

Acquisition and maintenance. The statistical power was not great enough to run accurate statistics for the first two daily sessions of cocaine self-administration, rather the percentage of rats that did meet the criterion within the first two daily sessions were reported. There were no significant differences observed for the maintenance phase of cocaine self-administration (see Table 1)

Progressive ratio of cocaine reinforcement. There were no significant differences in the progressive ratio phase of cocaine self-administration. (see Table 1).

Twenty-four-hour cocaine binge. Furthermore, a significant role for VTA activation during social stress was revealed by the effects of CP 154,526 microinfusion prior to each defeat on subsequent cocaine taking during the 24-hour "binge". The total number of cocaine infusions during the "binge" was significantly reduced in those rats receiving microinjections of CP 154,526 prior to each exposure to defeat compared to vehicle injected stressed rats, which contributed to an overall significant interaction between stress and drug treatment ($F_{1,26}$=4.628,p=0.041) (Fig. 4B).

The number of cocaine infusions during a 24-hour "binge" in vehicle-treated stressed rats was significantly higher compared to vehicle-treated controls (p=0.007). Intra-VTA infusions of CP 154,526 prior to intermittent social stress significantly reduced the total number of cocaine infusions over the 24-hour period as compared to the stressed vehicle-treated condition (p=0.032). Cumulative cocaine infusions represent the same statistics as the number of cocaine infusions during the 24-hour "binge" and we only did statistics at hour 24 during the cocaine

"binge". (Fig. 5B). Of the seventeen missed placements, one rat received both stress and microinjections of CP 154,526 that completed the "binge". This rat accumulated 181 infusions of cocaine which is below the group's average.

Discussion

Highlights of CRF-R1 in the VTA

The current results suggest that the CRF-R1 subtype in the VTA is critically involved in the development of stress-induced sensitization which can lead to escalated cocaine self-administration during continuous access in a 24-hour "binge". These results significantly extend previous demonstrations that antagonizing the CRF-R1 subtype in the VTA may be an important intervention for preventing behavioral and neural sensitization to repeated exposure to cocaine and in the escalation of cocaine self-administration (Goeders 2002a;Lodge and Grace 2005;Phillips et al. 2003;Specio et al. 2008). These data show that CRF-R1 antagonism in the VTA prevented locomotor sensitization to a cocaine challenge and prevented escalation of cocaine "binges" after experiencing brief intermittent episodes of social defeat stress. The current findings suggest that CRF may be activated by intermittent social stress episodes and, after repeated intermittent exposures, CRF may exert a significant role in inducing neuroplastic changes in the mesolimbic DA system primarily in the VTA (Miczek et al. 2008).

Social defeat stress, CRF, CRF receptors

Social defeat stress can increase the release of CRF throughout the brain and if repeated this may cause detrimental long-term effects at the behavioral and physiological level. One study examined the emotionality of a rat after being defeated. The measure of emotional behavior was described as decreased entry into the open, unprotected arms as opposed into entries in darkened

walled arms of the elevated Plus-Maze (Heinrichs et al. 1992). Rats that received i.c.v. administration of alpha-helical CRF antagonist post-defeat showed a reversal of the hyper-emotionality of one behavior in the elevated Plus-Maze as compared to rats that did not receive the CRF antagonist post-defeat. In addition, animals not exposed to stress, rather received different doses of alpha-helical CRF did not show any behavioral deficits in performing the task, which suggests that this measure is stress dependent. In attempt to specify exactly where in the brain the CRF antagonist had its effect on reversing the hyper-emotionality response post-defeat, this study aimed guide cannulae into the amygdala and the caudate putamen. Administration of alpha-helical CRF antagonist into the amygdala showed a more potent effect on reversing the hyper-emotionality response compared to vehicle treated animals post-defeat. On the contrary injections of alpha-helical CRF antagonist into the caudate putamen had no effect on the hyper-emotionality response post-defeat. These data suggest that the amygdala serves as a potential intervention site that acts upon the CRF receptors and exerts an anti-stress response that brings behavioral and physiological levels to their pre-stress, resting levels (Heinrichs et al. 1992).

As previously demonstrated exposure to social defeat stress can have detrimental effects on behavioral performances in the Plus-Maze that may be due to neuroplastic changes of CRF and CRF receptors. Other studies use a procedure called conditioned defeat (Potegal et al. 1993). Male Syrian hamsters are used in this procedure due to their normally aggressive characteristics to defend their home territory. However, if hamsters are exposed to even a mild social defeat experience they subsequently become highly submissive and lose their territorial aggression. Hamsters that are previously exposed to social defeat stress subsequently do not defend their home territory against smaller intruders or even to nonaggressive conspecifics, rather they avoid social interaction and submit to intruders without confrontation. Several lines of evidence point

to CRF as the major substrate that may be involved in conditioned defeat. I.C.V. administrations of the CRF receptor antagonist, D-Phe(12-41) attenuated defensive behaviors after being exposed to social defeat stress compared to hamsters receiving vehicle treatment (Jasnow et al. 1999). Further studies aimed to examine where in the brain this may be occurring and two sites of interest considered were the bed nucleus of the stria terminalis (BNST) and the central amygdala (CeA). These results demonstrated that bilateral injections of the non-specific CRF receptor antagonist, D-Phe(12-41) into the BNST prior to social defeat stress significantly attenuated the display of submissive-defensive behavior compared to vehicle treated hamsters. However, bilateral injections into the CeA prior to social defeat stress did not attenuate submissive-defensive behaviors post-stress as compared to vehicle treated hamsters (Jasnow et al. 2004). To confirm the neurocircuitry involved in conditioned defeat an experiment performed unilateral lesions to the CeA with simultaneous unilateral infusions of D-Phe(12-41) into the BNST which significantly reduced the submissive-defensive behaviors by previously defeated subjects. Further unilateral lesions of the CeA and contralateral injections of D-Phe(12-41) into the BNST significantly attenuated the display of submissive-defensive behaviors post-defeat compared to vehicle treated hamsters. These results suggest that CRF acts within the neural circuit that includes the BNST and the CeA that may regulate the behavioral responses to social defeat stress (Jasnow et al. 2004). However, CRF within the BNST, an important efferent to the amygdala may be the primary brain site containing CRF receptors in regulating conditioned defeat in regards to submissive-defensive behaviors. This CRF release throughout the brain during brief confrontations of social defeat stress can cause many downstream effects that can increase the release of many monoaminergic molecules such as norepinephrine, serotonin and dopamine.

Social stress, CRF, and monoamines

Peptidergic modulation of monoaminergic nuclei continue to be investigated as targets for therapeutic intervention in stress disorders. A brief experience of social defeat stress profoundly increases the release of aminergic and peptidergic transmitter substances in the brain, beginning with noradrenergic activation in the cortex after witnessing brief confrontations in mice (Hendley et al. 1973). Serotonin (5-HT) release is substantially increased as a result of an increase in the firing rate of 5-HT containing raphe cells recorded during intense defensive reactions by tree shews (Walletschek and Raab 1982). Other transmitter substances including dopamine in the limbic forebrain structures were increased in post-mortem assays in mice and rats that defended against attacks (Louilot et al. 1986;Puglisi-Allegra and Cabib 1990). In vivo microdialysis was able to measure a rise in accumbal and cortical dopamine release, but not in the striatum under threatening conditions by an aggressive opponent (Tidey and Miczek 1996).

