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Social Stress and Escalated Drug Self-administration in Mice II. Cocaine and Dopamine in Nucleus Accumbens

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Abstract

Rationale—Social defeat stress results in escalation of cocaine taking and long-term neural adaptations in rats. How the intensity and timing of social defeat stress determine these effects, particularly in mice, have not been well characterized.

Objective—This study investigated the effects of mild vs. moderate intensities and durations of social stress on intravenous cocaine self-administration as well as on dopamine (DA) release in nucleus accumbens shell (NAcSh) by using *in vivo* microdialysis.

Methods—Adult male CFW mice experienced 10 days of social defeat stress, either mild (15 attack bites in ca. 1.8 min) or moderate (30 attack bites in ca. 3.6 min), and compared to controls that were handled daily. Subsequently, the socially stressed mice were assessed for either (1) intravenous cocaine self-administration, using several unit doses (0, 0.3, 0.6, 1.0 mg/kg/infusion) under limited access conditions, or (2) neural sensitization, as determined by *in vivo* microdialysis of DA in the NAcSh in response to acute *d*-amphetamine challenge.

Results—Social defeat stress resulted in escalated cocaine self-administration in both mild and moderate socially stressed groups. In addition, social defeat stress led to increased DA release after *d*-amphetamine challenge.

Conclusions—These data suggest that both mild and moderate socially stressed mice exhibit increased cocaine taking compared to controls, and this increase is associated with escalated dopaminergic responses in the NAcSh.

Keywords

stress; cocaine; self-administration; dopamine; nucleus accumbens; intravenous; social defeat; microdialysis

Introduction

Stressful experiences contribute to the initiation and escalation of psychostimulant and alcohol use and may also trigger relapse (Shaham et al. 2000). Exposure to stressful life events can profoundly increase the vulnerability to psychostimulant and alcohol use in humans (Sinha 2001; Sinha 2008). Preclinical studies confirm that rats exposed to certain stressful events, such as mild foot shock, social defeat, or social isolation, self-administer increased amounts of cocaine and other drugs (Bossert et al. 2013; Piazza and Le Moal 1998), and this link extends also to laboratory mice and alcohol consumption (Norman et al. 2014 submitted). Understanding the underlying neurobiological mechanisms that link stress and drug intake is a critical first step in the development of effective treatments for stress-induced drug use disorders.

Social defeat stress triggers a cascade of sympathetic and neuroendocrine events and activates cells in mesocorticolimbic monoaminergic pathways (Miczek et al. 2008). In laboratory animals, brief intermittent social defeat stress immediately increases heart rate and temperature, and eventually activates the hypothalamic-pituitary-adrenocortical (HPA) axis, as indicated by increased plasma adrenocorticotrophic hormone and corticosterone levels (Tornatzky and Miczek 1993). In addition to activating the HPA axis, brief episodes of social defeat stress can also stimulate the dopaminergic mesolimbic system. Intermittent social defeat stress leads to persistently increased extracellular dopamine (DA) release in the nucleus accumbens shell (NAcSh) and prefrontal cortex (Tidey and Miczek 1996). These findings suggest that social defeat stress can induce a series of neurobiological effects including a sensitized HPA axis and neuroadaptations in the dopaminergic mesolimbic system, which may affect the vulnerability to drug use.

Still to be explored is how the activation of mesolimbic DA in anticipation and reaction to ostensibly aversive events such as social defeat stress is related to the experience of intensely rewarding cocaine infusions. A consistent and well-characterized pattern of DA activity during cocaine self-administration and other natural rewards establishes this monoamine as an essential component of the positive reinforcement process (Gratton and Wise 1994; Wise et al. 1992). For example, chronic cocaine administration increases the extracellular concentrations of DA in the nucleus accumbens (Pettit and Justice 1991). However, some elements of the dopaminergic system can also be activated by aversive stimuli such as social isolation (Han et al. 2012), foot shocks (Brischoux et al. 2009) and social defeat (Miczek et al. 2011; Tidey and Miczek 1996). In sum, the mesolimbic dopaminergic system may be a critical neural link between aversive stress experiences and rewarding drug taking. Here we begin to investigate how DA in the NAcSh, as a neural mediator of adaptations to social stress and cocaine reward, plays a role in subsequent cocaine self-administration.

