Published in final edited form as:

Neuropharmacology. 2012 August ; 63(2): 330–337. doi:10.1016/j.neuropharm.2012.03.012.

Differential Modulation of Cocaine's Discriminative Cue by Repeated and Variable Stress Exposure: Relation to Monoamine Transporter Levels

Stephen J. Kohut, Ph.D.¹, Kathleen L. Decicco-Skinner, Ph.D.², Shirin Johari, B.A.², Zachary E. Hurwitz, M.A.¹, Michael H. Baumann, Ph.D.⁴, and Anthony L. Riley, Ph.D.^{1,2}

¹Department of Psychology, American University, Washington, DC 20016

²Department of Biology, American University, Washington, DC 20016

⁴Chemical Biology Research Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD 21224

Abstract

Discriminative stimulus functions of drugs of abuse play an important role in the acquisition, maintenance and reinstatement of drug-taking behavior. The present study tested whether two different schedules of stressor presentation, i.e., repeated and variable, for 10 days, can modify the discriminative stimulus effects of cocaine in male rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline. Dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporter levels in mesocorticolimbic areas were also measured using western blotting after stress exposure to determine if the relative ratio of these proteins may explain differences in behavior. Rats exposed to both repeated and variable stress displayed shifts in the cocaine dose-response curve but with different patterns of responding. In handled controls, ED₅₀ values for cocaine-like responding were stable after 10 days of handling compared to baseline. Repeated stress produced a transient left-ward shift in cocaine-like responding, indicating increased sensitivity to the cocaine cue. ED₅₀ values after variable stress did not differ from baseline, although maximal cocaine-like responding was lower at the two highest doses of cocaine tested at which variably stressed rats switched from cocaine to saline-like responding. Alterations in DAT and NET were found in the Repeated stress group and DAT and SERT in the Variable stress group in select brain regions which may be responsible for differences in behavior.

Keywords

Cocaine; drug discrimination; stress; monoamine transporters; western blotting

© 2012 Elsevier Ltd. All rights reserved.

Correspondence to: Stephen J. Kohut.

¹Current address: Stephen J. Kohut, Ph.D., Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02478, TEL:+1-817-885-2167, FAX:+1-817-885-2195, steve.kohut@gmail.com

DISCLOSURE/CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. INTRODUCTION

Relapse is a major obstacle in the treatment of addiction. While many drug addicts are able to remain abstinent through early recovery, the risk for relapse remains high even after protracted periods of abstinence (McKay et al. 1999; Mendelson and Mello, 1996). Both clinical and preclinical studies suggest that stressful experiences precipitate relapse in some abstinent users (Kreek and Koob, 1997; Marlatt and Gordon, 1985; Shalev et al. 2000; Sinha, 2001). Understanding how stress interacts with the behavioral and neurochemical effects of drugs of abuse is important for developing prevention and treatment strategies for stress-induced relapse to drug addiction.

Several studies have suggested that “craving” is often elicited by stress exposure in abstinent drug users. Interestingly, stressors elicit qualitatively similar interoceptive cues as those produced by psychomotor stimulants such as cocaine (Back et al. 2010; Coffey et al. 2002; Sinha, 2001; Sinha et al. 1999). For example, many salient components of the subjective responses to psychostimulants such as “anxiety” and “jittery/nervousness” (Chait et al. 1986; Fischman et al. 1976) are also reported during stress tasks (Harris et al. 2005). This is in agreement with preclinical studies showing that exposure to stressors such as electric foot-shock (Mantsch and Goeders, 1999), restraint (or immobilization; Mantsch and Goeders, 1998a) and psychosocial stress (Miczek et al. 1999) reinstate extinguished cocaine responses and stress has also been shown to engender drug-like responding in drug discrimination experiments (Mantsch and Goeders, 1998b; Miczek et al. 1999). Further, clinical studies have found that abstinent drug users describe intense drug craving after exposure to a stressful situation (Sinha et al. 1999; 2007).

One possible mechanism that mediates behavioral responses between stress and drug abuse is the common or overlapping neurobiological substrates. The mesocorticolimbic system, while important for the behavioral effects of cocaine, is also a target for glucocorticoids (Harfstrand et al. 1986) and the interaction between stress and dopamine (Joels and De Kloet, 1994; Marinelli and Piazza, 2002; Rouge-Pont et al. 1995; Sarnyai et al. 2001). Evidence suggests that exposure to stressors provokes dopamine release in the mesolimbic system (Abercrombie et al. 1989), and alters dopamine receptor responsiveness to subsequent stimulation (Barrot et al. 2000; Biron et al. 1992; Joels and De Kloet, 1994) often leading to cross-sensitization between stress and psychostimulant administration (Antelman et al. 1980). Further, activity in striatal regions during stress tasks is significantly increased in cocaine users compared to healthy controls and this increase is related to reports of stress-induced cocaine craving (Li et al. 2005; Sinha et al. 2006).

Developing more effective pharmacotherapies for prevention of stress-induced relapse will require a better understanding of how stress alters cocaine’s interoceptive cue and the neurobiological correlates of these changes. To that end, the following experiments were designed to study the effects of chronic exposure to two different schedules of stressor presentation, repeated or variable, on the discriminative stimulus effects of cocaine. Drug discrimination is considered an animal model of the subjective effects of drugs that is sensitive to the distinct pharmacological effects of the training drug. Thus, testing animals before and after stress exposure will provide insight into changes to the pharmacological mechanisms responsible for the subjective effects of cocaine. Two schedules of stress were studied because prospective clinical reports have found that acute stressors and chronic adverse life events are both associated with subsequent drug relapse (Preston and Epstein, 2011; Sinha, 2001). Laboratory-based studies also show that the profile of subjective effects elicited varies depending on the type of stress administered (Back et al. 2010; Harris et al. 2005). For example, when subjective ratings on the Trier Social-Stress Test (TSST) and Stress Imagery Tests were compared in abstinent cocaine- and methamphetamine-dependent

subjects, the Stress Imagery Test produced significantly larger increases in the PANAS subscales Angry, Disturbed, Fearful, and Guilty, and in the Negative Affect Scale compared to the TSST. Interestingly, both tasks produced similar increases in ratings of “Desire for Stimulant.” Thus, it is conceivable that stressor exposure increases the likelihood of relapse regardless of the type of stress exposure, but through different mechanisms.