The current results suggest an additional target for intervention by focusing on the CRF-R1 subtype in the VTA. Brief intermittently scheduled social stress can cause long-term alterations that sensitize the mesolimbic DA pathway (Miczek et al. 2011;Tidey and Miczek 1996). Our results demonstrate that stress-escalated cocaine taking can be prevented by pretreatment with a CRF-R1 antagonist into the VTA prior to each social defeat episode. Thus, a CRF-R1 antagonist may be a promising therapeutic intervention for stress-escalated drug dependence. Once the side-effect profile of CRF-R1 antagonist is significantly improved, particularly at the human level, it is feasible to envision therapeutic interventions that rely primarily on sites of action at monoaminergic cells in the brain stem. This sensitized cellular activity might reflect a dysregulated cascade of intracellular processes that may promote the escalation of stress-induced drug-taking behavior. The previous studies provide a potential link

between dopamine, CRF, and CRF receptors in the VTA and this link may be relevant to a specific role in cocaine taking behaviors.

Cocaine, CRF and CRF receptors

Administration of cocaine can induce a number of changes in the expression of CRF and its receptors in the central nervous system. Neuroendocrine dysfunctions appear to be disrupted due to less CRF-like immunoreactivity in the hypothalamus after acute cocaine administration (Sarnyai 1993). In addition to the hypothalamus being affected by cocaine, changes in CRF-like immunoreativity has been shown in a number of other brain regions including the hippocampus, frontal cortex, basal forebrain, and amygdala (Gardi et al. 1997;Sarnyai 1993) One study reported that administration of cocaine can induce increases in CRF synthesis and release in the amygdala following acute injection of cocaine (Richter et al. 1995).

In addition to CRF being affected by cocaine administration, its receptors have been shown to be down-regulated in the amygdala following chronic cocaine administration (Goeders 1990). CRF activity in other extrahypothalamic areas including those of the mesolimbic dopaminergic system can also be affected after cocaine administration (Koob and Bloom 1988). Decreases in CRF-like immunoreativity (Gardi et al. 1997;Sarnyai 1993) and increases in CRF mRNA (Zhou et al. 1996) in the medial prefrontal cortex occur after acute cocaine deliveries. CRF receptor binding in the medial prefrontal cortex and in the nucleus accumbens is reduced after chronic cocaine administration (Ambrosio et al. 1997;Goeders 1990). In contrast to the terminal regions of the mesolimbic dopaminergic pathway, the VTA shows an increase in the CRF receptor binding, which may suggest that CRF has a modulatory effect on dopaminergic neurons (Ungless et al. 2003). In an attempt to target a particular neuropeptide that may be

activating dopamine release from cocaine injections, reports have indicated both acute and chronic blockade of the CRF-R1 attenuated cocaine-induced dopamine release in the NAC (Lodge and Grace 2005). Therefore it is highly likely that CRF and its receptors are involved in the initiation and maintenance of cocaine-taking behaviors and this may be relevant to stress-induced reinstatement of cocaine-seeking.

Stress-induced reinstatement, CRF and CRF receptors

As described above CRF and its receptors are directly involved in cocaine taking and in the response to a stressful stimulus which may cause susceptible characteristics to abuse drugs. Cocaine addiction has been associated with many neuroplastic changes in the mesolimbic dopamingic pathway and has many interactions with CRF, GLU, and DA that underlie the reward mechanisms in the brain. An early report using the procedure of cocaine self-administration, examined the role of a CRF-R1 antagonist on footshock induced reinstatement. Rats were trained to self-administer cocaine, extinguish there cocaine seeking behavior, subsequently reinstating the cocaine seeking behavior by footshock stress. Rats that were treated with a CRF-R1 antagonist, CP-154,526 before exposure to the footshock showed a significant decrease in the active lever response to cocaine seeking behavior compared to rats treated with saline injections (s.c.) (Shaham et al. 1998). This study is in accordance with a previous study that demonstrated i.c.v. injections of a non-selective CRF receptor antagonist, D-Phe(12-41) decreased the active lever responses to footshock-induced reinstatement of cocaine seeking behavior (Erb et al. 1998). However, a separate experiment using the non-selective CRF receptor antagonist failed to prevent cocaine-induced reinstatement of the seeking behavior, which suggests that the CRF antagonism seems to be stress-dependent. Further studies aimed to

examine if microinjections of a non-specific CRF antagonist into the BNST or the amygdala could reduce footshock-induced reinstatement. Intra-BNST infusions, but not intra-amygdala can prevent footshock-induced reinstatement of cocaine seeking behavior in rats. Administration of CRF into the BNST, but not into the amygdala successfully induced cocaine seeking to similar behavioral levels to those that experienced footshock-induced reinstatement. These results suggest that the BNST is a critical site for CRF receptor activation in regards to footshock-induced reinstatement of cocaine seeking behavior, whereas the amygdala seems to be not effective. This does not mean the amygdala is not part of the neurocircuit involved in cocaine-seeking behavior because the amygdala is a primary site for BNST efferents and afferents that contain CRF axon terminals. Therefore the amygdala cannot be excluded from the neural circuitry behind stress-induced relapse to cocaine. In addition to the amygdala and BNST, long-lasting alterations in the mesolimbic DA pathway have been proposed to increase the risk of stress-induced relapse to cocaine seeking (Wang et al. 2005). In a previous study, twenty-minute microdialysis samples from the VTA were collected during the footshock-induced reinstatement phase of the experiment. Footshock stress can increase endogenous CRF levels in the VTA in cocaine-treated and cocaine-naïve rats. Along with endogenous CRF increases, GLU and DA levels were increased in the cocaine-trained but not in the cocaine-naïve rats suggesting specific cellular signaling mediated by CRF release. Furthermore, microinjections of a non-specific CRF receptor antagonist prevented the increase in GLU and DA levels in the VTA, suggesting that CRF may exert control over VTA GLU and DA release projecting to other brain regions. The VTA seems to be a critical site for footshock-induced reinstatement as CRF application appears to elevate extracellular glutamate levels (Wang et al. 2007). Even though most research has

focused on the CRF-R1 subtype in the VTA some studies have reported a subpopulation of the VTA containing the CRF-R2 subtype and CRF-binding protein (CRF-BP) (Wang et al. 2007).