Intravenous (i.v.) drug self-administration has frequently been used in studies with rats and monkeys to evaluate the reinforcing effects of cocaine. This method has the advantage of more closely translating to human cocaine use (Schuster and Thompson 1969;). Since it is challenging to perform i.v. surgery and maintain catheter patency in mice, fewer studies have been conducted in this species (Caine et al. 1999; Rocha et al. 1998). Recently, even

though more success with i.v. methodology in mice has been achieved with cocaine self-administration (Ozburn et al. 2012; Ripley et al. 1999; Rocha et al. 1998; Ruiz-Durántez et al. 2006; Soria et al. 2005; 2008), to our knowledge, the present study is the first to examine the link between social stress and i.v. cocaine self-administration in mice.

Although the effects of stress on drug use have been intensively investigated, few studies have considered the intensity and duration of the stressor (Norman et al. 2014 submitted). The present study used Swiss Webster (CFW) mice as subjects since this outbred strain is characterized by substantial genetic and phenotypic variation, more similar to the human population than inbred strains. Since different intensities of stress play different roles in corticosterone concentration and alcohol drinking behaviors (Norman et al. 2014 submitted), in the current study we compared the effects of two different intensities and durations (mild vs. moderate) of social stress on intravenous cocaine self-administration under limited access conditions. We also used *in vivo* microdialysis to measure DA release in NAcSh, as an initial indicator of neuroadaptations to social stress.

Methods

Animals

Experimental male CFW mice (Charles River Laboratories; Wilmington, MA) weighing 23–25 g were singly housed in clear polycarbonate chambers (28 cm × 17 cm × 12 cm) with a stainless steel wire lid and pine shavings covering the floor. Mice were adapted to the facilities for at least 5 days before experiments. A separate group of adult male CFW mice were pair housed with females and served as aggressive stimulus mice (residents). All animals had unrestricted access to standard rodent chow and water. The room temperature was $21 \pm 1^\circ\text{C}$ with a 12 h reversed light/dark cycle (lights on from 18:00 to 06:00). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Tufts University and followed the NIH Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council 2011).

Drugs

Cocaine hydrochloride and *d*-amphetamine sulfate were obtained from the Research Technology Branch of the National Institute on Drug Abuse. Both were dissolved in sterile 0.9% saline.

Experimental Design

Upon arrival, mice were randomly assigned to three groups: mild social defeat (i.e. receiving 15 attack bites from a resident stimulus animal), moderate social defeat (30 attack bites) or unattacked controls. Mice in the control group were weighed daily, while the mice in the stress groups (mild and moderate) were weighed and then socially defeated for 10 consecutive days (Day 1–10). Six days after the last defeat, animals were assessed for cocaine self-administration (experiment I) or assessed for DA in the NAcSh in response to acute *d*-amphetamine (experiment II, see Fig. 1).

Social Defeat Procedure

We used the resident-intruder paradigm for social defeat previously described for rats and mice (Tornatzky and Miczek 1993; Yap and Miczek 2007). Experimental mice experienced social defeat stress for 10 consecutive days. Each resident mouse participated in only one intruder encounter per day. Each intruder encountered the same resident only once, which prevented habituation and counterbalanced the stimulus animals across intruders.

Phase 1: Pre-defeat Threat—The intruder was weighed and then placed into a perforated plastic protective cage ($18 \times 6 \times 6$ cm). Once the female was removed from the resident cage, the protective cage containing the intruder was placed into the resident cage. The protective cage allowed unrestricted auditory, olfactory, and visual contact between the two animals but protected the intruder from potentially injurious bites.

Phase 2: Defeat—After 5 min of pre-defeat threat, the intruder was removed from the protective cage and immediately placed back into the resident's home cage for another 5 min or until it received 15 (mild social stress group) or 30 attack bites (moderate social stress group).

Phase 3: Post-defeat Threat—After the defeat, the intruder was placed back in the protective cage and returned to the resident's home cage for an additional 5 min of protected threat, after which the intruder was returned to its home cage and the female mouse was returned to the resident cage.