Adult, male rats were trained to discriminate cocaine (10 mg/kg, IP) from saline and then exposed to either handling (control), repeated stress or variable stress with cumulative cocaine dose-effect curves determined before and after control or stress exposure. Further, we were interested in determining which neurotransmitter systems may be differentially regulated during repeated or variable stress that would be associated with different patterns of cocaine-like responding. To test this, we focused on the monoamine transporters for dopamine (DAT), serotonin (SERT), and norepinephrine (NET) in the prefrontal cortex (PFC), nucleus accumbens (NAc), caudate-putamen (CPu), and ventral tegmental area (VTA). The monoamine transporters were targeted because cocaine has direct pharmacological interactions with these proteins and these brain areas have been shown to be important for the behavioral effects of cocaine (Wise et al. 1998) and because stress (particularly increases in corticosterone) has been shown to alter monoamine systems (Nestler et al. 2002; Morilak et al. 2005; Sarnyai et al. 2001). Serotonin and norepinephrine also have modulatory effects on dopamine neurotransmission which may impact discriminative performance.

2. Materials

2.1 Subjects

Adult male, Long-Evans rats (Harlan, Indianapolis, IN) were housed in standard polycarbonate shoebox bins with woodchip bedding. All behavioral assessments took place between 1600h and 1800h in the light phase of a 12:12 h light:dark cycle. Upon arrival to the colony, rats were maintained on *ad libitum* food and water until body weight reached 350 (± 10) g and were then fed sufficient chow (approximately 12–15 g) daily to maintain this weight for the remainder of the experiment unless otherwise noted. All procedures were in compliance with National Institutes of Health and National Research Council guidelines (2003) and were approved by the Institutional Animal Care and Use Committee at American University.

2.2 Drugs

Cocaine hydrochloride (generously supplied by the National Institute on Drug Abuse) was prepared in 0.9% saline vehicle. All doses were administered intraperitoneally (IP) at a volume of 1 ml/kg.

3. Methods

3.1 Drug Discrimination

3.1.1 Behavioral Apparatus—Experimental sessions were conducted with subjects placed in custom designed operant-conditioning chambers measuring 27.7 cm $\times$ 19.8 cm $\times$ 20.0 cm. Two empty graduated Nalgene drinking bottles were mounted 13 cm from each side of a center-mounted food hopper on one wall. The metal lick spout of each bottle was situated such that it was flush with the outer wall of the chamber. Bottle contact was detected by a drinkometer (Lafayette Instruments Model 58008). A 28-V cue-light centered above the food cup was illuminated during all sessions except where noted below. All events were programmed on a desktop Dell PC connected to the boxes via a Med Associates Interface that also recorded all lick responses. A white noise generator masked extraneous

sounds during experimental sessions, and a desk lamp with a red light bulb was used to illuminate the room.

3.1.2 Drug Discrimination Procedures—Training began following 7 days of daily handling and weighing. During training sessions, rats were administered saline or cocaine and placed into the chamber with only the non-drug (ND) or drug (D) associated bottle present, respectively. After 15 min, the center cue light was illuminated and rats were trained to make contact with the metal lick spout under a fixed-ratio 1 (FR1) schedule of reinforcement for 45-mg food pellets (BioRad). A 10 sec time-out during which the cue light became dark and bottle licks had no programmed consequences followed delivery of each pellet. The assignment of ND and D associated bottles was counterbalanced across subjects. Saline and cocaine sessions alternated with the requirement that no condition was repeated for more than three consecutive sessions. As responding stabilized, the response requirement for each pellet was increased across 15 min sessions until an FR 10 was reached. At this point, both ND and D associated bottles were present during the sessions and the response requirement was gradually increased again to FR15. A consecutive response contingency was in effect such that licking the condition-inappropriate bottle prior to completing the response requirement reset the count toward completion of the response requirement for pellet delivery to zero.

Individual performance criteria for the discrimination were that (1) the first FR15 and (2) at least 95% of all total responses must have been completed on the stimulus-appropriate bottle. Before beginning cumulative dose testing (described below), three component tests within a single session were given to assess bottle shifting between components and stimulus control by the training conditions. That is, three successive 5 min test components were preceded by 15-, 10- and 10 min timeout intervals, respectively, where either saline or 10 mg/kg cocaine was injected prior to each component. This gave tests of (1) ND-D-ND, (2) ND-ND-ND and (3) ND-ND-D.

Subsequently, a cumulative dose-effect procedure was used for testing which allowed an assessment of multiple doses of cocaine each day for each rat. The dose-effect test sessions consisted of six 5 min test components to determine a six-point cumulative dose-effect curve for cocaine. During dose-effect tests, a vehicle injection was administered 15 min before the first component to establish a baseline level of responding. After the first component, cocaine (1.0 mg/kg) was administered 10 min before the next 5 min component. This procedure was repeated for four more cycles to obtain the full dose-effect curve. The doses used were 1.0 (cumulative dose: 1.0), 2.2 (3.2), 2.4 (5.6), 4.4 (10) and 8.0 (18) mg/kg of cocaine. Dose-effect tests were administered for 3–4 consecutive tests to establish a stable baseline level of responding for each rat. Stability was defined as individual ED₅₀ values not varying more than 20%.