VTA, CRF receptor subtype 2, and CRF-binding protein

In addition to the CRF-R1 subtype in the VTA, CRF-R2 and CRF binding protein have been identified in a subpopulation of VTA neurons (Ungless et al. 2003;Wang et al. 2007). Pharmacological and electrophysiologcal data suggest participation of CRF-R2 and CRF-BP in VTA interactions with glutamate signaling to dopamine neurons. Mixed results using RT-PCR and in-situ hybridization reveal detectible evidence for CRF-R2 mRNA in the VTA of cocaine-naïve mice (Ungless et al. 2003), but other studies did not replicate this finding and could not detect CRF-R2 mRNA in cocaine-naïve mice. Our ongoing research aims to characterize the CRF-R2 subtype in the VTA by microinfusion of a selective CRF-R2 antagonist, Astressin-2B. Our preliminary results regarding the CRF-R2 subtype seem to reveal an amplified response to a cocaine challenge after brief intermittent exposure to social stress, but CRF-R2 antagonism seems to prevent escalated increases in cocaine taking during a continuous access 24-h “binge”. To date the current literature has at least two hypotheses regarding the CRF-R2 subtype. One hypothesis supports CRF-R2 as an additive receptor that can exert similar cellular signaling to that of CRF-R1 (Risbrough et al. 2003;Risbrough et al. 2004;Risbrough et al. 2009). The second hypothesis is that the CRF-R2 subtype exerts opposite functions to those of the CRF-R1 (Makino et al. 2002;Müller and Wurst 2004;Wang et al. 2007). Although further research needs to be done to examine the role of CRF-R2 subtype in regards to social stress and cocaine taking behaviors our preliminary results suggests that the VTA may be a critical intervention site that may decrease the susceptibility to escalated cocaine taking behaviors during a 24-h “binge”.

Conclusion

Epidemiological evidence and neurobiological data converge to link social stress and drug use (Sinha et al. 2006;Sinha 2009;Substance Abuse and Mental Health Services Administration 2010). Some specific types of social stress can promote drug abuse and trigger relapse, whereas others do not, each stressor activating discrete neurobiological mechanisms (Miczek et al. 2008). Social stress, depending on intensity and duration, may increase the susceptibility to anxiety, post traumatic stress syndrome, panic disorders or depression. In spite of several promising leads no effective pharmacological intervention has been approved for the treatment of stress-induced escalated cocaine self-administration.

The transition from initial drug use to escalated drug taking behavior is a complex issue that likely involves neural sensitization in specific mesolimbic DA neurons (Covington, III et al. 2008;Miczek et al. 2008;Wang et al. 2009). Based on the current results, we have replicated our previous finding that brief intermittent social stress can induce locomotor sensitization which in turn can escalate the reinforcing effects of cocaine self-administration during a 24-hour "binge" (Covington, III et al. 2008;Yap et al. 2005). Overall, we prevented stress-induced locomotor sensitization to cocaine by administering a CRF-R1 antagonist into the VTA prior to each episode of social defeat. These results indicate that brief intermittent episodes of social stress can activate the mesolimbic DA circuit via CRF-R1 subtype, which contribute to long-lasting neural adaptations that may influence the reinforcing effects of cocaine.

Future directions

Future studies should consider the role of stress-induced sensitization in regards to neurochemical release. For example, previous research exposed rats to intermittent social defeat

stress and showed an increase in dopamine release to a subsequent cocaine challenge 10 days post-stress in the nucleus accumbens. One question of interest is to identify a link between the VTA and the accumbens in regards to CRF and CRF receptors after being exposed to intermittent social stress. One proposed experiment is to submit rats to intermittent social defeat stress subsequently implanting a unilateral guide cannulae into the VTA and a unilateral guide cannulae into the accumbens. This experiment would allow us to administer CRF antagonists into the VTA while quantifying dopamine levels in the accumbens after a cocaine challenge. These results would lead to information about neurocircuitry and neurochemical release. It is anticipated that dopamine release in the nucleus accumbens should be blunted by administration of a CRF antagonist into the VTA prior to a cocaine injection. Another study should involve the analysis of quantifying CRF levels after being exposed to intermittent social defeat stress. In addition to the PVN being a primary site for CRF neurons, it would be of interest to examine extrahypothalamic regions that include the BNST, CeA, PFC, NAC, and the VTA. The micropunch technique would be very useful to examine these different regions and it would be of interest to see if intermittent social defeat stress can elevate CRF levels. It is anticipated that intermittent social defeat stress should increase CRF levels in comparison to contemporary controls. If this hypothesis stands to be true than we could proceed to see how an increase in CRF levels may contribute to escalated cocaine taking behaviors.

Reference List

1. 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

2. Ambrosio E, Sharpe LG, Pilotte NS (1997) Regional binding to corticotropin releasing factor receptors in brain of rats exposed to chronic cocaine and cocaine withdrawal. Synapse 25:272-276

3. Antelman SM, Eichler AJ, Black CA, Kocan D (1980) Interchangeability of stress and amphetamine in sensitization. Science 207:329-331

4. Badiani A, Anagnostaras SG, Robinson TE (1995a) The development of sensitization to the psychomotor stimulant effects of amphetamine is enhanced in a novel environment. Psychopharmacology 117:443-452

5. Badiani A, Browman KE, Robinson TE (1995b) Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of cocaine and amphetamine. Brain Research 674:291-298

6. Bannon MJ, Roth RH (1983) Pharmacology of mesocortical dopamine neurons. Pharmacological Reviews 35:53-68

7. Barnett SA (1975) The Rat. A Study in Behavior. University of Chicago Press, Chicago

8. Bhatnagar S, Vining C (2003) Facilitation of hypothalamic-pituitary-adrenal responses to novel stress following repeated social stress using the resident/intruder paradigm. Hormones and Behavior 43:158-165

9. Björkqvist K (2001) Social defeat as a stressor in humans. Physiology & Behavior 73:435-442

10. Blanchard RJ, Blanchard DC, Flannelly KJ (1985) Social stress, mortality and aggression in colonies and burrowing habitats. Behavioural Processes 11:209-213

11. Brady KT, Myrick H, McElroy S (1998) The relationship between substance use disorders, impulse control disorders, and pathological aggression. Am J Addict 7:221-230

12. Brady KT, Sinha R (2005) Co-occurring mental and substance use disorders: The neurobiological effects of chronic stress. Am J Psychiatry 162:1483-1493

13. Brain PF (1972) Mammalian behavior and the adrenal cortex -- a review. Behavioral Biology 7:453-477

14. Bronson FH, Marsden HM (1973) The preputial gland as an indicator of social dominance in male mice. Behavioral Biology 9:625-628

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

16. Cabib S, Orsini C, Le Moal M, Piazza PV (2000) Abolition and reversal of strain differences in behavioral responses to drugs of abuse after a brief experience. Science 289:463-465

17. 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

18. Covington HE, III, Kikusui T, Goodhue J, Nikulina EM, Hammer RP, Jr., Miczek KA (2005) Brief social defeat stress: long lasting effects on cocaine taking during a binge and zif268 mRNA expression in the amygdala and prefrontal cortex. Neuropsychopharmacology 30:310-321

19. Covington HE, III, Miczek KA (2001) Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". Psychopharmacology (Berl) 158:388-398

20. Covington HE, III, Miczek KA (2005) Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. Psychopharmacology (Berl) 183:331-340

21. Covington HE, III, 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

22. Dazzi L, Motzo C, Imperato A, Serra M, Gessa GL, Biggio G (1995) Modulation of basal and stress-induced release of acetylcholine and dopamine in rat brain by abecarnil and imidazenil, two anxioselective gamma-aminobutyric acidA receptor modulators. J Pharmacol Exp Ther 273:241-247

23. DiChiara G, Imperato A (1988) Drugs Abused by Humans Preferentially Increase Synaptic Dopamine Concentrations in the Mesolimbic System of Freely Moving Rats. Proceedings of the National Academy of Sciences of the United States of America 85:5274-5278

24. Dunn AJ, Berridge CW (1990) Physiological and Behavioral Responses to Corticotropin- Releasing Factor Administration - Is CRF a Mediator of Anxiety or Stress Responses. Brain Research Reviews 15:71-100