Experiment I: i.v. Cocaine Self-administration

I.v. Catheterization Surgery—Six days after the last social defeat, mild socially defeated ($n = 18$), moderate socially defeated ($n = 28$) and control ($n = 20$) mice were anesthetized with a combination of ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and were permanently implanted with an indwelling catheter (Silastic silicone tubing, Dow Corning, Midland, MI; ID: 0.30 mm, OD: 0.64 mm) into the right jugular vein, extending 1.2 cm from insertion point to the tip. The distal end of the catheter passed subcutaneously from the insertion point to the back where it was affixed to a small plastic pedestal (Plastics One, Roanoke, VA). After catheter surgery, mice were allowed 3–5 days to recover, and catheters were flushed with heparin (30 IU/ml) daily to prevent clotting.

Cocaine Self-administration—For cocaine self-administration, a panel equipped with cue lights and 2 nose-poke operanda was inserted vertically into the home cage of the mouse (Miczek and de Almeida 2001). A spring-supported arm holding a liquid swivel (Instech Laboratories, Plymouth Meeting, PA) was affixed to the top of the panel, and a house light illuminated the cage. A computer-controlled syringe pump held a 3-ml syringe which was connected to the swivel and pedestal by PE20 tubing. One of the operanda was identified as the “active” hole by a green cue light. The active side was counterbalanced across subjects, and remained constant throughout the experiment. When the mouse responded on the active side, an infusion of cocaine was administered intravenously and the stimulus light was deactivated for 30 seconds, indicating the post-infusion interval. This time-out period was

used to prevent overdose. All responses and infusions were recorded automatically using a computer interface and software from Med Associates (St. Albans, VT).

Following recovery from surgery, mice were trained to self-administer cocaine (1.0 mg/kg/infusion) under a fixed ratio 1 (FR1) schedule. Each daily session lasted for a maximum of 2 h or until the mouse received 20 cocaine infusions. Acquisition was defined as intake of at least 6 infusions in 2 h and a 3:1 ratio of active to inactive nose poking on 2 consecutive days. Six mice were excluded for failing to meet the acquisition criterion.

After acquisition, mice were tested under an FR1 schedule with descending unit doses (1.0, 0.6 and 0.3 mg/kg/infusion) receiving each dose for two consecutive 2 h daily sessions. Following dose response testing, saline was substituted for cocaine on 3 consecutive days. Seventeen mice failed to finish the cocaine self-administration phase because of loss of catheter patency or leaks.

Experimental II: in vivo Microdialysis

Surgery—Extracellular DA levels in NAcSh in response to acute *d*-amphetamine injection (1.5 mg/kg, i.p.) were measured in a separate cohort of mild socially defeated ($n=10$), moderate socially defeated ($n=10$) and control mice ($n=8$). Mice were anesthetized and a unilateral guide cannula (CMA Microdialysis Inc, North Chelmsford, MA) aimed at the NAcSh was implanted using the following coordinates: AP: + 1.8mm from bregma; ML: + 0.8 mm from midline; and DV: – 3.8 from skull (Paxinos and Franklin 2001).

In vivo Microdialysis—DA levels in the NAcSh were determined by using a modified version of methods described previously (Shimamoto et al. 2011; Takahashi et al. 2010). Briefly, on the afternoon before microdialysis sample collection, the dummy probe was removed and a microdialysis probe (CMA/7; CMA Microdialysis Inc, North Chelmsford, MA) with 1-mm Cuprophane membrane was inserted into the guide cannula. Each probe was connected to a syringe filled with artificial cerebrospinal fluid (aCSF). The flow rate was set to 0.5 μ l/min overnight and was increased to 2.0 μ l/min the next day. One hour after the flow rate adjustment, dialysis samples were collected every 10 min using a refrigerated fraction collector (CMA 142, CMA Microdialysis Inc., North Chelmsford, MA). Each sample vial contained 5 μ l antioxidant, consisting of 20 mM phosphate buffer including 25 mM EDTA-2Na and 0.5 mM of ascorbic acid (pH 3.5). After 5 baseline samples were collected, all mice first received an i.p. injection of saline, followed by the collection of 2 samples, then an injection of *d*-amphetamine (1.5 mg/kg, i.p.) followed by the collection of 12 additional samples, all at 10-min intervals.