3.1.3 Handling/Stress Procedures—On the day after baseline responding was established, the handling/stress procedures began. During this procedure, training and testing were suspended and animals remained in their home cages for 10 days. Rats continued on the restricted food schedule to maintain weights at approximately 350 ($\pm$ 10) g during this phase and received regular cage maintenance. Rats in the Handled group were removed from their home-cage and handled for about 60 sec twice each day and placed back in their home-cage with no other disruptions for the entire 10 day period. Over the same period, rats in the Repeated Stress group were weighed each morning and then restrained in custom plastic restraint tubes (6.5 cm I.D. $\times$ 21.5 cm L) for 60 min in the afternoon (about 1530 h). The restraint stress was performed in a separate room adjacent to the housing room. After the stress, rats were kept in the experimental room for 30 min before being returned to the housing room. Rats in the Variable Stress group were exposed to various stressors presented

randomly, two per day, over the 10 day period. The variable stress procedure was based on published methods (Ortiz et al. 1996) and details are shown in Table 1. Animals in all three conditions were handled twice daily to equate the amount of experimenter contact in all groups.

3.1.4 Post-Handling/Stress Testing—Cumulative cocaine drug discrimination tests identical to those administered prior to the initiation of handling/stress were completed on the day immediately following completion of the handling/stress procedure (Post 1) and one day later (Post 2).

3.2 Determination of Corticosterone and Monoamine Transporter Levels

For determination of corticosterone and monoamine transporter levels, a separate group of adult, male Long-Evans rats (n=5/group) were trained to discriminate 10 mg/kg cocaine (IP) from saline and were then exposed to one of the three stress manipulations exactly as described above. However, instead of discrimination testing on Post 1, blood was collected at 1000–1100 h via tail-vein to measure baseline corticosterone levels after stress. Three hours later, rats were given an IP injection of 10 mg/kg cocaine followed 15 min later by decapitation. Plasma for corticosterone measurement and brains for western blotting were collected and immediately stored at -80°C .

3.2.1 Corticosterone—Plasma corticosterone concentrations were determined by radioimmunoassay using commercially available kits (MP Biomedicals LLC, Irvine, CA, USA). Plasma samples were run in one assay using the standard dilution method (sample dilution=1:200). Average intra- and inter-assay variability across assays were 5.6 and 8.8%, respectively.

3.2.2 Western Blot—As noted, brains were rapidly collected after decapitation and immediately stored at -80°C . At processing, 60 micron coronal slices of brain regions according to the coordinates from Paxinos and Watson (1998) were made using a cryostat and collected onto glass slides. The slides were then kept at -80°C before regional dissection. Frozen slides were observed at 40 $\times$ magnification on an Olympus SZX16 Research Stereo Microscope and isolation and removal of the PFC, NAc, CPu, VTA was done under the microscope without thawing. Dissection was completed using a procedure similar to that described by Palkovits (1973). Once collected, tissue was again stored at -80°C .

Tissue was homogenized on ice with 3 ml of 1x Radio Immuno Precipitation Assay (RIPA) Buffer/g tissue. Samples were centrifuged at 13000 rpm for 10 min at 4°C , the pellet discarded, lysates transferred to a new microtube and re-centrifuged under the same conditions. Lysates from the second centrifugation were used for western blotting. Protein concentrations were calculated using Biorad Protein Assay Dye (Pierce, Rockford, IL), and the amount of lysate needed for 20 μg of protein was added to 6.3 μl of 4X dye with the remaining volume of RIPA buffer added for a total of 25 μl loaded in each well. Samples were then heated at 70°C for 10 min before loading onto 4–12% Bis-Tris gels and electrophoresed for 80 min at 125 volts in 20X MES SDS running buffer (Invitrogen). As seen in Figures 5 and 6, each brain area from individual subjects was loaded onto a separate gel. Protein was then transferred onto nitrocellulose membrane using 1x transfer buffer (Invitrogen), for 80 min at 25V. After transfer, the membrane was blocked in 5% milk in 1x TBST overnight at 4°C . The following day, membranes were brought to room temperature and then incubated with primary antibody. The following primary antibodies were used: dopamine transporter (DAT; 1:200, Santa Cruz, CA), serotonin transporter (SERT; 1:1000, Santa Cruz, CA), norepinephrine transporter (NET; 1:1000, Millipore), cFos (1:1000, Cell

Signaling) and β -actin (1:2000; Cell Signaling). Antibodies were diluted in 5% milk and rocked for 1 h at room temperature. After incubation, membranes were washed three times for 10 min each with TBST and then incubated in secondary antibody for 45 minutes (anti-goat HRP (1:2000; Cell Signaling) for NET and anti-rabbit HRP (1:2000; Cell Signaling) for all other antibodies. After additional washes in TBST, membranes were developed using Pierce West Pico Chemilluminescence substrate. Bands were visualized on a UVP Biosystem Imaging system. Densitometry for protein bands was performed using NIH Image J program. Beta-actin served as the housekeeping gene.