25. Ellinwood EH (1967) Amphetamine Psychosis .I. Description of Individuals and Process. Journal of Nervous and Mental Disease 144:273-&

26. 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

27. Erb S, Stewart J (1999) A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. Journal of Neuroscience 19:RC35

28. Fibiger HC, Fibiger HP, Zis AP (1973) Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. Br J Pharmacol 47:683-692

29. Fleshner M, Laudenslager ML, Simons L, Maier SF (1989) Reduced serum antibodies associated with social defeat in rats. Physiology and Behavior 45:1183-1187

30. 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

31. Goeders NE (1990) The effects of chronic cocaine administration on brain nuerotransmitter receptors. Drug Development Research 20:349-357

32. Goeders NE (2002a) Stress and cocaine addiction. J Pharmacol Exp Ther 301:785-789

33. Goeders NE (2002b) The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 27:13-33

34. Gould E, Reeves AJ, Graziano MS, Gross CG (1999) Neurogenesis in the neocortex of adult primates. Science 286:548-552

35. Griffith JD, Cavanaugh J, Held J, Oates JA (1972) Dextroamphetamine. Evaluation of psychomimetic properties in man. Arch Gen Psychiatry 26:97-100

36. Hahn B, Zacharko RM, Anisman H (1986) Alterations of amphetamine elicited perseveration and locomotor excitation following acute and repeated stressor application. Pharmacol Biochem Behav 25:29-33

37. 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

38. Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF (1992) Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Research 581:190-197

39. Hendley ED, Moisset B, Welch BL (1973) Catecholamine uptake in cerebral cortex: Adaptive change induced by fighting. Science 180:1050-1052

40. Higelin J, Py-Lang G, Paternoster C, Ellis GJ, Patel A, Dautzenberg FM (2001) 125I-Antisauvagine-30: a novel and specific high-affinity radioligand for the characterization of corticotropin-releasing factor type 2 receptors. Neuropharmacology 40:114-122

41. Hodos W (1961) Progressive Ratio as a Measure of Reward Strength. Science 134:943-944

42. Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev 31:6-41

43. Imperato A, Puglisi-Allegra S, Casolini P, Angelucci L (1991) Changes in Brain Dopamine and Acetylcholine Release During and Following Stress Are Independent of the Pituitary-Adrenocortical Axis. Brain Research 538:111-117

44. Iwasaki-Sekino A, Mano-Otagiri A, Ohata H, Yamauchi N, Shibasaki T (2009) Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. Psychoneuroendocrinology 34:226-237

45. Jasnow AM, Banks MC, Owens EC, Huhman KL (1999) Differential effects of two corticotropin-releasing factor antagonists on conditioned defeat in male Syrian hamsters (Mesocricetus auratus). Brain Res 846:122-128

46. Jasnow AM, Davis M, Huhman KL (2004) Involvement of central amygdalar and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral responses to social defeat. Behav Neurosci 118:1052-1061

47. Kalivas PW (1993) Neurotransmitter Regulation of Dopamine Neurons in the Ventral Tegmental Area. Brain Research Reviews 18:75-113

48. Kalivas PW, Duffy P (1989) Similar effects of daily cocaine and stress on mesocorticolimbic Dopamine Neurotransmission in the rat. Biological Psychiatry 25:913-928

49. Kalivas PW, Duffy P (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Res 675:325-328

50. 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

51. Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413

52. Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research 94:507-522

53. Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715-723

54. Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217-238

55. Kosten TR, Gawin FH, Rounsaville BJ, Kleber HD (1986) Cocaine abuse among opioid addicts: demographic and diagnostic factors in treatment. Am J Drug Alcohol Abuse 12:1-16

56. Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y (2000) The role of corticotropin-releasing factor instress-induced relapse to alcohol-seeking behavior in rats. Psychopharmacology 150:317-324

57. Leyton M (2007) Conditioned and sensitized responses to stimulant drugs in humans. Prog Neuropsychopharmacol Biol Psychiatry 31:1601-1613

58. Liu X, Weiss F (2003) Stimulus conditioned to foot-shock stress reinstates alcohol-seeking behavior in an animal model of relapse. Psychopharmacology (Berl) 168:184-191

59. 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

60. Louilot A, Le Moal M, Simon H (1986) Differential reactivity of dopaminergic neurons in the nucleus accumbens in response to different behavioral situations. An in vivo voltammetric study in free moving rats. Brain Research 397:395-400

61. Makino S, Hashimoto K, Gold PW (2002) Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. Pharmacol Biochem Behav 73:147-158

62. Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci 16:387-394

63. McFarland K, Davidge SB, Lapish CC, Kalivas PW (2004) Limbic and Motor Circuitry Underlying Footshock-Induced Reinstatement of Cocaine-Seeking Behavior. Journal of Neuroscience 24:1551-1560

64. Meaney MJ, Brake W, Gratton A (2002) Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? Psychoneuroendocrinology 27:127-138

65. Meerlo P, de Boer SF, Koolhaas JM, Daan S, van den Hoofdakker RH (1996) Changes in daily rhythms of body temperature and activity after a single social defeat in rats. Physiology and Behavior 59:735-739

66. Meerlo P, Sgoifo A, de Boer SF, Koolhaas JM (1999) Long-lasting consequences of a social conflict in rats: Behavior during the interaction predicts subsequent changes in daily rhythms of heart rate, temperature, and activity. Behavioral Neuroscience 113:1283-1290

67. Miczek KA (1979) A new test for aggression in rats without aversive stimulation: Differential effects of d-amphetamine and cocaine. Psychopharmacology 60:253-259

68. Miczek KA, Mutschler NH (1996) Activational effects of social stress on IV cocaine self-administration in rats. Psychopharmacology 128:256-264

69. Miczek KA, Nikulina EM, Shimamoto A, Covington HE, III (2011) Escalated or suppressed cocaine reward, tegmental BDNF and accumbal dopamine due to episodic vs. continuous social stress in rats. J Neurosci (in press):

70. Miczek KA, Thompson ML, Tornatzky W (1991) Subordinate animals: Behavioral and physiological adaptations and opioid tolerance. In: Brown MR (ed) Stress: Neurobiology and Neuroendocrinology. Marcel Dekker, New York, pp 323-357

71. Miczek KA, Yap JJ, Covington HE, III (2008) Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol Ther 120:102-128

72. Millan MA, Jacobowitz DM, Hauger RL, Catt KJ, Aguilera G (1986) Distribution of corticotropin-releasing factor receptors in primate brain. Proc Natl Acad Sci U S A 83:1921-1925

73. Moghaddam B (2002) Stress activation of glutamate neurotransmission in the prefrontal cortex: Implications for dopamine-associated psychiatric disorders. Biological Psychiatry 51:775-787

74. Mos J, Van Valkenburg CFM (1979) Specific effect on social stress and aggression on regional dopamine metabolism in rat brain. Neurosci Lett 15:325-327

75. Müller MB, Wurst W (2004) Getting closer to affective disorders: the role of CRH receptor systems. Trends Mol Med 10:409-415

76. Nikulina EM, Covington HE, III, 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

77. Oades RD, Halliday GM (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 434:117-165

78. Paulson PE, Camp DM, Robinson TE (1991) Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology 103:480-492

79. Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates, 3rd edition. Academic Press, San Diego

80. Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. Nature 422:614-618

81. Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vunerability to amphetamine self- administration. Science 245:1511-1513

82. Pickering C, Gustafsson L, Cebere A, Nylander I, Liljequist S (2006) Repeated maternal separation of male Wistar rats alters glutamate receptor expression in the hippocampus but not the prefrontal cortex. Brain Research 1099:101-108

83. Pontieri FE, Tanda G, Di Chiara G (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the ''shell'' as compared with the ''core'' of the rat nucleus accumbens. Proceedings of the National Academy of Sciences of the United States of America 92:12304-12308

84. Potegal M, Huhman K, Moore T, Meyerhoff J (1993) Conditioned defeat in the syrian golden hamster (Mesocricetus- auratus). Behavioral and Neural Biology 60:93-102

85. Puglisi-Allegra S, Cabib S (1990) Effects of defeat experiences on dopamine metabolism in different brain areas of the mouse. Aggress Behav 16:271-284

86. Raab A, Dantzer R, MIchaud B, Mormede P, Taghzouti K, Simon H, Lemoal M (1986) Behavioural, physiological and immunological consequences of social status and aggression in chronically coexisting resident-intruder dyads of male rats. Physiology and Behavior 36:223-228

87. Remie R, van Dongen JJ, Rensema JW (1990) Permanent cannulation of the jugular vein (acc. to Steffens). In: van Dongen JJ (ed) Manual of microsurgery on the laboratory rat. Elsevier, Amsterdam, pp 159-169

88. Richardson NR, Roberts DCS (1996) Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. Journal of Neuroscience Methods 66:1-11

89. Richter RM, Pich EM, Koob GF, Weiss F (1995) Sensitization of cocaine-stimulated increase in extracellular levels of corticotropin-releasing factor from the rat amygdala after repeated administration as determined by intracranial microdialysis. Neurosci Lett 187:169-172

90. Risbrough VB, Geyer MA, Hauger RL, Coste S, Stenzel-Poore M, Wurst W, Holsboer F (2009) CRF1 and CRF2 receptors are required for potentiated startle to contextual but not discrete cues. Neuropsychopharmacology 34:1494-1503

91. Risbrough VB, Hauger RL, Pelleymounter MA, Geyer MA (2003) Role of corticotropin releasing factor (CRF) receptors 1 and 2 in CRF-potentiated acoustic startle in mice. Psychopharmacology (Berl) 170:178-187

92. 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

93. 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

94. Robinson TE, Berridge KC (2008) Review. The incentive sensitization theory of addiction: some current issues. Philos Trans R Soc Lond B Biol Sci 363:3137-3146

95. 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

96. Rodd ZA, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ (2005) Intracranial self-administration of cocaine within the posterior ventral tegmental area of Wistar rats: evidence for involvement of serotonin-3 receptors and dopamine neurons. Journal of Pharmacology and Experimental Therapeutics 313:134-145

97. Sarnyai Z (1993) Measurement of cocaine-induced stereotyped behavior in response to neuropeptides. Methods in Neurosciences 14:153-165

98. 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

99. Sawchenko PE, Imaki T, Potter E, Kovacs K, Imaki J, Vale W (1993) The functional neuroanatomy of corticotropin-releasing factor. Ciba Found Symp 172:5-21

100. Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley FD, III, 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

101. Schuurman T (1980) Hormonal correlates of agonistic behavior in adult male rats. In: McConnel PS, Boer GJ, Romijn HJ, Van de Poll NE, Corner MA (eds) Progress in Brain Research, Vol. 53: Adaptive Capabilities of the Nervous System. Elsevier Biomedical Press, Amsterdam, pp 415-420

102. 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

103. Shaham Y, Funk D, Erb S, Brown TJ, Walker C, Stewart J (1997) Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. Journal of Neuroscience 17:0-4

104. Sharp T, Zetterstrom T, Ljungberg T, Ungerstedt U (1987) A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res 401:322-330

105. Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology 158:343-359

106. Sinha R (2009) Stress and addiction: a dynamic interplay of genes, environment, and drug intake. Biol Psychiatry 66:100-101

107. 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. Archives of General Psychiatry 63:324-331

108. Sorg BA, Kalivas PW (1991) Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. Brain Research 559:29-36

109. 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

110. Stam R, Bruijnzeel AW, Wiegant VM (2000) Long-lasting stress sensitisation. European Journal of Pharmacology 405:217-224

111. Stefanski V (2001) Social stress in laboratory rats. Behavior, immune function, and tumor metastasis. Physiol Behav 73:385-391

112. Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behavioural Pharmacology 4:289-312

113. Substance Abuse and Mental Health Services Administration (2010) Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of National Findings. US Department of Health and Human Services, Rockville, MD

114. 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

115. 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

116. Tidey JW, Miczek KA (1996) Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study. Brain Res 721:140-149

117. Tornatzky W, Miczek KA (1993) Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiology and Behavior 53:983-993

118. 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

119. 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

120. Vanderschuren LJMJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151:99-120

121. Von Holst D (1998) The concept of stress and its relevance for animal behavior. In: Moller AP, Milinski M, Slater PJB (eds) Advances in the Study of Behavior, Vol. 27: Stress and Behavior. Academic Press, New York, pp 1-131

122. Walletschek H, Raab A (1982) Spontaneous activity of dorsal raphe neurons during defensive and offensive encounters in the tree-shrew. Physiology and Behavior 28:697-705

123. 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

124. 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

125. 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

126. Wang B, You ZB, Wise RA (2009) Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: independence from the local corticotropin-releasing factor network. Biol Psychiatry 65:857-862

127. Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP, Valdez GR, Ben Shahar O, Angeletti S, Richter RR (2001) Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. Ann N Y Acad Sci 937:1-26

128. Wise RA, Rompre PP (1989) Brain dopamine and reward. Annual Review of Psychology 40:191-225

129. Wolf ME, White FJ, Hu XT (1994) MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. Neuroscience 14(3):1735-1745

130. Yap JJ, Covington HE, III, Gale MC, Datta R, Miczek KA (2005) Behavioral sensitization due to social defeat stress in mice: antagonism at mGluR5 and NMDA receptors. Psychopharmacology (Berl) 179:230-239

131. 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. Journal of Pharmacology and Experimental Therapeutics 279:351-358

132. Zislis G, Desai TV, Prado M, Shah HP, Bruijnzeel AW (2007) Effects of the CRF receptor antagonist D-Phe CRF(12-41) and the alpha2-adrenergic receptor agonist clonidine on stress-induced reinstatement of nicotine-seeking behavior in rats. Neuropharmacology 53:958-966

