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Escalated cocaine “binges” in rats: enduring effects of social defeat stress or intra-VTA CRF

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Abstract

Rationale—Exposure to intermittent social defeat stress elicits CRF release into the VTA, and induces long-term modulation of mesocorticolimbic dopamine activity in rats. These adaptations are associated with an intense cocaine-taking phenotype, which is prevented by CRF receptor antagonists.

Objective—The present studies examine whether infusion of CRF into the VTA is sufficient to escalate cocaine-taking behavior, in the absence of social defeat experience. Additionally, we aimed to characterize changes in cocaine valuation that may promote binge-like cocaine intake.

Methods—Male Long-Evans rats were microinjected into the VTA with CRF (50 or 500 ng/side), vehicle, or subjected to social defeat stress, intermittently over 10 days. Animals were then trained to self-administer IV cocaine (FR5). Economic demand for cocaine was evaluated using a within-session behavioral-economics threshold procedure, which was followed by a 24-hour extended-access “binge”.

Results—Rats that experienced social defeat or received intra-VTA CRF microinfusions (50ng) both took significantly more cocaine than controls over the 24-hour “binge”, but showed distinct patterns of intake. Behavioral economic analysis revealed that individual demand for cocaine strongly predicts binge-like consumption, and demand elasticity (i.e. α) is augmented by intra-VTA CRF, but not by social defeat. The effects of CRF on cocaine-taking were also prevented by intra-VTA pretreatment with CP376395, but not Astressin-2B.

Conclusions—Repeated infusion of CRF into the VTA persistently alters cocaine valuation, and intensifies binge-like drug intake in a CRF-R1-dependent manner. Conversely, the persistent pattern of cocaine bingeing induced by social defeat stress may suggest impaired inhibitory control, independent of reward valuation.

Introduction

Repeated exposure to stressful events is associated with an increased vulnerability to drug abuse (Sinha 2001; Goeders 2002; Koob and Kreek 2007). This relationship has been well-

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Conflict of Interest

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established in the clinical population (Karlsgodt et al. 2003; Sinha 2008), and can be recapitulated in animal models of drug self-administration, where stress has been shown to augment all phases of drug-taking; spanning the initiation of drug-taking (Goeders and Guerin 1994; Haney et al. 1995; Miczek and Mutschler 1996), escalation of consumption (Covington and Miczek 2001; 2005a; Mantsch and Katz 2007) and models of relapse (Erb et al. 1996; Ahmed and Koob 1997; Mantsch et al. 2016). Stressful experiences are thought to facilitate these effects through the induction of long-term neuroadaptations that ultimately influence the physiological and behavioral response to drug exposure later in life.

Evidence for such stress-induced pathophysiology has been established in rodents using a social defeat procedure, which is an ethologically relevant model of social stress (Miczek et al. 2008). Social defeat captures many of the physiological and behavioral consequences of social stress in humans (Björkqvist 2001), including increased circulating corticosterone (Covington and Miczek 2005) and persistent dysregulation of core temperature and cardiovascular function (Tornatzky and Miczek 1993). In drug-naïve rats, intermittent exposure to a social defeat also induces long-lasting neural and behavioral cross-sensitization to psychostimulants (Nikulina et al. 2004; Covington et al. 2005; Boyson et al. 2014); animals show a neurochemical and motoric response to cocaine that is typical to animals with an extensive history of drug exposure (Kalivas and Stewart 1993; Pierce and Kalivas 1997). This augmented response is accompanied by escalated cocaine self-administration during a 24-hour 'binge', long after the initial stress experience (Covington and Miczek 2001; 2005; Boyson et al., 2011; 2014; Yap et al., 2015). Clarifying the neurobiological mechanisms underlying these stress-induced neuroadaptations is a critical step towards the development of therapeutic interventions.

The stress neuropeptide corticotropin releasing factor (CRF) has emerged as a potential target for the interaction between stress and drug abuse (Sarnyai et al. 2001; Corominas et al. 2010; Zorilla et al. 2014). CRF plays a critical role in organizing the physiological and behavioral response to stress through both the CRF-R1 and CRF-R2 receptors, localized in discrete brain regions (Vale et al. 1981; Swanson et al. 1983). First identified for its role in initiating hypothalamic-pituitary-adrenal (HPA) neuroendocrine activity via release from the hypothalamus into the anterior pituitary, CRF is also released extrahypothalamically to mediate behavioral and autonomic components of stress response throughout the brain (Swanson et al. 1983; Primus et al. 1997; Sánchez et al. 1999). Although CRF is released diffusely throughout the brain during stress response, much focus has been placed on its interactions with mesolimbic dopamine circuitry. In particular, the ventral tegmental area (VTA) has been identified as a candidate target due to its dual role in mediating both reward and aversion (Brischoux et al. 2009; Lammel et al. 2012). Indeed, CRF appears to regulate excitability of VTA dopamine neurons (Korotkova et al. 2006; Wanat et al. 2008), and repeated cocaine exposure can potentiate its activational effects (Wang et al. 2005; Hahn et al. 2009). It was recently demonstrated that CRF is released in the VTA during acute social defeat, and CRF remains tonically elevated within the VTA long after stress exposure (Holly et al. 2016a). Therein, repeated social stress may contribute to an enduring dysregulation of mesolimbic dopamine circuitry via CRF-dependent plasticity.