3.3 Data Analysis

One rat in the Handled group was removed from the final analysis because no cocaine-like responding was produced during the cumulative testing procedure, and another rat in the Repeated Stress group was removed because it responded exclusively on the cocaine-associated lever during all baseline test sessions. This left five, five and six subjects in the Handled, Repeated Stress and Variable Stress groups, respectively. To determine generalization profiles, group means were calculated for cocaine-appropriate responding at each dose. ED₅₀ values (with 95% confidence limits; CL) on cocaine-appropriate responding were determined using linear interpolation with individual subjects on the group data prior to and following the handling/stress manipulations. Individual ED₅₀ values were also compared on Post 1 and Post 2 to their own baseline using paired t-tests. Response rates were calculated by dividing the total responses on both bottles when the cue lights were illuminated by the total session time (in sec) and expressed as responses per second. Values were then transformed to a percent of vehicle responding and were subjected to a repeated measures ANOVA with Tukey's HSD post-hocs. Rate of responding for each group was analyzed using a $2 \times 3 \times 6$ mixed ANOVA. Changes in body weight were determined by dividing each animal's weight on Post 1 by its weight on the last day prior to handling/stress and then transformed to a percentage. One-way ANOVAs, with Tukey's HSD post-hocs were used to compare changes in body weight. Corticosterone levels were analyzed using separate one-way ANOVAs for the baseline and cocaine-induced data because blood was collected using different sampling techniques (i.e., tail clip and trunk blood). After western blot density was quantified for each protein in each brain region using the ImageJ Method (Miller 2010), values of individual animals in the Repeated Stress and Variable Stress groups were transformed to a percent of Handled controls and were subjected to one-way ANOVA with Tukey's HSD post-hocs. All statistical and graphing procedures were performed using GraphPad Prism v.5.0 or SPSS v.16.

4. RESULTS

4.1 Cocaine-like Responding: Baseline

Cocaine engendered dose-dependent cocaine-like responding in all rats during baseline conditions (see Figure 1; Top Row: Baseline). There were no differences in baseline ED₅₀ values between the Handled (ED₅₀: 2.82; CL: 1.59–5.02), Repeated (ED₅₀: 4.28; CL: 2.92–6.26), and Variable (ED₅₀: 3.60; CL: 2.28–5.69) Stress groups.

4.2 Cocaine-like Responding: Post Handling/Stress

Cocaine continued to engender dose-dependent responding after 10 days of handling in the control group. ED₅₀ values were stable throughout all testing in that baseline and post-handling dose-response curves did not differ significantly on Post 1 (ED₅₀: 2.36; CL: 1.68–3.33) or 2 (ED₅₀: 3.57; CL: 2.55–5.02; see Figure 1; top left). Cocaine-like responding after 10 days of repeated stress was shifted to the left compared to the baseline (see Figure 1; top middle). While group ED₅₀ values did not differ in the repeated stress group on Post 1 (ED₅₀: 2.29; CL: 1.28–4.11) or Post 2 (ED₅₀: 2.67; CL: 1.56–4.57), paired t-tests showed that

there was a significant decrease in ED₅₀ values in 4/5 individuals on Post 1 ($t(4)=2.348$; $p<0.05$) but not on Post 2. ED₅₀ values for cocaine-like responding after variable stress (Post 1 ED₅₀: 2.64; CL: 1.83–3.61; Post 2 ED₅₀: 4.00; CL: 1.76–9.06) did not differ from the baseline level of responding (Figure 1; top right). Interestingly, maximal cocaine-like responding was lower than the baseline maximum and while cocaine still engendered dose-dependent increases in cocaine-like responding, there was a trend toward decreased cocaine-like responding that reached a maximum of 89% before falling to 82% on Post 1 and a maximum of 85% and decreasing to 72% on Post 2 at the higher cocaine doses (i.e., 10 and 18 mg/kg).

4.3 Rate of Responding

Rate of responding during cumulative dose cocaine testing is shown in Figure 1 (lower panels). The $2 \times 3 \times 6$ mixed ANOVA with the within-subject factor of Day (Post 1 and 2) and the between-subjects factors of Group (Handled, Repeated Stress and Variable Stress) and Dose (0, 1.0, 3.2, 5.6, 10, 18 mg/kg) revealed no significant main or interaction effects.

4.4 Body weight

The ANOVA on body weight revealed a significant effect [$F(2,17)=12.883$; $p<0.01$]. Post-hoc tests showed that the Variable Stress group gained significantly less weight than both the Handled and Repeated Stress groups (both p 's <0.05). Change in body weight during the 10-day handling/stress procedure is shown in Figure 2.

4.5 Corticosterone

Mean corticosterone levels are shown in Figure 3. The ANOVAs revealed that there were no differences in corticosterone levels between Handled, Repeated Stress and Variable Stress groups 24 hr after handling/stress (see Figure 3A) or 15 min after a 10 mg/kg, IP cocaine injection (Figure 3B).

4.6 Western blots

The ANOVA on density of DAT revealed significant differences in the PFC [$F(2,14)=10.320$; $p<0.05$], NAc [$F(2,14)=4.149$; $p<0.05$], CPu [$F(2,14)=18.450$; $p<0.001$] and VTA [$F(2,14)=10.977$; $p<0.01$]. Post-hoc analyses found that the Repeated Stress and Variable Stress groups had lower levels of DAT in the PFC (p 's <0.05). In the CPu and VTA, the Variable Stress group had higher levels of DAT compared to both the Repeated Stress group and Handled controls (both p 's <0.05).

ANOVA on density of SERT revealed significant differences in the NAc [$F(2,14)=22.094$; $p<0.001$] and VTA [$F(2,14)=16.112$; $p<0.001$] but not in the PFC or CPu. Post-hoc analyses found that SERT levels in the NAc and VTA was greater in the Variable Stress group compared to the Repeated Stress group and Handled controls (all p 's <0.01 ; Figure 4B).

ANOVA on density of NET revealed significant differences in the NAc [$F(2,14)=5.431$; $p<0.05$] and CPu [$F(2,14)=4.731$; $p<0.05$]. There was no NET detectable in the VTA (not shown). Post-hoc analyses found that the Repeated Stress group had higher levels of NET in the NAc compared to the Variable Stress group ($p<0.05$). In the CPU, the Repeated Stress group had lower levels of NET compared to the Handled controls ($p<0.05$; Figure 4C).