Figures and Tables

Fig. 1

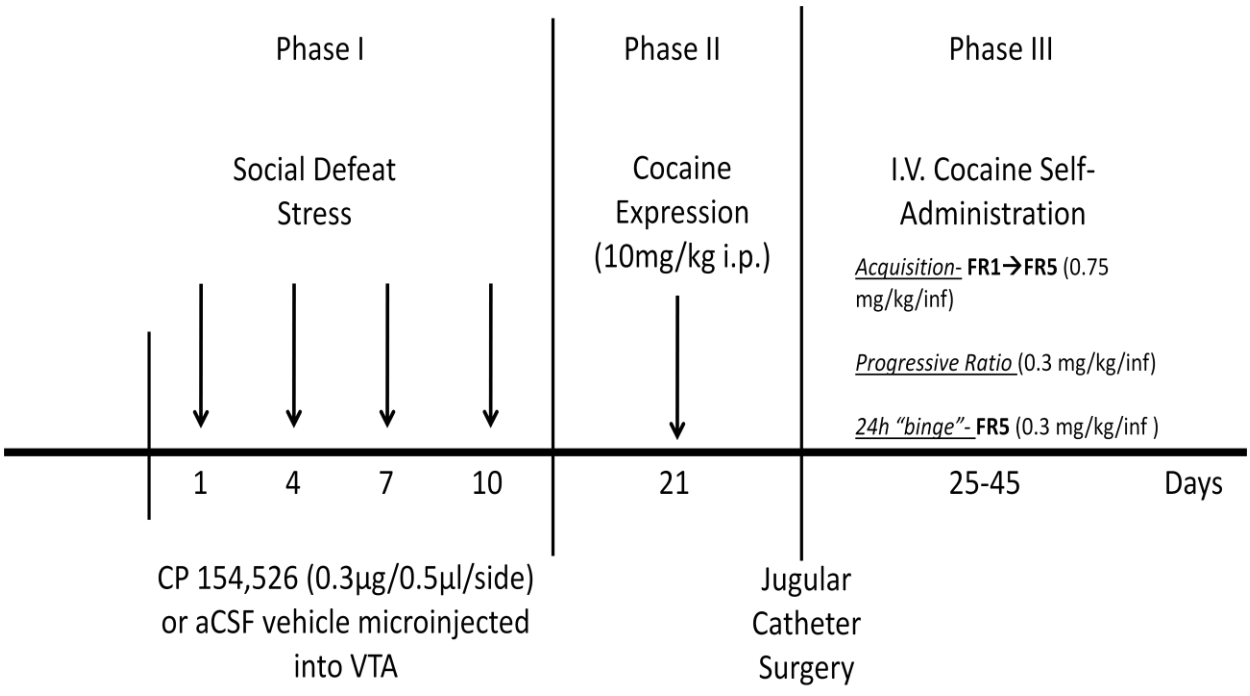

Fig. 1 Experimental design, timeline, and drugs used in phase I-III

Fig. 2

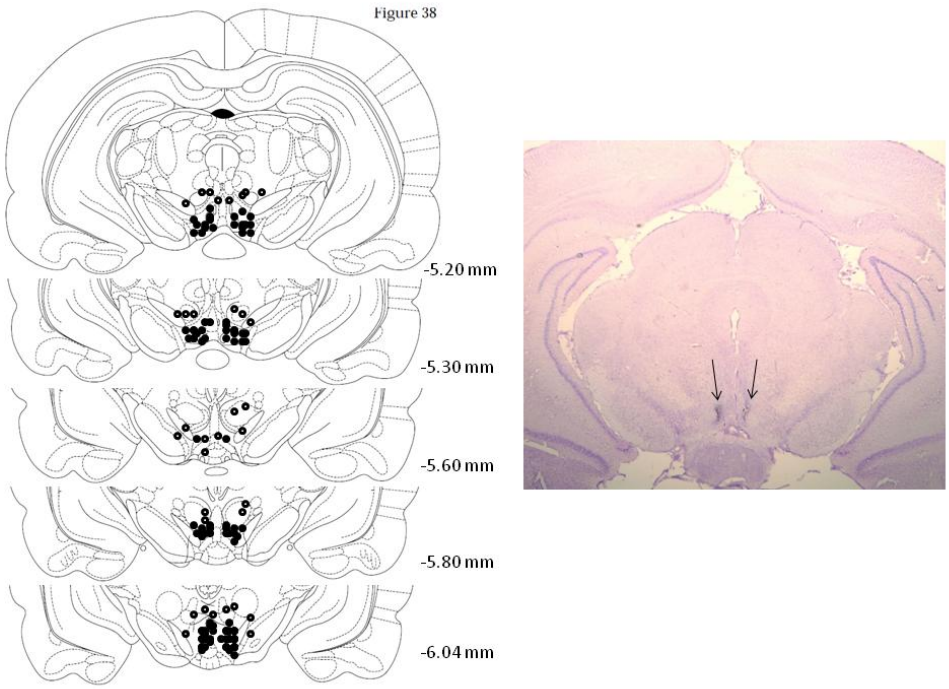

Fig. 2 *Left.* Schematic portrayal of accurately placed intra-VTA sites. Each figure corresponds to coronal sections of the rat brain from -5.2 to -6.04 mm from bregma. Filled circles represent the average location of each pair of bilateral cannulae. The injection sites from seventeen rats were inaccurate placements and are shown as open circles. *Right.* Photomicrograph of an intra-VTA injection site