Dialysate DA content was determined using a high-performance liquid chromatography system consisting of an LC10-AD pump (Shimadzu, Columbia, MD), a manual injector (model 7, 125; Rheodyne, Cotati, CA) with a 100 μ l sample loop, and an electrochemical detection (ECD) system (DECADE II, Antec Leyden BV, Zoeterwoude, Netherland). Monoamines were separated using a cation-exchange column (CAPCELL PAK, 1.5 $\times$ 250 mm, 5 μ m I.D., Shiseido, Tokyo, Japan) with temperature set at 30°C. The mobile phase consisted of 150 mM ammonium acetate, 50 mM citric acid, 27 μ M EDTA, 10 % methanol, and 1 % acetonitrile with pH adjusted to 4.6 and was pumped at a flow rate of 0.2 ml/min.

The concentration of DA was determined by using standard curves with known amounts of monoamines in a range of 0.5–5 pg and detection limit for DA was approximately 0.18 pg. Samples from two mice with undetectable levels of DA were excluded from analyses.

Histology

After microdialysis sample collection, mice were deeply anesthetized with an overdose of ketamine and xylazine combination and were perfused with 0.9 % saline and 4 % paraformaldehyde (PFA) solution prior to removal of the brain for examination of probe placements. The fixed brains were sliced in 50 μ m coronal sections using a Cryostat (Leica CM 1900, Bannockburn, IL). The brain sections were mounted on gelatin-coated slides and stained with cresyl violet. Animals with incorrect placements into the NAcSh were excluded from the analysis (n= 4).

Statistical analysis

Data analyses were performed using Sigma Stat V11.0 (Systat Software Inc., San Jose, CA). For cocaine self-administration, the average number of infusions received over 2 cocaine self-administration sessions was analyzed using a two-way repeated-measures analysis of variance (ANOVA) with stress (mild social defeat stress, moderate social defeat stress or control) as the between-subject factor and cocaine dosage (0, 0.3, 0.6, 1.0 mg/kg/infusion) as the within-subject factor. For microdialysis, percent change from tonic DA concentrations during baseline was analyzed by two-way repeated-measures ANOVA, followed by a Bonferroni test for *post hoc* comparisons when there were significant main effects. Data are presented as mean $\pm$ SEM. The criterion for statistical significance was $p < 0.05$.

Results

Experiment I: Effects of social defeat stress on cocaine self-administration

Mice in the mild social stress group received 15 bites within 1.80 minutes (SEM = $\pm$ 0.40), and mice in the moderate social stress group received 30 bites within 3.64 minutes (SEM = $\pm$ 0.37). Previous experience with either mild or moderate social defeat stress engendered subsequently higher rates of cocaine self-administration, particularly at low cocaine doses. A repeated-measures two-way ANOVA revealed a significant effect of cocaine dosage ($F_{3, 114} = 44.19$; $p < 0.001$) (Fig. 2). The interaction between stress and cocaine dosage was also significant ($F_{6, 114} = 2.58$; $p < 0.05$). However, there was no significant main effect of stress.

Post hoc analysis showed that mice, which received 15 bites, self-administered significantly more cocaine infusions at the 0.3 mg/kg/infusion dosage of cocaine ($p < 0.05$) compared to controls (Fig. 2). Similarly, mice that received 30 bites also escalated cocaine self-administration at a low 0.3 mg/kg/infusion dosage of cocaine ($p < 0.05$, Fig. 2). The cumulative patterns of cocaine self-administration are illustrated in Fig. 3.

However, neither mild nor moderate social defeat stress had any significant effect on cocaine self-administration at a higher dose (0.6 or 1.0 mg/kg/infusion). Moreover, all three groups showed increased responses at 0.6 mg/kg/infusion and 0.3 mg/kg/infusion dosages compared to saline condition ($p < 0.05$).

Experiment II: In vivo Microdialysis

Schematic representations of probe placements in the NAcSh and a Nissl-stained tissue showing the location of the probe are presented in Fig. 4. Since we found no significant difference in average tonic DA concentration among treatment groups, absolute levels of DA in the microdialysis samples were converted to percent of baseline dialysate DA content in order to reduce the intergroup variability. We did observe highly significant effects of stress ($F_{2, 187} = 6.99, p < 0.001$), time ($F_{11, 187} = 52.14, p < 0.001$), and a significant stress $\times$ time interaction ($F_{36, 187} = 2.99, p < 0.001$).