ANOVA on density of cFos revealed significant differences in the NAc [$F(2,14)=39.49$; $p<0.001$] and CPu [$F(2,14)=58.668$; $p<0.001$] but no differences in PFC or VTA. Post-hoc analyses found that in the NAc all groups were different from each other with the Variable Stress group having the lowest density of cFos, Handled controls having the highest density and Repeated Stress being intermediate (all p 's <0.01). In the CPU, Repeated and Variable

stress groups had lower levels of cFos compared to the Handled controls ($p's < 0.001$). Data are shown in Figure 5A.

The ANOVA on density of β -actin found that there was no difference in PFC, NAc or VTA [all $F's < 0.768$; $p's < 0.05$]. An overall significant difference in the CPu was found, but post-hocs revealed that no group was significantly different from the others, suggesting that differences found in the other proteins described above were due to experimental manipulations and not protein loading onto the gels. β -actin is shown in Figure 5B.

6. DISCUSSION

In the present study, we used an animal model of the subjective effects of drugs (drug discrimination) to study changes to cocaine's interoceptive cue after a period of forced abstinence when animals were exposed to two schedules of stressor presentation, repeated or variable. Further, monoamine transporter density was measured to investigate the effects of these stressors on cocaine's direct pharmacological targets that may give insight into changes in cocaine's discriminative stimulus. Understanding how stressors are able to alter the subjective effects of drugs of abuse during periods of abstinence at the behavioral and neurochemical level may lead to more effective treatment and relapse-prevention strategies.

We demonstrated for the first time that exposure to repeated or variable stress, produced distinct alterations in cocaine's interoceptive cue which may be related to activation of different neurotransmitter systems. Specifically, exposure to repeated stress produced a transient left-ward shift in the cocaine dose-effect curve, indicating increased sensitivity to the cocaine cue. In the Variable stress group, ED_{50} values after stress did not differ from baseline although an interesting pattern of decreased maximal levels of cocaine-like responding at the two highest doses of cocaine was found (see Figure 1). Specifically, cocaine-like responding reached a maximum of 89 – 85% at 5.6 mg/kg which decreased to 82 – 72% at 10 and 18 mg/kg during Post Stress testing. This was in contrast to both Handled and Repeated stress groups where complete generalization was maintained after it had been reached (regardless of dose). These differences were not the result of general disruptions in operant responding as there were no differences between the groups in rate of responding. The different patterns of cocaine-like responding between the Repeated Stress and Variable Stress groups were paralleled by changes in different monoamine transporters found in mesolimbic brain regions. The increased cocaine-like responding in the Repeated Stress group may be the result of differences found in DAT in the PFC and NET in the NAc and CPu. In contrast, the Variable Stress group showed DAT alterations in PFC, CPU, and VTA and SERT in NAc and VTA.

While repeated stress did produce statistically significant left-ward shifts on Post 1, these were perhaps smaller than might have been anticipated. Unpublished observations from our laboratory have found that in a subset of rats trained under similar conditions to the present study, a single restraint stress presented 15 min prior to the drug discrimination session produced an almost 10-fold left-ward shift in the cocaine dose-effect curve (Kohut, unpublished). This suggests that adaptive changes may have occurred with repeated exposure to the same stressor. In support of this idea, we found reduced c-Fos density (a measure of neuronal activation) in NAc and CPu in the Repeated stress group. This is in contrast to the changes found in norepinephrine (Florin et al. 1995; Rosario et al. 1999; Wong et al. 2011) activity in the NAc and CPU (see also de Boer et al. 1989), which suggest greater activation. McClung and Nestler (2003) have shown that *chronic* exposure to drugs of abuse and stress (Berton et al. 2007; Ohnishi et al. 2011) decrease levels of immediate early gene markers of neuronal activation (i.e., c-Fos) in favor of more stable markers such as Δ FosB, c-Jun, and/or CREB. Anecdotally, behavioral changes were also noted during the

repeated stress procedure that was not seen in the Handled or Variable stress groups. Specifically, defensive-burying behaviors (De Boer and Koolhaas, 2003; Woods-Kettelberger et al. 1997) were prominent in the Repeated stress group when rats were moved to the room associated with stress application after about 3 days of exposure (see also Pinel and Treit, 1978). More work investigating the effects of chronic repeated stress should shed light onto the behavioral and physiological mechanism responsible for this effect.

The influence of variable stress on the discriminative stimulus effects of cocaine was surprisingly different from repeated stress. In fact, while cocaine-like responding was enhanced after repeated stress, variable stress exposure decreased cocaine's efficacy in eliciting cocaine-like responding. The maximal level of cocaine-like responding after variable stress was attenuated when compared with baseline levels (shown in Figure 1) despite a cumulative dose that was nearly 2-fold greater than the training dose. One possible explanation for a decrease in efficacy is that variable stress could produce insurmountable tolerance to the discriminative stimulus effects of cocaine, an effect previously reported after chronic high-dose cocaine exposure (Wood and Emmett-Oglesby, 1987). In support of this idea, variable stress has been shown to attenuate DA efflux in the NAc (Ahmad et al. 2010; Bekris et al. 2005; DiChiara et al. 1999; Mangiavacchi et al. 2001; Tata et al. 2007). Attenuated DA release in the NAc might prevent the maximal level of intrinsic activity needed by DA receptor activation to be achieved to fully reproduce the cocaine discriminative stimulus (c.f., Colpaert, 1988). Further support comes from studies showing that serotonin has an inhibitory effect on cocaine-induced dopamine release in rats (Navailles et al. 2007) and non-human primates (Czoty et al. 2002). Callahan and Cunningham (2001) found that in cocaine discriminating rats, a pre-session administration of serotonin agonists (buspirone and gepirone) resulted in attenuated cocaine-like responding similar to the present study. We found that Variable Stress rats showed increased SERT density in NAc and VTA suggesting possible compensatory effects of elevated serotonin in these regions. While a general increase in serotonin neurotransmission would be expected to activate multiple serotonin receptor subtypes it is possible that activation of specific receptor subtypes are primarily involved in the decreased efficacy found in the present study (Navailles et al. 2007).