Selective antagonists for both the CRF-R1 and CRF-R2 protect against the behavioral consequences of social defeat when infused into the VTA prior to defeat exposure, and attenuate the later escalation of binge-like cocaine consumption (Boyson et al. 2011;2014), as well as dopaminergic cross-sensitization to psychomotor stimulants (Boyson et al. 2014). Similarly, intra-VTA blockade of CRFR₁ is sufficient to attenuate stress- and context-induced reinstatement of cocaine-seeking (Wang et al. 2005; Blacktop et al. 2011; Chen et al. 2015; Holly et al. 2016a). Together, these data provide support for intra-VTA CRF activity as a necessary element in both the development and expression of stress-induced drug-seeking and -taking behaviors. This raises the question of whether CRF alone is sufficient to facilitate neuroadaptations within VTA circuitry that enhance drug-abuse vulnerability. Therefore, one major goal of this study is to determine if exogenously administered CRF, in the absence of stress, is sufficient to potentiate the long-term escalation of cocaine-taking behavior, when selectively infused into the VTA of drug-naïve rats.

In a second set of experiments, we aim to uncover the specific behavioral dysfunction that facilitates escalated drug-taking in individuals with a stress history. Given the interactions between stress and mesolimbic dopamine circuitry, it has been hypothesized that addictive-like behaviors are driven by a dysregulation of reward and/or motivation (Piazza and Le Moal 1998; Sinha 2001; Koob and Kreek 2007). There remains inconsistent evidence for long-term effects of stress on motivation and reward processing, and their relative effects depend heavily on stress modality, severity and frequency. Behavioral economics has emerged as a valuable approach for the behavioral analysis of drug use, and is particularly useful for dissociating the motivational and hedonic components of drug-taking (Hursh 1991; Bickel et al. 1993; Hursh and Roma 2015). Here, we use a recently validated within-session threshold procedure which allows us to produce individual demand curves for cocaine in a single test session (Oleson et al. 2011; Bentzley et al. 2013). Using this model we aim to identify the consequences of social defeat stress or intra-VTA manipulation of CRF on demand for cocaine.

In the present study, we explore the hypothesis that intra-VTA CRF critically facilitates the binge-like cocaine-taking phenotype induced by social defeat stress, in rats. Therefore, we microinfused CRF (50 or 500ng) into the VTA according to an intermittent schedule, and examined later cocaine self-administration during a 24-hour “binge” (Experiment 1). Using behavioral economics, we further evaluated the long-term consequences of social defeat or CRF microinfusion on cocaine reward valuation, in order to clarify the behavioral maladaptation that drives binge-like drug consumption under an extended access to cocaine (Experiment 2). Finally, we determine the extent to which CRF-potentiated drug-taking behaviors could be prevented by infusion of CRF-R1 or CRF-R2 antagonists (Experiment 3).

Materials and Methods

Subjects

Experimental Animals—Male Long-Evans rats (n=62; Charles River Laboratories, Wilmington, MA) weighing 225–250g at arrival were individually housed in custom-built clear acrylic chambers (30 × 30 × 24.5cm) that were lined with cellulose pellet bedding

(Cellu-Dri™, Shepherd Specialty Papers, Kalamazoo, MI). Animals were maintained on a reverse light/dark cycle (lights on: 20:00–8:00) within a temperature-controlled vivarium, and provided food and water *ad libitum*. All rats were allowed to acclimate for 1 week prior to experiments. Experimental procedures were approved by the Tufts University Institutional Animal Care and Use Committee, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011)

Residents—Ten additional male Long-Evans rats were pair-housed with females in a separate vivarium under similar conditions. They were housed in large stainless steel chambers (45.7 × 71.1 × 45.7cm) that were lined with sawdust bedding. These “resident” rats served as aggressive stimulus animals for the social defeat protocol, and were screened regularly for aggressive behavior using naïve “intruder” males.

Intra-VTA Microinjections

Intracranial Cannula Implant—Under ketamine HCl (100mg/kg, i.p.) and Xylazine (6mg/kg, i.p.) anesthesia, rats were bilaterally implanted with stainless steel guide cannulae (24G, 11mm projection; Plastics One, Roanoke, VA) for intracranial microinjection. Target coordinates were calculated from a stereotaxic atlas (Paxinos and Watson 1997) such that cannula tips were aimed 1.0mm above the VTA (at a 10° angle from midline; AP: –5.2mm from bregma, ML: ± 2.0mm from midline, DV: –7.3mm from skull surface). Obturators were inserted between microinjections in order to maintain cannula patency. After approximately 1 week of recovery, animals were randomly assigned to experimental conditions and began microinjection or social defeat protocols (see Fig. 1).

CRF Microinjections—In order to parallel the intermittent schedule of the previously described social defeat protocol (Covington et al. 2008; Tornatzky and Miczek 1993; details below), rats received intra-VTA microinjections of either CRF (500ng or 50ng/side; n=22) or vehicle (aCSF; n=16) on experimental days 1, 4, 7 and 10 (see Fig. 1; Experiment 1). An infusion volume of 0.50µl was delivered over 3 minutes by CMA/100 microinfusion pump (CMA Microdialysis, Sweden) via 31G injectors that projected 1mm beyond the tip of the cannulae. Injectors were left in place for one minute following the infusion in order to facilitate diffusion and reduce backflow.