All groups showed significant increases in DA from baseline in response to *d*-amphetamine (Fig. 5). The peak increase in DA was significantly greater in both stress groups than the non-stressed controls (Fig. 5). In the mild stress group, a significant effect of stress was observed from 10 to 30 min following the *d*-amphetamine injection ($p < 0.05$), compared to controls. However, in the moderate stress group, the significant increase lasted longer, from 10 to 50 min post *d*-amphetamine ($p < 0.05$), as shown in Fig. 5.

Discussion

The present findings characterize specific social stress conditions that escalate the rate of cocaine self-administration and intensify the DA response in NAcSh to a stimulant challenge. The reinforcing effects of cocaine are enhanced by previous exposure to a broader range of social stress conditions than those that are necessary to increase alcohol drinking (Norman et al. 2014 submitted). Although previous studies showed that social stress escalated subsequent cocaine intake in rats, the current data represent the first evidence that intermittent episodes of social defeat stress in male mice engender substantial neuroadaptations in the mesolimbic dopaminergic system and persistently escalate the rate of cocaine self-administration.

Social defeat stress experience can escalate drug seeking and leads to intense cocaine taking in rats (Cruz et al. 2011; Miczek et al. 2011; Tidey and Miczek 1996). For example, rats that experienced social defeat stress self-administered more i.v. cocaine under a progressive ratio schedule and during binge-like 24 h access (Quadros and Miczek 2009; Covington, III and Miczek 2001). While previous studies have focused on social defeat stress in rats, the current study provides the first evidence identifying intermittent social defeat conditions (both mild and moderate stress intensities) that increase cocaine self-administration at low unit doses in mice.

Although social defeat stress can interact with the reinforcing effects of cocaine, episodes of social defeat stress escalate cocaine self-administration in a dose-dependent manner, with 0.3 mg/kg/infusion showing the largest escalations in the current study. These results are consistent with earlier findings in rats, which demonstrated that mild social stress, in the form of non-injurious threats, increased the rate of self-administration at low to moderate doses of cocaine (ca. 0.031–0.125 mg/kg/infusion) compared to controls (Miczek and Mutschler 1996). It is possible that the higher doses of cocaine could produce a ceiling effect, such as dopamine release in the NAcSh, which overlaps with the potential stress effects. These findings indicate that the stress-induced increase in cocaine intake may be

most prominent at lower unit doses that are part of the ascending limb of the dose-response curve, which coincide with stress-induced DA release in the NAcSh.

The intensity and duration of stress appear to be important parameters determining the interaction between stress and cocaine self-administration. Stressors, especially when acute, lead to increased cocaine self-administration only within a limited range of intensities. For example, high intensity unpredictable foot shocks increases the acquisition of cocaine self-administration (Goeders and Guerin 1994), but mild shocks do not (Ramsey and van Ree 1993). Similarly, other physical stressors of very short duration, such as exposure to a hot plate for 30 s before the self-administration session, fail to modify cocaine intake (Ramsey and van Ree 1993). Here we have designated these two intensities as mild and moderate, respectively, in contrast to more severe procedures (Golden et al. 2011; Miczek et al. 1982). The present study demonstrates that cocaine self-administration can be influenced by exposure to either mild or moderate social defeat stress, suggesting that repeated social defeat stress may induce neuroadaptations that are particularly important for cocaine self-administration.