A major finding in the present study was that exposure to the two stressors produced differential alterations of neurotransmitter systems in a brain-region specific manner. We chose to measure monoamine transporters because they are highly regulated by changes in the synaptic environment (Lin et al. 2011; Zahniser and Sorkin, 2005). Interestingly, the only similarity in transporter alterations between the two stress groups was in DAT density in the PFC where both Repeated and Variable stress groups showed significantly lower levels compared to Handled controls. The Variable Stress group also had higher levels of DAT in the CPu and VTA compared to the Repeated stress and Control groups. It is not surprising that variable stress produced more robust effects on DA in the areas measured as it has been suggested that mesolimbic DA is particularly susceptible to the effects of variable (compared to repeated) stress because of the alterations in cocaine responsivity and intracellular pathways that have previously been demonstrated in this region (Haile et al. 2001; Ortiz et al. 1996). While extracellular neurotransmitter levels were not measured in the present study, the absence of an effect of variable stress on NAc DAT is similar to previous reports showing that baseline extracellular DA levels are not different from controls (Di Chiara et al. 1999; Raudensky and Yamamoto, 2007). The most relevant changes in monoamine transporters to the present study lie in the differences found in the NAc and VTA. Nakajima et al. (2004) reported that the discriminative stimulus effects of psychostimulants appear to be directly related to neuronal activation in NAc and VTA (but not 13 other brain regions). Thus, the fact that the Variable stress group showed increased SERT density in NAc and VTA and increased DAT in VTA while repeated stress showed

only increases in NET in the NAc may explain the differences found in cocaine's interoceptive cue. The absence of differences in NAc DA at baseline suggests that the mesolimbic response to a stimulus such as cocaine administration (see also Raudensky and Yamamoto, 2007) is modulated by altered serotonin or norepinephrine inputs from unknown brain regions.

The basis for the changes in dopamine, norepinephrine and serotonin between Repeated and Variable Stress groups is unknown and worth further investigation. We found the typical delay in body weight increases over the stress period that is seen in variable stress protocols but absent with repeated stress, suggesting differences in physiological responses between the two stress procedures. However, we did not find differences in absolute corticosterone levels (Figure 2) which differs from previous reports with variable stress (Haile et al. 2001; though see Bielksja et al. 2004). A possible explanation may be that the chronic cocaine exposure used in the present study altered HPA-axis function (Borowsky and Kuhn, 1991; Mello and Mendelson, 2003), which wasn't seen in drug-free or acute procedures used in other studies. An intriguing series of recent studies found that alterations in the pattern of corticosterone release elicits different neuronal responses at the molecular level (Sarabdjitsingh et al. 2010a,b; see also Sarnyai et al. 2001). It has previously been shown that repeated and variable stress produce different patterns of corticosterone release with sustained elevations characterizing the response to variable stress and more rapid after repeated stress suggesting that it may be the pattern of corticosterone increases rather than the absolute levels. Clearly, more work is needed to clarify the molecular mechanisms that underlie the behavioral and neurochemical differences between different types of stressors.

The results of the present study have major implications for prevention of stress-induced relapse and suggest novel targets for prevention. Stress-induced relapse has received considerable attention recently by preclinical and clinical researchers. Currently, a number of adrenergic medications are being investigated as prevention of relapse with varying levels of efficacy (Erb et al. 2000; Jobes et al. 2011; Kampman et al. 2001). This is consistent with the current results in predictably stressed rats. However, our data in the Variable stress group suggests that serotonin may also be an important target for future investigations. Serotonergic medications such as buspirone and several selective serotonin reuptake inhibitors are FDA-approved and would facilitate clinical use. Further, mixed monoamine releasers or monoamine transport inhibitors (Negus et al. 2007; Rothman et al. 2008) during early relapse could possibly be effective in modulating (or normalizing) both systems and preventing stress-induced relapse regardless of the types of stressor encountered. Future clinical studies may determine whether the subjective effects of different stressors are attenuated by pretreatment with antidepressants in drug-experienced subjects and shed more light on this important issue.

Acknowledgments

This work was supported by a grant from the Mellon Foundation to Anthony L. Riley, Ph.D. and Kathleen Decicco-Skinner, Ph.D. and NIH Intramural funds to Michael H. Baumann, Ph.D.

References

- Abercrombie ED, Keef KA, DiFrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem.* 1989; 52:1655–1658. [PubMed: 2709017]
- Ahmad A, Rasheed N, Banu N, Palit G. Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic Variable stress. *Stress.* 2010; 13(4):356–365.