CRF-R1 and CRF-R2 Antagonism—To investigate the role of CRF-R1 and CRF-R2 in mediating the behavioral effects of intra-VTA CRF infusion, a separate group of animals received bilateral infusions of either the selective CRF-R1 antagonist CP376395 (500ng/side; n=7) or CRF-R2 antagonist Astressin-2B (1000ng/side; n=7) 15 minutes prior to CRF microinjections (see Fig. 1; Experiment 3). Antagonists were delivered in a volume of 0.25µl over 1.5 minutes according to the described procedure above. Post-mortem histology verified that additional microinjections did not result in significant damage within the injection region (i.e. neurodegeneration, gliosis, bleeding).

Social Defeat Stress

A separate cohort of rats (n=10) were exposed to episodic social defeat as described previously (Tornatzky and Miczek 1993; Covington and Miczek 2001; Boyson et al. 2014),

on experimental days 1, 4, 7 and 10 (see fig 1; Experiment 2). Briefly, animals were placed in a wire mesh protective cage positioned within a resident male home-cage for 10 minutes. After this initial “instigation phase”, rats were removed from the protective cage and reintroduced to the resident’s home-cage, where an aggressive encounter ensued. Fights were terminated after 6 seconds of supine posture exhibited by the intruder, 10 attack bites by the resident, or 5 minutes after the first attack bite, whichever came first. Immediately following the fight, intruder rats were returned to the wire mesh protective cage and placed within the resident home-cage, once again, for a 10 minute period of “social threat”. All social defeat encounters were observed by the experimenter, and attack latency, total attack bites and fight duration were recorded.

Cocaine Self-administration

Intravenous Catheter Surgery—One day after the final microinjection or defeat, rats were anesthetized with Ketamine (100mg/kg, i.p.) and Xylazine (6mg/kg, i.p.) and implanted with an indwelling catheter (Silastic® silicon tubing, ID 0.63mm, OD 1.17 mm) into the right jugular vein, using sterile surgical procedures. Catheter tubing was passed subcutaneously from the jugular entry site to the rat’s back, where it exited through a small incision between the scapulae. Here, tubing was affixed to a plastic pedestal mounted within an adjustable harness (SAI Infusion Technologies). Animals were allowed to recover for 5 days in their home-cage prior to self-administration training. Upon recovery, rats were housed in IV self-administration chambers, dimensionally identical to the standard home-cage, for the duration of experiments. Each morning, catheters were flushed with 0.20mL saline and 0.20mL of heparinized saline (20 IU/mL) and, overnight, saline was delivered in .17ml pulses every 30 minutes in order to maintain patency.

Acquisition and Maintenance—Self-administration sessions were carried out in the modified home-cage, which was outfitted with a removable panel containing two retractable levers and two stimulus lights (Med Associates Inc.). Chambers were located within sound- and light-attenuating enclosures, equipped with a ventilation fan and house light. During acquisition of self-administration, each response on the active lever (fixed ratio; FR1) was reinforced with 0.75mg/kg cocaine infusion, which was accompanied by illumination of a green stimulus light, and followed by a 30-second post-infusion timeout period. Responses on the inactive lever and responses during the timeout were recorded, but had no programmed consequence. All sessions were terminated after 15 infusions or 5 hours, whichever came first. Criteria for acquisition were defined as 15 infusions obtained on 2 consecutive days. Rats that did not acquire within the first 2 days of training were behaviorally shaped with female urine on the active lever, beginning on the third training session. Due to this intervention, differences in acquisition rates across treatment conditions could not be evaluated. After acquisition, the response requirement was gradually increased to FR5 (i.e. every fifth response resulted in cocaine infusion) over the course of 2–3 days. Self-administration performance was then maintained on an FR5 schedule of reinforcement for a minimum of 5 sessions prior to testing on the threshold procedure (Experiment 2; see below) or a 24-hour extended access “binge (Experiment 1).

24-hour “binge”—Beginning 2-hours into the dark-phase (10:00), rats were given unlimited access to cocaine (FR5, 0.3mg/kg/infusion) for 24-hours, where the total number of infusions was recorded as the primary dependent measure across groups. Immediately upon completion of the binge, catheter patency was evaluated by administering 0.20mL propofol (10mg/kg). Rats were subsequently perfused, and brains were extracted to confirm injector placement.

Within-Session Threshold Procedure

Procedure—Once rats demonstrated stable self-administration behavior, they were tested on a recently described threshold procedure in order to evaluate individual differences in demand for cocaine (España et al. 2010; Oleson et al. 2011; Bentzley et al. 2013). The threshold procedure measures the extent to which price (i.e. lever presses per mg cocaine) influences drug consumption (i.e. demand) within a single experimental session. Briefly, rats were presented with a series of descending cocaine doses (316, 178, 100, 56, 32, 18, 10, 5.6, 3.2, 1.8 µg/infusion) over the course of a 100-minute session, where successive doses were available in 10 minute bins according to an FR1 schedule of reinforcement. Dose manipulation was achieved by reducing the duration of infusion (1.07ml/min × infusion duration) of a fixed drug concentration (2.0mg/mL). No post-infusion timeouts were applied, but active-lever responses during the infusion had no consequence.

Animals performed this procedure for a minimum of 4 daily sessions, and continued until performance reached stability; i.e. when $\log_{10}(\alpha)$ varied by <15% for 3 consecutive sessions. Individual demand parameters, α and Q_0 , were ultimately determined by averaging daily values over the final three sessions for each rat. The only modification to this procedure to the dose range; the current protocol only includes fluid volumes that could be reliably delivered by our drug infusion system (PMH100, Med Associates; with 10mL syringe, BD Medical Supplies), while also maintaining a quarter-log scale. Therefore, a range of 10 doses was studied, as opposed to the 11 used by Oleson et al. (2011) and others (Bentzley et al. 2014; Calipari et al. 2015; Groblewski et al. 2015).