The mesolimbic dopaminergic system has been implicated in the rewarding effects of drugs of abuse (Koob and Le Moal 2001; Wise and Koob 2014), as well as in responses to aversive stress (Brischoux et al. 2009). The dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens are the key pathways in drug intake and stress (Nestler 2005). In the present study, we focused on the NAcSh, which has been postulated to be more important than the core portion of the nucleus accumbens for drug reward (Ikemoto et al. 2005; Pontieri et al. 1995). For example, lesions of DA terminals in the shell, but not in the core, can attenuate conditioned place preference induced by i.v. administration of cocaine or *d*-amphetamine (Sellings et al. 2006; Sellings and Clarke 2003). The current study shows that episodic social defeat stress induced greater increases in DA release in NAcSh in response to an acute *d*-amphetamine challenge compared to controls. This coincides with escalations in cocaine self-administration in defeated mice. This pattern may indicate that social defeat stress induces neuroadaptation in NAcSh DA transmission, contributing to the subsequently increased cocaine intake. It will be a future option to use cocaine as a challenge. However, the use of amphetamine to demonstrate stress-induced sensitization is congruent with earlier work (Yap et al. 2007). Interestingly, mice that experienced moderate defeat stress showed an even larger and longer lasting increase in DA response after *d*-amphetamine challenge than those who experienced less severe brief stress.

In line with the intensive research in rats (Covington, III and Miczek 2005; Schuurman 1980), social defeat stress results in large increases in corticosterone in mice (Norman et al. 2014 submitted). In particular, both mild and moderate social defeat stress escalate corticosterone, without indication of habituation over the course of repeated stress experiences (Norman et al. 2014 submitted). Moreover, after 10 days of social defeat stress, moderate social defeated mice show an even greater corticosterone concentration compared to mild social defeat group (Norman et al. 2014 submitted).

Previous studies demonstrate that corticosterone may interact with dopaminergic systems. For example, corticosterone administration increases extracellular DA concentration in the

nucleus accumbens and induces DA dependent locomotor activity (Piazza et al. 1996). Hence, it is possible that corticosterone released during social defeat stress may influence the activity of DA neurons, which results in increased extracellular DA release in NAcSh, and ultimately contributes to the stress-induced escalation of cocaine intake.

In conclusion, social defeat stress stimulates mesolimbic dopaminergic activity, and increases cocaine self-administration in male mice. While both mild and moderate social defeat stress increased low-dose cocaine self-administration, moderate social defeat stress led to a higher and longer DA release than mild defeat stress. These findings indicate that stress intensity may be a critical factor in stress-induced drug use disorders. In addition to the role of corticosterone-DA interactions in the mesolimbic system during social defeat stress we are now investigating how extra-hypothalamic corticotropin releasing factor (CRF) contributes to the stress-escalated cocaine intake.

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References

- Bossert JM, Marchant NJ, Calu DJ, Shaham Y. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology (Berl)*. 2013; 229:453–476. [PubMed: 23685858]
- Brischoux F, Chakraborty S, Brierley DI, Ungless MA. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *Proc Natl Acad Sci U S A*. 2009; 106:4894–4899. [PubMed: 19261850]
- Caine SB, Negus SS, Mello NK. Method for training operant responding and evaluating cocaine self-administration behavior in mutant mice. *Psychopharmacology*. 1999; 147:22–24. [PubMed: 10591861]
- Covington HE III, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration “binges”. *Psychopharmacology (Berl)*. 2001; 158:388–398. [PubMed: 11797060]
- Covington HE III, Miczek KA. Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. *Psychopharmacology (Berl)*. 2005; 183:331–340. [PubMed: 16249907]
- Cruz FC, Quadros IM, Hogenelst K, Planeta CS, Miczek KA. Social defeat stress in rats: escalation of cocaine and “speedball” binge self-administration, but not heroin. *Psychopharmacology*. 2011; 215:165–175. [PubMed: 21197616]
- Goeders NE, Guerin GF. Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. *Psychopharmacology*. 1994; 114:63–70. [PubMed: 7846208]
- Golden SA, Covington HE III, Berton O, Russo SJ. A standardized protocol for repeated social defeat stress in mice. *Nat Protoc*. 2011; 6:1183–1191. [PubMed: 21799487]
- Gratton A, Wise RA. Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. *Journal of Neuroscience*. 1994; 14:4130–4146. [PubMed: 8027767]
- Han X, Li NX, Xue XF, Shao F, Wang WW. Early social isolation disrupts latent inhibition and increases dopamine D2 receptor expression in the medial prefrontal cortex and nucleus accumbens of adult rats. *Brain Research*. 2012; 1447:38–43. [PubMed: 22341870]