- Antelman SM, Eichler AJ, Black CA, Kocan D. Interchangeability of stress and amphetamine in sensitization. *Science*. 1980; 207(4428):329–331. [PubMed: 7188649]
- Back SE, Hartwell K, DeSantis SM, Saladin M, McRae-Clark AL, Spratt EG, et al. Reactivity to laboratory stress provocation predicts relapse to cocaine. *Drug Alcohol Depend*. 2010; 106:21–27. [PubMed: 19726138]
- Barrot M, Marinelli M, Abrous DN, Rouge-Pont F, Le Moal M, Piazza PV. The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *European J Neuroscience*. 2000; 12:973–979.
- Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. *Behavioural Brain Research*. 2005; 161:45–59. [PubMed: 15904709]
- Berton O, Covington HE 3rd, Ebner K, Tsankova NM, Carle TL, Ulery P, et al. Induction of deltaFosB in the periaqueductal gray by stress promotes active coping responses. *Neuron*. 2007; 55:289–300. [PubMed: 17640529]
- Bielajew C, Konkle ATM, Merali Z. The effects of chronic mild stress on male Sprague-Dawley and Long Evans rats I. Biochemical and physiological analyses. *Behavioural Brain Research*. 2004; 136:583–592. [PubMed: 12429420]
- Biron D, Dauphin C, Di Paolo T. Effects of adrenalectomy and glucocorticoids on rat brain dopamine receptors. *Neuroendocrinology*. 1992; 55:468–476. [PubMed: 1314340]
- Borowsky B, Kuhn CM. Monoamine mediation of cocaine-induced hypothalamo-pituitary-adrenal activation. *J Pharmacol Exp Ther*. 1991; 256:204–210. [PubMed: 1671094]
- Callahan PM, Cunningham KA. Modulation of the discriminative stimulus properties of cocaine: comparison of the effects of fluoxetine with 5HT1A and 5HT1B receptor agonists. *Neuropharmacology*. 2000; 36:373–381. [PubMed: 9175616]
- Chait LD, Uhlenhuth EH, Johanson CE. The discriminative stimulus and subjective effects of phenylpropanolamine, mazindol, and d-amphetamine in humans. *Pharmacol, Biochem, Behav*. 1986; 24:1665–1672. [PubMed: 3737634]
- DiChiara G, Loddo P, Tanda G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol Psychiatry*. 1999; 46:1624–16–33. [PubMed: 10624543]
- Coffey SF, Saladin ME, Drobos DJ, Brady KT, Dansky BS, Kilpatrick DG. Trauma and substance cue reactivity in individuals with comorbid posttraumatic stress disorder and cocaine or alcohol dependence. *Drug Alcohol Depend*. 2002; 65:115–127. [PubMed: 11772473]
- Colpaert, FC. Intrinsic activity and discriminative effects of drugs. In: Colpaert, FC.; Balster, RI., editors. *Transduction mechanisms of drug stimuli*. Berlin: Springer Verlag; 1988. p. 154-160.
- Czoty PW, Ginsburg BC, Howell LL. Serotonergic attenuation of the reinforcing and neurochemical effects of cocaine in squirrel monkeys. *J Pharmacol Exp Ther*. 2002; 300(3):831–837. [PubMed: 11861788]
- de Boer SF, de Beun R, Slangen JL, van der Gugten J. Plasma catecholamine and corticosterone responses to predictable and unpredictable noise stress in rats. *Physiology & Behavior*. 1989; 45:789–795. [PubMed: 2780849]
- de Boer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. *Eur J Pharmacol*. 2003; 463:145–161. [PubMed: 12600707]
- DiChiara G, Loddo P, Tanda G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli and chronic mild stress: Implications for the psychobiology of depression. *Biol Psychiatry*. 1999; 46:1624–1633. [PubMed: 10624543]
- Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y, Stewart J. Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. *Neuropsychopharmacology*. 2000; 23:138–150. [PubMed: 10882840]
- Fischman MW, Schuster CR, Resnekov L, Shick JFE, Krasnegor NA, Fennel W, Freedman DX. Cardiovascular and subjective effects of intravenous cocaine administration in humans. *Arch Gen Psychiatry*. 1976; 33:983–989. [PubMed: 949232]

- Florin SM, Kuczenski R, Segal DS. Effects of reserpine on extracellular caudate dopamine and hippocampus norepinephrine responses to amphetamine and cocaine: mechanistic and behavioral considerations. *J Pharmacol Exp Ther*. 1995; 274:231–241. [PubMed: 7616403]
- Haile CN, GranPre T, Kosten TA. Chronic Variable stress, but not chronic Repeated stress, enhances the sensitivity to the behavioral effects of cocaine in rats. *Psychopharmacology*. 2001; 154:213–220. [PubMed: 11314684]
- Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, et al. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. *Proc Natl Acad Sci*. 1986; 83:9779–9783. [PubMed: 2879285]
- Harris DS, Reus VI, Wolkowitz OM, Mendelson JE, Jones RT. Repeated psychological stress testing in stimulant-dependent patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 2005; 29:669–677. [PubMed: 15913869]
- Jobes ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ, Preston KL. Clonidine blocks stress-induced craving in cocaine users. *Psychopharmacology*. in press.
- Joels M, De Kloet ER. Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. *Progress in Neurobiology*. 1994; 43:1–36.
- Kampman KM, Volpicelli JR, Mulvaney F, Alterman AI, Cornish J, Gariti P, et al. Effectiveness of propranolol for cocaine dependence treatment may depend on cocaine withdrawal symptom severity. *Drug Alc Depend*. 2001; 63:69–78.
- Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Glugge G, Korte SM, et al. Stress revisited: A critical evaluation of the stress concept. *Neurosci Biobeh Rev*. 2011; 35:1291–1301.
- Kreek MJ, Koob GF. Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Depend*. 1997; 51:23–47. [PubMed: 9716928]
- Li CS, Kosten TR, Sinha R. Sex differences in brain activation during stress imagery in abstinent cocaine users: a functional magnetic resonance imaging study. *Biol Psychiatry*. 2005; 57:487–494. [PubMed: 15737663]
- MacLennan AJ, Maier SF. Coping and the stress-induced potentiation of stimulant stereotypy in the rat. *Science*. 1983; 219:1091–1093. [PubMed: 6681679]
- Mangiavacchi S, Masi F, Scheggi S, Leggio B, De Montis MG, Gambarana C. Long-term behavioral and neurochemical effects of chronic stress exposure in rats. *J Neurochem*. 2001; 79:1113–1121. [PubMed: 11752052]
- Mantsch JR, Goeders NE. Generalization of a restraint-induced discriminative stimulus to cocaine in rats. *Psychopharmacology*. 1998; 135:423–426. [PubMed: 9539268]
- Mantsch JR, Goeders NE. Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: relationship to the discriminative stimulus effects of cocaine. *Psychopharmacology*. 1999; 142:399–407. [PubMed: 10229065]
- Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *European Journal of Neuroscience*. 2002; 16:387–394. [PubMed: 12193179]
- Marlatt, GA.; Gordon, JR. Relapse prevention: maintenance strategies in the treatment of addictive behaviors. Guilford Press; New York: 1985.
- McClung CA, Nestler EJ. Regulation of gene expression and cocaine reward by CREB and FosB. *Nature Neuroscience*. 2003; 6:1208–1215.
- McKay JR. Studies of factors in relapse to alcohol, drug and nicotine use: a critical review of methodologies and findings. *J Stud Alcohol*. 1999; 60:566–576. [PubMed: 10463814]
- Mello NK, Mendelson JH. Cocaine's effects on neuroendocrine systems: clinical and preclinical studies. *Pharmacol Biochem Behav*. 2003; 57:571–599. [PubMed: 9218281]
- Mendelson JH, Mello NK. Management of cocaine abuse and dependence. *N Engl J Med*. 1996; 334:965–972. [PubMed: 8596599]
- Miczek KA, Mutschler NH, van Erp AMM, Blank AD, McInerney SC. d-Amphetamine “cue” generalizes to social defeat stress: behavioral sensitization and attenuated accumbens dopamine. *Psychopharmacology*. 1999; 147:190–199. [PubMed: 10591887]
- Morilak DA, Barrera G, Echevarria DJ, Garcia AS, Hernandez A, Ma S, Petre CO. Role of brain norepinephrine in the behavioral response to stress. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005; 29:1214–1224. [PubMed: 16226365]