Analysis—Each threshold session produced a unique dose-response curve for cocaine consumption (depicted in Figure 5). Demand curves were fitted to consumption data with an exponential demand equation (Hursh and Silberberg, 2008) in order to produce values for α and Q_0 :

$$\ln Q = \ln Q_0 + k(e^{-\alpha Q_0 C} - 1)$$

In the above equation, Q and C are defined as consumption (mg cocaine) and cost (responses/mg), respectively. Additionally, there are 3 free variables Q_0 , k and α . K represents the total range of consumption, and was set at 3.5 for all animals based on maximum observed intake (in $\log_{10}$ units). The remaining parameters, Q_0 and α , were estimated to achieve best-fit using standard techniques, and serve as the dependent measures of demand for cocaine. However, the first data point was omitted from all analysis, as it represents a ‘loading dose’ in which cocaine consumption can be highly variable (Oleson et al. 2011; Bentzley et al. 2013).

Q_0 estimates peak consumption, which corresponds to the highest level of demand for a given reinforcer (Hursh and Silberberg, 2008). This value specifies the magnitude of cocaine consumption at a minimally constraining price (i.e. when effort requirement is lowest). Under the assumption that a rat will maintain stable levels of cocaine intake when consumption is unrestricted (Tsibulsky and Norman, 1999; Zittel-Lazarini et al. 2007), the Q_0 parameter most closely relates to the animals' optimal level of consumption.

Alpha (α) indicates the rate at which consumption declines with increasing price (i.e. "elasticity of demand"), when controlling for individual differences in Q_0 . The sensitivity of demand to changes in price is considered to be inversely related to the reinforcing efficacy of a drug, where a strong reinforcer will maintain behavior at high cost. Moreover, α should remain constant for a given reinforcer, regardless of dose or cost manipulation (Hursh and Silberberg, 2008). Therefore, changes in α may reflect variation in the motivation to obtain a reinforcer (Christensen et al. 2008), or in the capacity to adjust to alteration in contingencies between behavior and its consequences. Together, Q_0 and α allow us to independently evaluate the consummatory and appetitive processes that contribute to individual differences in cocaine self-administration behavior.

Histology

Upon completion of experiments, rats were transcardially perfused with saline and 4% PFA under pentobarbital anesthesia (100mg/kg, i.p.). Brains were extracted and fixed in PFA for a minimum of 24-hours. Tissue samples were later sectioned into 50 μ m slices, mounted on gel-coated slides, and Nissl stained using cresyl violet. Photomicrographs were obtained for the stained sections in order to confirm injector placement within the VTA (Fig. 2). Missed placements were identified, and data from these animals (n=2) were removed from the present analysis.

Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Park, NC), and prepared in sterile 0.9% saline solution for intravenous administration. CRF (rat/human; Sigma Aldrich), CP376395 (Tocris Bioscience) and Astressin-2B (Tocris Bioscience) were prepared in aCSF, and stored in 20 μ L aliquots at -20°C until use. CRF doses were selected based on previous *in vivo* studies in rats (Kalivas et al. 1987; Blacktop et al., 2011; Wanat et al. 2013). A lower dose of CRF (50ng) was initially used in order to characterize the dose-response relationship, however, because the 500ng dose of CRF was ineffective in modulating cocaine-taking behavior during the binge-access procedure, only the 50ng dose was further examined (i.e. Experiments 2 and 3). Doses of antagonists were determined based on previous studies conducted in our laboratory (Boyson et al. 2014; Holly et al. 2015; 2016a)

Statistical Analysis

All statistical analyses were conducted using Prism version 5.0 (Graphpad Software Inc.). Cocaine intake patterns across the "binge" were assessed using two-way ANOVA, with stress/drug condition and time (2-hour bin) as factors. Group-level effects of CRF treatment conditions (alone, after CP, or after A2B) or social defeat on total binge intake and economic

demand parameters were analyzed using one-way ANOVAs. All significant results were followed by *post hoc* analysis with Bonferroni corrections for multiple comparisons.

Consumption data obtained during the threshold procedure were fit with the exponential demand equation by modifying an existing analysis and graphing template (Institutes for Behavior Resources, Baltimore, MD). Logarithmic transformations of both Q_0 and α were used for all regression analyses, and these produced a normal distribution (Shapiro-Wilk, $p > 0.05$). Simple linear regression was used to assess the extent to which demand parameters predict binge-like cocaine intake, where $\log(Q_0)$ and $\log(\alpha)$ were set as independent variables, and total infusions during the binge was the dependent variable. Pearson's correlation coefficients (r) were also generated to determine the relationship between α and Q_0 .

Results

Experiment 1

Effects of intra-VTA microinfusions on cocaine intake during 24-hour “binge”

—After establishing stable cocaine self-administration, rats that had previously received CRF microinjections into the VTA took significantly more drug during a 24-hour “binge” than aCSF-treated controls (Fig. 3; $F_{2,29} = 11.33$, $p < 0.001$). Interestingly, *post hoc* analysis revealed that heightened drug-taking behavior was only produced by infusions of 50ng/side (50ng CRF vs aCSF; $t = 4.306$, $p < 0.001$), and the higher CRF dose (500ng/side) did not alter cocaine bingeing (500ng CRF vs aCSF; $t = 0.1099$, $p > 0.05$).