- Ikemoto S, Qin M, Liu ZH. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid? *J Neurosci*. 2005; 25:5061–5065. [PubMed: 15901788]
- Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*. 2001; 24:97–129. [PubMed: 11120394]
- Miczek KA, de Almeida RMM. Oral drug self-administration in the home cage of mice: alcohol-heightened aggression and inhibition by the 5-HT1B agonist anpirtoline. *Psychopharmacology (Berl)*. 2001; 157:421–429. [PubMed: 11605102]
- Miczek KA, Mutschler NH. Activational effects of social stress on IV cocaine self-administration in rats. *Psychopharmacology*. 1996; 128:256–264. [PubMed: 8972545]
- Miczek KA, Nikulina EM, Shimamoto A, Covington HE III. Escalated or suppressed cocaine reward, tegmental BDNF and accumbal dopamine due to episodic vs. continuous social stress in rats. *J Neurosci*. 2011; 31:9848–9857. [PubMed: 21734276]
- Miczek KA, Thompson ML, Shuster L. Opioid-like analgesia in defeated mice. *Science*. 1982; 215:1520–1522. [PubMed: 7199758]
- Miczek KA, Yap JJ, Covington HE III. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacol Ther*. 2008; 120:102–128. [PubMed: 18789966]
- National Research Council. Guide for the Care and Use of Laboratory Animals. 8. National Academy Press; Washington DC: 2011.
- Nestler EJ. Is there a common molecular pathway for addiction? *Nat Neurosci*. 2005; 8:1445–1449. [PubMed: 16251986]
- Norman KJ, Seiden JS, Klickstein JA, Han X, Hwa LS, Debold JF, Miczek KA. Social Stress and Escalated Drug Self-Administration in Mice I. Alcohol and Corticosterone. *Psychopharmacology*. 2014 submitted.
- Ozburn AR, Larson EB, Self DW, McClung CA. Cocaine self-administration behaviors in Clock^{-/-} mice. *Psychopharmacology (Berl)*. 2012; 223:169–177. [PubMed: 22535308]
- Paxinos, G.; Franklin, KBJ. The Mouse Brain in Stereotaxic Coordinates. 2. Academic Press; San Diego: 2001.
- Pettit HO, Justice JB. Effect of Dose on Cocaine Self-Administration Behavior and Dopamine Levels in the Nucleus Accumbens. *Brain Research*. 1991; 539:94–102. [PubMed: 2015506]
- Piazza PV, Le Moal M. The role of stress in drug self-administration. *Trends in Pharmacological Sciences*. 1998; 19:67–74. [PubMed: 9550944]
- Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M. Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. *Neurobiology*. 1996; 93:8716–8720.
- Pontieri FE, Tanda G, Di Chiara G. 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*. 1995; 92:12304–12308. [PubMed: 8618890]
- Quadros IM, Miczek KA. Two modes of intense cocaine bingeing: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. *Psychopharmacology (Berl)*. 2009; 206:109–121. [PubMed: 19513697]
- Ramsey NF, van Ree JM. Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naïve rats. *Brain Research*. 1993; 608:216–222. [PubMed: 8495356]
- Ripley TL, Rocha BA, Oglesby MW, Stephens DN. Increased sensitivity to cocaine, and over-responding during cocaine self-administration in tPA knockout mice. *Brain Res*. 1999; 826:117–127. [PubMed: 10216203]
- Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, Miller GW, Caron MG. Cocaine self-administration in dopamine-transporter knockout mice. *Nature Neuroscience*. 1998; 1:132–137.
- Ruiz-Durántez E, Hall SK, Steffen C, Self DW. Enhanced acquisition of cocaine self-administration by increasing percentages of C57BL/6J genes in mice with a nonpreferring outbred background. *Psychopharmacology (Berl)*. 2006; 186:553–560. [PubMed: 16586086]