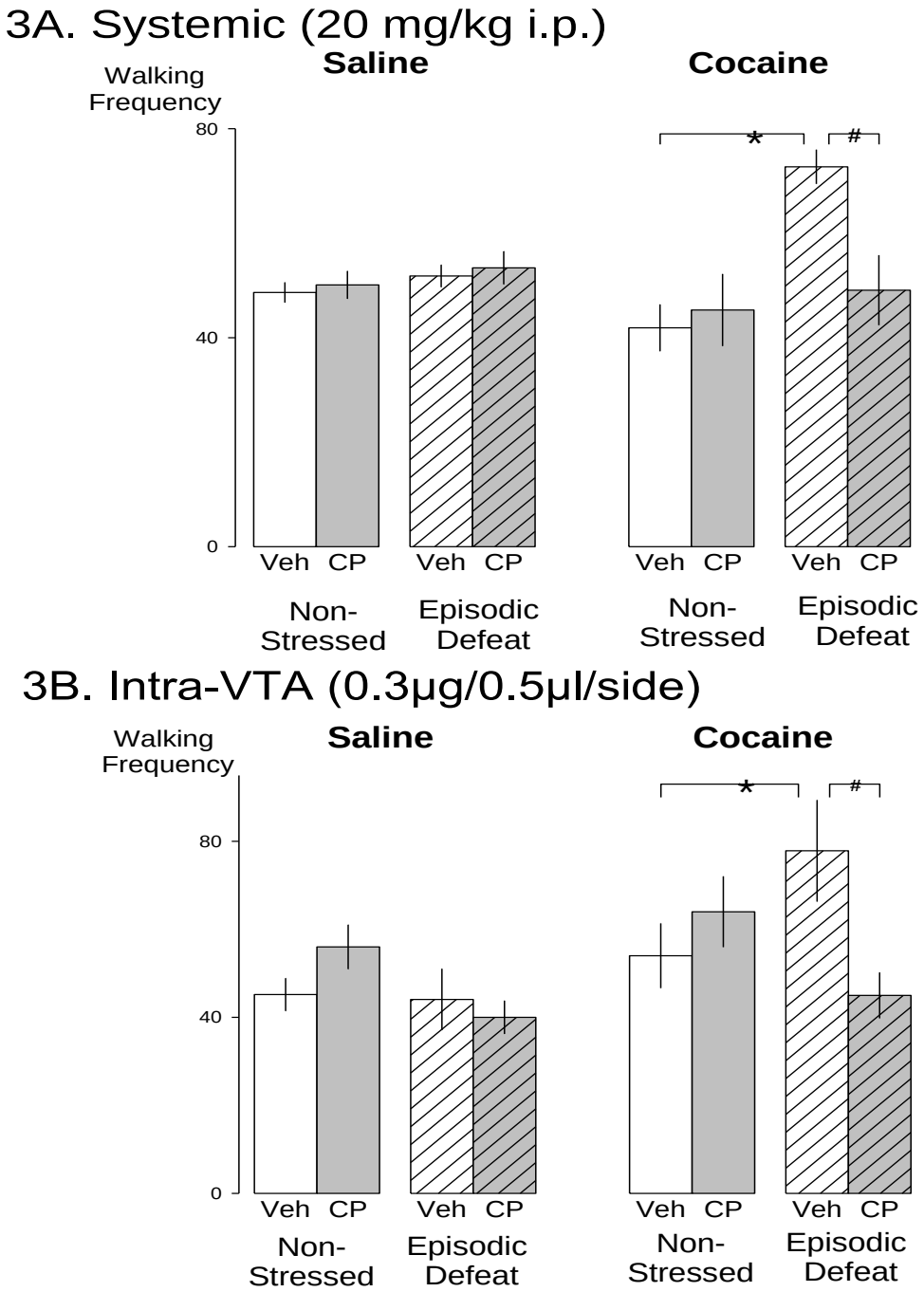

Fig. 3 Systemic or intra-VTA injections of a CRF-R1 receptor antagonist blocked and prevented stress-induced locomotor sensitization in rats. A. portrays the mean (±SEM) walking frequency during a saline challenge (*left)* and a cocaine challenge (*right*) (10 mg/kg i.p.) 11 days after the

social defeat phase with or without prior defeat stress. Non-stressed vehicle n=17; Non-stressed CP 154,526 n=10; Episodic defeat vehicle n=20; Episodic defeat CP 154,526 n=11. B. portrays mean (±SEM) walking frequency after 10 mg/kg i.p. cocaine challenge 11 days after episodic defeat stress modified by microinjection of CRF-R1 antagonist CP 154,526 (0.3µg/0.5µL /side) into the VTA of non-defeated and episodically defeated rats. Non-stressed vehicle n=11; Non-stressed CP 154,526 n=9; Episodic defeat vehicle n=14; Episodic defeat CP 154,526 n=13. The asterisks sign indicate groups that were significantly different from the non-defeated vehicle condition at the $p< 0.05$ level. The pound sign indicate the groups that were significantly different from the stressed-vehicle condition compared to the stressed drug condition at the $p< 0.05$ level

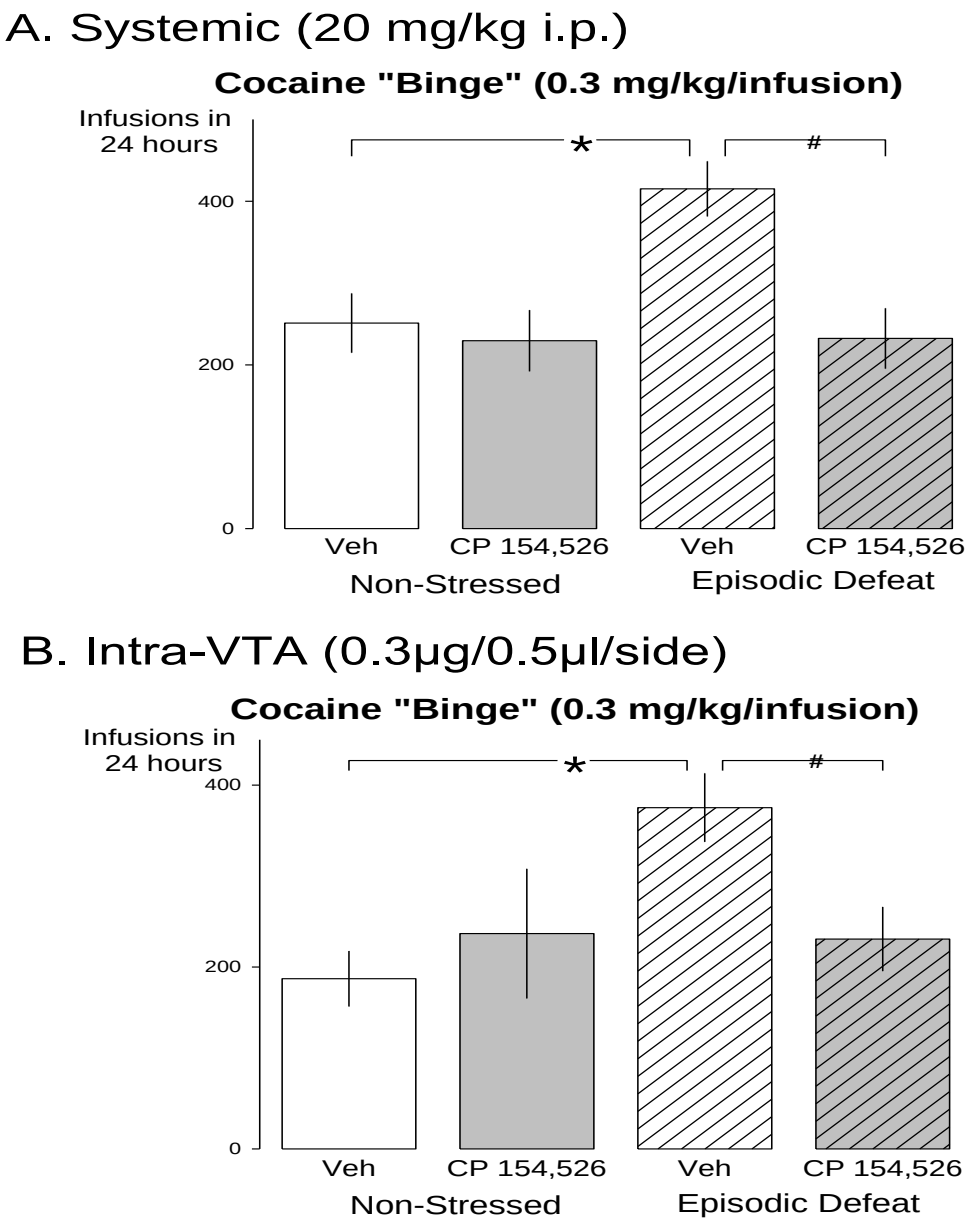

Fig. 4 Effects of systemically administered or intra-VTA infusions of CRF-R1 antagonists prior to each episode of social defeat on subsequent cocaine taking behavior during a 24-hour continuous access "binge". The mean (±SEM) total number of cocaine infusions self-administered by non-stressed control rats (*left bars)* and episodically stressed rats (*right bars).* A. portrays data from rats that received systemic injections of saline (*open bars*) or CP 154,526 (*gray bars*) during the social defeat phase of the experiment. Non-stressed vehicle n=9; Non-

stressed CP 154,526 n=8; Episodic defeat vehicle n=9; Episodic defeat CP 154,526 n=9. B. portrays data from rats that received acsf (*open bars)* prior to each episode of social defeat, and rats that received CP 154,526 (*gray bars).* Non-stressed vehicle n=8; Non-stressed CP 154,526 n=7; Episodic defeat vehicle n=7; Episodic defeat CP 154,526 n=8. The asterisks indicate groups that were significantly different from the non-defeated vehicle condition at the $p< 0.05$ level. The pound sign indicates the groups that were significantly different from the stressed-vehicle condition compared to the stressed drug condition at the $p< 0.05$ level