Experiment 2

Differential effects of intra-VTA CRF and social defeat on cocaine bingeing—

In accordance with previous findings, animals exposed to intermittent social defeat also showed elevated cocaine intake during the 24-hour binge (Fig. 4C; $F_{2,31} = 12.47$, $p < 0.001$), where the total number of infusions exceeded non-stressed controls (Bonferroni $t = 3.097$, $P < 0.05$), but did not significantly differ from CRF-treated animals (Bonferroni $t = 1.594$, $p > 0.05$). Although we find similar effects of both CRF and social defeat on the total number of infusions obtained during the binge, further analysis suggests that the pattern of self-administration was markedly different between conditions (Fig. 4). CRF-treated animals consume cocaine at an elevated rate early in the binge period, but typically stopped responding after 18–20 hours. Defeated animals, on the other hand, took cocaine at similar rate to controls through the early stages of the binge, but exhibited a longer duration of self-administration; most persisted for the entire 24-hours. To highlight these temporal differences in behavior, we assessed cocaine intake during the first six (hour 0–6) and final six hours of access (hour 18–24). CRF-treated animals took substantially more cocaine than either the defeated or vehicle-control rats during the first 6 hours of bingeing ($F_{2,31} = 9.329$, $p < 0.001$; CRF vs. defeat, $t = 3.519$, $p < 0.001$; CRF vs aCSF, $t = 3.880$, $p < 0.01$). Conversely, stressed animals accumulated more cocaine over the final 6 hours of the binge ($F_{2,31} = 9.021$, $p < 0.001$; defeat vs. CRF, $t = 2.911$, $p < 0.05$; defeat vs. vehicle, $t = 4.180$, $p < 0.01$). A two-way ANOVA for treatment condition X binge time (2-hour bins) confirms a significant interaction ($F_{22,372} = 2.790$, $p < 0.01$), whereby CRF-treated rats exhibit

increased responding (infusions/2-hr) over the first 10 hours of the extended access binge ($p < 0.01$), and defeated animals maintain elevated cocaine intake from hours 18–24 ($p < 0.05$).

Effects of intra-VTA CRF and social defeat on economic demand for cocaine—

Demand curves were obtained from 44 rats, each of which produced stable performance within 4–7 sessions. Of the individual demand curves generated, seven sessions were discarded due to poor model-fit, based on the previously defined exclusion criteria of $R^2 \leq .30$ (Murphy et al., 2009; Bentzley et al., 2013). All other data met this criterion, with an R^2 range of 0.47–0.97. Across all animals, we found no significant correlation between Q_0 and α values ($r = -.2190$, $p = 0.4152$), which supports the assumption that these measures represent independent and dissociable components of demand.

At the group-level, we demonstrate that a series intra-VTA CRF microinjections is sufficient to augment demand for cocaine by selectively altering measures of elasticity (α). In figure 4 we show that average Q_0 did not differ between experimental groups ($F_{2,24} = 0.5329$, $p = 0.5493$), but a main effect of treatment condition on α was observed ($F_{2,24} = 3.54$, $p < 0.05$). *Post hoc* analysis reveals that CRF microinjections significantly reduced α (Bonferroni $t = 3.572$, $p < 0.05$), but contrary to our initial hypothesis, social defeat experience did not influence the elasticity of demand for cocaine (Bonferroni $t = .3048$, $p = 0.7642$). It appears that intra-VTA CRF infusions induce long-lasting effects on cocaine valuation that are not produced by exposure to defeat stress.

Experiment 3

Effects of CRF-R1 and -R2 antagonists on CRF-induced cocaine-taking phenotype—

Intra-VTA pretreatment with the selective CRF-R1 antagonist CP376395 (500ng/side) was sufficient to prevent the effects of repeated CRF (50ng/side) infusions on cocaine-taking behavior (Fig. 6). A one-way ANOVA shows a significant effect of drug treatment on the total number of infusions during the 24-hour access “binge” ($F_{3,34} = 10.52$, $p < 0.0001$). As demonstrated by Bonferroni *post hoc* comparisons, animals given CP before CRF ($n=7$) took significantly less cocaine than CRF alone ($t = 4.606$, $p < 0.001$), and did not differ from aCSF-controls ($t = 0.8151$, $p > 0.05$). Similarly, CRF-R1 blockade prevented the persistent effects of intra-VTA CRF on motivation to obtain cocaine ($F_{3,34} = 5.09$, $p < .01$). Rats pretreated with CP exhibit similar α values to aCSF-controls ($t = 0.3532$, $p > 0.05$), and were significantly higher than CRF-treated counterparts ($t = 3.146$, $p < 0.05$). None of the manipulations had a significant effect on Q_0 ($p = 0.1924$)

CRF-R2 blockade with Astressin-2B (1000n/side), however, was ineffective in preventing the effects of intra-VTA CRF microinjections (Fig. 6). Compared to controls, rats pretreated with A2B ($n=7$) took significantly more cocaine during the extended access binge (Bonferroni $t = 3.195$, $p < 0.01$), and showed a reduction in α that mimicked infusion of CRF alone ($t = 3.093$, $p < 0.05$). Although, on average, α was lowest in rats that received A2B+CRF, this was not significantly different from rats that received CRF alone ($t = 0.7927$, $p > 0.05$)

Economic demand differentially predicts cocaine binge phenotypes

In order to better understand the behavioral process underlying binge-like cocaine consumption we assessed the extent to which demand parameters were predictive of total intake during the extended access protocol. Across conditions, simple linear regression reveals that α reliably predicts total infusions accumulated during the 24-hour binge ($r = -0.4721$, $p < .05$), and Q_0 does not ($r = -0.0558$, $p > 0.05$). This aligns with the findings of Bentzley et al. (2014), which demonstrates that α (demand elasticity) is a strong predictor of many drug-seeking and -taking behaviors in non-manipulated rats, and that Q_0 (free consumption) was less frequently related.