- Schuster CR, Thompson T. Self administration of and behavioral dependence on drugs. *Annu Rev Pharmacol.* 1969; 9:483–502. [PubMed: 4978013]
- Schuurman, T. Hormonal correlates of agonistic behavior in adult male rats. In: McConnell, PS.; Boer, GJ.; Romijn, HJ.; Van de Poll, NE.; Corner, MA., editors. *Progress in Brain Research*, Vol. 53: Adaptive Capabilities of the Nervous System. Elsevier Biomedical Press; Amsterdam: 1980. p. 415-420.
- Sellings LH, McQuade LE, Clarke PB. Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats. *Neuroscience.* 2006; 141:1457–1468. [PubMed: 16753267]
- Sellings LHL, Clarke PBS. Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *Journal of Neuroscience.* 2003; 23:6295. [PubMed: 12867514]
- Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Research Reviews.* 2000; 33:13–33. [PubMed: 10967352]
- Shimamoto A, DeBold JF, Holly EN, Miczek KA. Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: exploring a link between depression and drug abuse. *Psychopharmacology (Berl).* 2011; 218:271–279. [PubMed: 21638221]
- Sinha R. How does stress increase risk of drug abuse and relapse? *Psychopharmacology.* 2001; 158:343–359. [PubMed: 11797055]
- Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann N Y Acad Sci.* 2008; 1141:105–130. [PubMed: 18991954]
- Soria G, Barbano MF, Maldonado R, Valverde O. A reliable method to study cue-, priming-, and stress-induced reinstatement of cocaine self-administration in mice. *Psychopharmacology (Berl).* 2008; 199:593–603. [PubMed: 18488200]
- Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, Maldonado R, Valverde O. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology.* 2005; 30:1670–1680. [PubMed: 15742004]
- Takahashi A, Shimamoto A, Boyson CO, DeBold JF, Miczek KA. GABA(B) receptor modulation of serotonin neurons in the dorsal raphe nucleus and escalation of aggression in mice. *J Neurosci.* 2010; 30:11771–11780. [PubMed: 20810897]
- Tidey JW, Miczek KA. Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study. *Brain Res.* 1996; 721:140–149. [PubMed: 8793094]
- Tornatzky W, Miczek KA. Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior.* 1993; 53:983–993. [PubMed: 8511216]
- Wise RA, Bauco P, Carlezon WA, Trojnar W. Self-Stimulation and Drug Reward Mechanisms. *Neurobiology of Drug and Alcohol Addiction.* 1992; 654:192–198.
- Wise RA, Koob GF. The development and maintenance of drug addiction. *Neuropsychopharmacology.* 2014; 39:254–262. [PubMed: 24121188]
- Yap JJ, Miczek KA. Social defeat stress, sensitization, and intravenous cocaine self-administration in mice. *Psychopharmacology (Berl).* 2007; 192:261–273. [PubMed: 17297635]

Fig. 1.

Experimental design. Mild or moderate social defeats (indicated by arrows) were conducted daily for 10 days. Mice in Experiment I were catheterized six days after the last defeat and allowed to self-administer cocaine on Day 20. A separate cohort in Experiment II was implanted with intracerebral cannulae on Day 11 and underwent *in vivo* microdialysis to determine DA on Day 16–20.

Number of reinforcements

Fig. 2.

Intravenous cocaine dose-effect curves (0, 0.3, 0.6, 1.0 mg/kg/infusion) in mild socially defeated ($n = 18$), moderate socially defeated ($n = 11$) and control ($n = 14$) mice. Individual data were averaged over 2 sessions. Data are expressed as mean $\pm$ SEM; * $p < 0.05$ vs. controls. # $p < 0.05$ vs. saline.

Fig. 3. Cumulative records of cocaine intake at 0.3 mg/kg/infusion in control, mild socially defeated mice and moderate socially defeated mice. Records are shown for the individual mice with the maximum, minimum and median total number of infusions in each group.

Fig. 4. Placements of intra-NAcSh probes for the *in vivo* microdialysis experiment. a. Each figure corresponds to a coronal section of the mouse brain from + 1.70 to + 1.10 mm from bregma (Paxinos and Franklin 2001). b. A photomicrograph of correct placement after Nissl staining.

DA in NAcSh (%BL)

Fig. 5.

DA levels in the NAcSh in response to i.p. injections of saline and *d*-amphetamine (1.5 mg/kg) in mild socially defeated ($n = 7$), moderate socially defeated ($n = 7$) and control ($n = 6$) mice. All data are expressed as percent change from individual baseline and are represented as mean $\pm$ SEM; * $p < 0.05$ vs. controls.