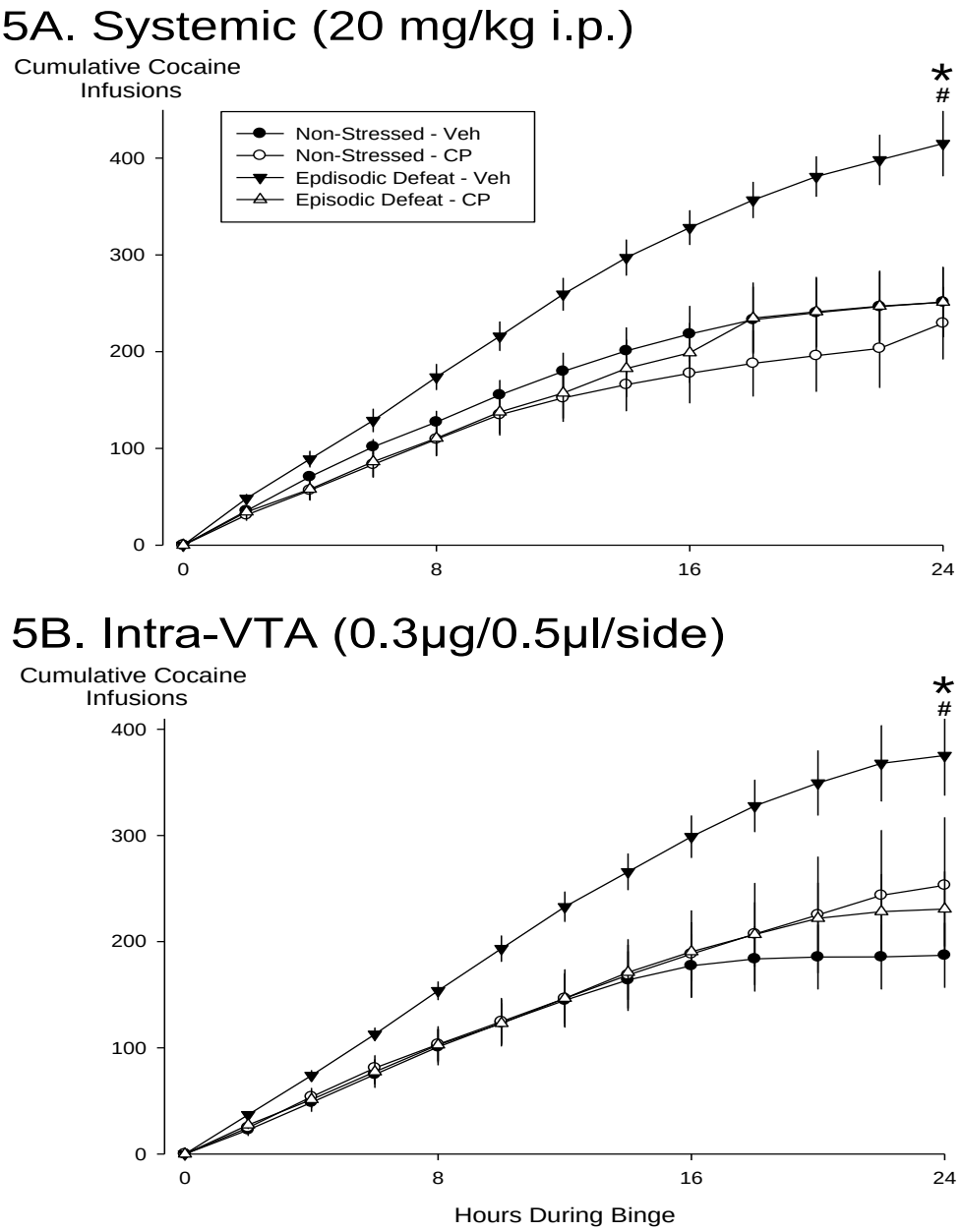

Fig. 5 Effects of systemically administered or intra-VTA infusions of CRF-R1 antagonists during episodic social defeat on subsequent cocaine taking behavior during a 24-hour continuous access "binge". The mean (±SEM) cumulative number of cocaine infusions self-administered over 24 hours by non-stressed control rats (*circles)* and episodically stressed rats (*triangles).* A. portrays the data from rats that received systemic injections of saline (*black*) or CP 154,526 (white) prior to each social defeat. Non-stressed vehicle n=9; Non-stressed CP 154,526 n=8;

Episodic defeat vehicle n=9; Episodic defeat CP 154,526 n=9. B. portrays the data from rats that received aCSF (black*)* prior to each social defeat, and rats that received CP 154,526 (white). Non-stressed vehicle n=8; Non-stressed CP 154,526 n=7; Episodic defeat vehicle n=7; Episodic defeat CP 154,526 n=8. The asterisks indicate groups that were significantly different from the non-defeated vehicle condition at the $p< 0.05$ level. The pound sign indicates the groups that were significantly different from the stressed-vehicle condition compared to the stressed drug condition at the $p< 0.05$ level

Table 1 Effects of CP 154,526 on acquisition, maintenance fixed ratio (FR), and progressive ratio (PR) cocaine self-administration

Systemic (20 mg/kg)
CP154,526

Condition	Treatment		Acquisition within two daily sessions		Maintenance		PR break point (infusions obtained)
Control	Vehicle	n=10	30%	n=10	**0.840 ± 0.085** [1]	n=9	**10.7 ± 0.627** [1]
Control	CP 154,526	n=10	10%	n=10	0.548 ± 0.081	n=8	9.3 ± 0.665
Stress	Vehicle	n=13	7%	n=10	**0.990 ± 0.085** [1 2]	n=9	**11.9 ± 0.627** [1]
Stress	CP 154,526	n=11	27%	n=9	**0.891 ± 0.089** [2]	n=9	10.2 ± 0.627

Intra-VTA (0.3µg/0.5µl/side) CP154,526

Condition	Treatment		Acquisition within two daily sessions		Maintenance		PR break point (infusions obtained)
Control	Vehicle	n=12	8%	n=12	0.630 ± 0.086	n=8	9.7 ± 0.590
Control	CP 154,526	n=10	10%	n=8	0.639 ± 0.109	n=7	9.9 ± 0.631
Stress	Vehicle	n=10	30%	n=10	0.870 ± 0.098	n=7	10.1 ± 0.631
Stress	CP 154,526	n=10	10%	n=10	0.630 ± 0.058	n=8	10.6 ± 0.590

The superscript 1 and bold type indicate a significant drug effect between vehicle and CP 154,526 groups
The superscript 2 and bold type indicate a significant stress effect between stress and control groups
[1] $p<0.05$
[2] $p<0.05$

Table 1 Effects of CP 154,526 on acquisition of cocaine self-administration, and maintenance of cocaine taking according to either a fixed ratio (FR) or progressive ratio (PR) schedule