Since defeated rats exhibit high cocaine intake during 24-hour access despite control-like demand measures, we presumed that demand parameters may not be indicative of binge-like self-administration in these animals. Therefore, we evaluated stressed and non-stressed animals separately in order to validate this hypothesis. For this analysis, CRF- and vehicle-treated rats were pooled in order to comprise a single group of animals that were not exposed to social defeat stress. Indeed, when analyzed independently (Fig. 7), we found that neither Q_0 nor α predicts the persistent cocaine bingeing exhibited by stressed animals ($n=10$; α : $r = 0.0598$, $p = 0.881$; Q_0 : $r = 0.0298$, $p = 0.944$). Alternatively, in non-stressed rats, the relationship between α and binge-intake was strengthened by the removal of stressed animals from the analysis. Q_0 was unchanged ($n=14$; α : $\beta = -0.6540$, $p = 0.0163$; Q_0 : $\beta = 0.0501$, $p = 0.6714$). Together, these results indicate that intra-VTA CRF infusions may heighten binge-like cocaine consumption by augmenting the reinforcing effectiveness of cocaine, as measured by α . Whereas the persistent drug-taking phenotype induced by social defeat stress is not fully explained by our demand measures.

Discussion

The current studies demonstrate that repeated, intermittent exposure to either social defeat stress or intra-VTA microinfusion of CRF produce intense, but distinct patterns of cocaine-taking across a 24-hour binge, long after their initial application. On the one hand, treatment with CRF selectively reduced demand elasticity (α) for cocaine, while free consumption (Q_0) remained unchanged. These changes may signal a state of heightened motivation that drives high levels of drug consumption in the early stages of an unrestricted-access “binge”. Social defeat stress, on the other hand, facilitates a persistent bingeing phenotype that is characterized by prolonged, steady intake; often lasting for 24-hours. Interestingly, this phenotype was not accompanied by a shift in demand for cocaine. These phenotypes appear to reflect enduring neuroadaptive processes that may contribute to drug abuse vulnerability, but by separate mechanisms.

Measures of economic demand have been associated with several experimental metrics of addiction-like behavior. Here, we establish demand elasticity for cocaine (α) as a strong predictor of heightened consumption during continuous access conditions (“binge”), particularly during the early hours of prolonged (24-hr) access. In the context of our procedure, the α -value is an assessment of demand elasticity, and may indicate motivation to consume cocaine. An alternative interpretation portrays the shifts in demand elasticity as a reflection of cognitive or behavioral inflexibility. Prolonged exposure to either stress or

cocaine may constrain the ability to adjust to changing contingencies between behavior and its consequences (Schoenbaum and Setlow 2005; Dias-Ferreira et al. 2009; Ersche et al. 2016). Recently, α has been shown to predict multiple drug-seeking behaviors such as resistance to extinction, cue- and drug-induced reinstatement, and punished drug-taking (Galuska et al. 2011; Bentzley et al. 2014). Although Q_0 is considered to most effectively quantify consummatory behavior (Oleson et al. 2011), we did not find a significant association between the Q_0 parameter and binge-like cocaine consumption.

Intra-VTA CRF promotes escalated cocaine bingeing via augmented demand

The effect of intra-VTA CRF infusion on cocaine taking was dependent on CRF-R1. These results provide further evidence for the importance CRF-R1 in drug-related behaviors, and potentially as a key modulator of motivation to obtain drug. The transition from controlled to excessive drug-seeking and -taking is thought to be facilitated, in part, by long-lasting neuroplastic changes in the VTA that are dependent on CRF release (Corominas et al. 2010). CRF-dependent plasticity in the VTA may potentiate motivated behavior by augmenting dopaminergic response to rewards and their predicting stimuli. Using behavioral economics, we demonstrate that repeated infusions of CRF into the VTA prior to self-administration training significantly reduces demand elasticity for cocaine (α), without altering peak consumption (Q_0). These findings are consistent with the proposal that CRF contributes to motivational processes, but not hedonic valuation. Acute CRF infusion into the VTA profoundly reduced high-effort responding for a preferred food reward, in favor of a smaller reward obtained at a lower response-cost (Bryce and Floresco 2016). In this study, the observed effort-discounting was selectively associated with motivational impairments (i.e. reduced PR breakpoint), while subjective reward valuation remained intact; at equal response-cost, CRF pretreatment did not affect preferential consumption of the larger reward. It is possible that repeated recruitment of these circuits by CRF may promote neural adaptations that persistently alter effortful reward-seeking behavior.

The present studies show that repeated intra-VTA CRF infusions not only engenders heightened responding for cocaine, but that this is associated with intense cocaine 'bingeing'. Under continuous access conditions, CRF-treated animals consumed nearly twice as much drug as non-manipulated controls, and display a pattern of escalated drug-taking that has, up until this point, primarily been described as a consequence of extended daily access to cocaine (i.e. Long-Access; LgA; Ahmed and Koob 1998; Quadros and Miczek 2009). Extended daily access to cocaine (6-hour sessions) typically produces a heightened rate of drug consumption which emerges during the first few days of access, and is most prominent during the early hours of self-administration (Ahmed and Koob 1998; 1999). This elevated rate of cocaine intake, characterized by short inter-infusion intervals, has been demonstrated in 6-hour (Liu et al. 2005), 12-hour (Wee et al. 2007) and 24-hour protocols (Quadros and Miczek 2009). Indeed, rats trained under long-access conditions or microinjected with CRF exhibit a strikingly similar pattern of consumption over a 24-hour binge, despite limited cocaine experience in CRF-treated rats (11.25mg/kg/day) compared to long-access (25–30mg/kg/day; Ahmed and Koob 1998, 1999; Zimmer et al. 2012). Interestingly, rats that have been exposed to long-access procedures have increased CRF immunoreactivity in discrete brain nuclei (Zorilla et al. 2012) and, moreover, escalated

cocaine self-administration is attenuated by the CRF-R1 antagonist antalarmin (Specio et al. 2008). In fact, rats exposed to LgA also have diminished demand elasticity that parallels our CRF-R1-dependent effects (Christensen et al. 2008; Bentzley et al. 2014; Zimmer et al. 2012). Together, these findings bolster the hypothesis that excessive drug consumption may manifest as a consequence of dysregulated extrahypothalamic CRF, where a sensitized CRF system drives escalated drug-seeking behavior (Koob and Le Moal 2005).

Social defeat stress promotes persistent cocaine self-administration, independent of demand

The persistent, enduring pattern of cocaine self-administration during binges is a robust feature of prior social stress experience, and has been repeatedly described in both male and female rats (Covington and Miczek 2001; 2005; Covington et al. 2005; 2008; Quadros and Miczek 2009; Boyson et al. 2010; Holly et al. 2012; Yap et al. 2015). Defeat stress persistently alters mesolimbic dopamine tone and phasic response to stimulant challenge. However, whether or not the extended duration of cocaine bingeing is indicative of alterations in reward value remains unclear (Boyson et al. 2014; Holly et al. 2015). The present studies show that stress-induced persistence of cocaine-bingeing is clearly dissociated from intrinsic demand, and may be mediated by processes independent of reward valuation. Under conditions of escalating “price”, stressed animals did not significantly differ from controls in their free (Q_0) or effortful drug consumption (α) in order to maintain consistent levels of intake, which suggests that the acute reinforcing effects of cocaine remain unchanged by social stress experience. Although it should also be noted that different stressors, contexts or genetic background likely contribute to stress-related behavioral outcomes. It was recently reported that exposure to forced swim test increases demand (P_{max}) in rats, using a similar behavioral economics preparation (Groblewski et al. 2015). In the case of this latter study, however, it is possible that prior cocaine history may have influenced the effects of stress on subsequent drug-taking. Previous cocaine experience can critically modulate the behavioral response to stress, as it pertains to drug-related or affective-like behaviors (Mantsch et al. 2008; Covington et al. 2011; Mejias-Aponte et al. 2015).

Behavioral effects of VTA-CRF may be stimulus-dependent

Previous work from our lab has demonstrated that social defeat stress persistently alters bingelike cocaine-taking through a CRF-dependent mechanism in the VTA (Boyson et al. 2011; 2014). Both CRF-R1 and CRF-R2 are recruited during stressful encounters, and contribute to lasting changes in local CRF tone as well as DA release in response to stress or psychomotor stimulants (Holly et al. 2016b). Here, we show that repeated infusion of exogenous CRF into the VTA produces robust, CRF-R1-dependent changes in cocaine self-administration that may be dissociated from the effects of intermittent social stress, alone. The present results highlight the complexity of CRF's interactions within the VTA, and support the view that its effects may be stimulus-dependent (Wanat et al. 2013).

Notably, the effects of CRF were also dose-dependent, as only lower concentrations of CRF (50ng/side) elicited lasting behavioral effects on drug taking, whereas a higher dose (500ng/side) was ineffective in this capacity. CRF has been previously reported to exert different,

and sometimes opposite effects on both physiological and behavioral endpoints at high and low concentrations within the VTA (Williams et al. 2014), as well as the dorsal raphe (Price et al. 1998; Kirby et al. 2000; Snyder et al. 2015) and locus coeruleus (Snyder et al. 2012). These biphasic effects are likely determined by preferential binding at CRF-R1 at low concentrations, where higher CRF concentrations appear necessary to engage CRF-R2. Within the VTA, CRF-R2 may serve to modulate dopamine activity via GABAergic heterosynaptic regulation of glutamate release (Williams et al. 2014). At high extracellular concentrations, CRF-R2-mediated GABA release can attenuate glutamate release onto VTA dopamine neurons, and thereby counteract the excitatory actions evoked by CRF-R1. Therefore, a high dose (i.e. 500ng) may mitigate CRF-R1-dependent neuroplasticity through actions at CRF-R2.

It is also important to consider the origin and composition of CRF-containing inputs that may “gate” patterns of neural plasticity in the VTA, and organize long-term behavioral outcomes. The VTA receives CRF from terminals projecting from the central amygdala (CeA), the bed nucleus of the stria terminalis (BNST), the paraventricular nucleus (PVN), as well as from local CRF-producing neurons within the VTA (Rodaros et al. 2007; Tagliaferro and Morales 2008; Grieder et al. 2014). These diverse inputs, in part, contribute to functional heterogeneity of VTA-CRF, however, the relative contribution of these inputs during stressful encounters, or otherwise, remains unclear and perhaps complex. For example, Wanat et al. (2013) demonstrate that intra-VTA CRF can provoke opposing effects in an input-specific manner. Concurrent stimulation the PPT-VTA pathway in the presence of CRF application decreases NAc DA efflux. On the other hand, stimulation of the BNST permits exogenous CRF to elicit DA release in the NAc. CRF-containing inputs to the VTA may be differentially recruited across stress modalities, in order to facilitate distinct behavioral responses. Similarly, the behavioral consequences of CRF signaling may be guided by interactions with glucocorticoids released during stress, or drug exposure. Elevated CRF transmission within the VTA is sufficient to initiate reinstatement of cocaine-seeking behavior in rats, however, this function only emerges in a subset of animals that have been exposed to high-intake self-administration conditions (i.e. LgA; Blacktop et al. 2011) or social defeat stress (Holly et al. 2016a). Indeed, elevated glucocorticoids have been implicated in the development of both stress- and drug-induced plasticity within mesolimbic dopamine circuits, which might facilitate later drug-related behaviors (Marinelli and Piazza 2002; Saal et al. 2003; Raftary et al. 2009). Adrenalectomized rats are not susceptible to CRF-induced reinstatement (Graf et al. 2011), and glucocorticoid receptor blockade prevents both the enhancement of LTP in the VTA, as well as cocaine CPP in rats previously exposed to social defeat stress (Stelly et al. 2016). Ultimately, orchestration of stress-specific inputs may be critical to the cellular response of VTA neurons to CRF during defeat. By the same token, the absence of stress-eliciting stimuli during CRF microinfusions likely influenced the outcome of this manipulation, where exogenous CRF may engage separate mechanisms, and perhaps, engender more pronounced behavioral consequences.

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Figure 1.
Experimental timeline.

Figure 2. Histology. Closed circles represent injection sites within the VTA. X's represent missed placements (outside VTA). These schematics are also representative of injection locations for antagonist studies (placements not shown).

Figure 3.

Effects of intra-VTA CRF microinjections (0, 50 or 500ng/side) on the number of cocaine infusions taken during a 24-hour “binge”. **(a)** Average cumulative infusions per two-hour period across extended-access “binge”. **(b)** Average total infusions ($\pm$ SEM) during 24-hour “binge” was significantly enhanced by microinfusions of 50ng, but not 500ng, CRF ($n=12$, $p<.001$). * $p<0.05$; ** $p<0.01$

Figure 4.

Pattern of cocaine intake over a 24-hour “binge”. **(a)** Mean ($\pm$ SEM) cocaine infusions per 2-hr bin across 24-hour “binge”. **(b)** Total infusions ($\pm$ SEM) during the first six hours of the binge (*left*), and during the last 6-hours of the binge (*right*). **(c)** Total number of cocaine infusions across 24-hours. Rats that received intra-VTA CRF microinjections show an elevated rate of intake during initial access to cocaine, and took significantly more cocaine than defeated or control animals across the first 6 hours ($n=12$, $p < 0.01$). Rats exposed to social defeat stress show steady cocaine intake that persists throughout the “binge”, where they took significantly more infusions in the final 6-hours of the “binge” ($n=10$, $p < 0.05$).

Both manipulations produced elevated total intake, relative to control ($p < 0.01$). ** $p < 0.01$;
*CRF-effect ($p < 0.001$); #Stress-effect ($p < 0.05$)

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Figure 5.

Economic demand is augmented by intra-VTA CRF microinjections. (a) Representative demand curves for rats treated with either intra-VTA CRF (*left*) or Vehicle (*right*) microinjections. Cocaine consumption (mg per 10 min. bin; Circles) is plotted on the left *y-axis* (ordinate) as a function of unit price (response/mg cocaine); data is fit with a demand curve. Corresponding responses (Triangles) are plotted on the right *y-axis*. (b) Treatment with CRF produced a significant decrease in α ($n=8$; $p<0.05$). There was no observed effect of social defeat ($n=10$). (c) Neither intra-VTA CRF infusions, nor social defeat exposure altered the Q_0 parameter, relative to the vehicle condition. * $p<0.05$; ** $p<0.01$

Figure 6.

Effects of repeated intra-VTA CRF microinfusion are prevented by local pretreatment with CP376395 (500ng/side), but not Astressin-2B (1000ng/side). (a) Mean number of cocaine infusions ($\pm$ SEM) per 2-hr bin across 24-hour "binge" (b) Total number of infusions during "binge" (c) Mean α . (d) Mean Q_0 . * p <0.05; ** p <0.01

Figure 7.

Economic demand is predictive of binge-like cocaine intake in non-stressed rats (CRF and aCSF treated). α predicts total number of infusions taken during a 24-hour “binge” in non-stressed rats [(n=14) (α : $r = -0.6464$, $p = 0.0125$)]. In defeated rats (n=10) α was not predictive of drug-taking during the binge (α : $r = -0.0598$, $p = 0.8687$)