- Nakajima A, Yamada K, He J, Zeng N, Nitta A, Nabeshima T. Anatomical substrates for the discriminative stimulus effects of methamphetamine in rats. *J Neurochem.* 2004; 91:308–317. [PubMed: 15447664]
- National Research Council. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Academy Press; Washington, D.C: 2003.
- Navailles S, Moison D, Cunningham KA, Spampinato U. Differential regulation of the mesoaccumbens dopamine circuit by serotonin_{2C} receptors in the ventral tegmental area and the nucleus accumbens: An in vivo microdialysis study with cocaine. *Neuropsychopharmacology.* 2007; 33:1–10.
- Negus SS, Mello NK, Blough BE, Baumann MH, Rothman RB. Monoamine releasers with varying selectivity for dopamine/norepinephrine versus serotonin release as candidate “agonist” medications for cocaine dependence: studies in assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. *J Pharmacol Exp Ther.* 2007; 320:627–636. [PubMed: 17071819]
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron.* 2002; 34:13–25. [PubMed: 11931738]
- Ohnishi YN, Ohnishi YH, Hokama M, Nomaru H, Yamazaki K, Tominaga Y, et al. FosB is essential for the enhancement of stress tolerance and antagonizes locomotor sensitization by deltaFosB. *Biol Psychiatry.* in press.
- Ortiz J, Fitzgerald LW, Lane S, Terwilliger R, Nestler EJ. Biochemical adaptations in the mesolimbic dopamine system in response to repeated stress. *Neuropsychopharmacology.* 1996; 14:443–452. [PubMed: 8726755]
- Palkovits M. Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Research.* 1973; 59:449–450. [PubMed: 4747772]
- Pinel JPJ, Treit D. Burying as a defensive response in rats. *J Comp Physiol Psychol.* 1978; 92:708–712.
- Preston KL, Epstein DH. Stress in the daily lives of cocaine and heroin users: relationship to mood, craving, relapse triggers, and cocaine use. *Psychopharmacology.* in press.
- Raudensky J, Yamamoto BK. Effects of chronic variable stress on monoamine transporter immunoreactivity and methamphetamine-induced dopamine release in the nucleus accumbens shell. *Synapse.* 2007; 61:353–355. [PubMed: 17318887]
- Rosario LA, Abercrombie ED. Individual differences in behavioral reactivity: correlation with stress-induced norepinephrine efflux in the hippocampus of Sprague-Dawley rats. *Brain Res Bull.* 1999; 48:595–602. [PubMed: 10386839]
- Rothman RB, Blough BE, Baumann MH. Dopamine/serotonin releasers as medications for stimulant addictions. *Prog Brain Res.* 2008; 172:385–406. [PubMed: 18772043]
- Rouge-Pont F, Marinelli M, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. *J Neurosci.* 1995; 15:7189–7195. [PubMed: 7472473]
- Sarabdjitsingh RA, Isenia S, Polman A, Mijalkovic J, Lachize S, Datson N, de Kloet ER, Meijer OC. Disrupted corticosterone pulsatile patterns attenuate responsiveness to glucocorticoid signaling in rat brain. *Endocrinology.* 2010a; 151:1177–1186. [PubMed: 20080870]
- Sarabdjitsingh RA, Conway-Campbell BL, Leggett JD, Waite EJ, Meijer OC, de Kloet ER, Lightman SL. *Endocrinology.* 2010b; 151:5369–5379. [PubMed: 20861228]
- Samyay Z, Mckittrick CR, McEwen BS, Kreek MJ. Selective regulation of dopamine transporter binding in the shell of the nucleus accumbens by adrenalectomy and corticosterone-replacement. *Synapse.* 2001; 30:334–337. [PubMed: 9776137]
- Shalev U, Highfield D, Yap J, Shaham Y. Stress and relapse to drug seeking in rats: studies on the generality of the effect. *Psychopharmacology.* 2000; 150:337–346. [PubMed: 10923762]
- Sinha R. How does stress increase risk of drug abuse and relapse? *Psychopharmacology.* 2001; 158:343–359. [PubMed: 11797055]
- Sinha R. The role of stress in addiction relapse. *Current psychiatry reports.* 2007; 9:388–395. [PubMed: 17915078]

- Sinha R, Catapano D, O'Malley S. Stress-induced craving and stress response in cocaine dependent individuals. *Psychopharmacology*. 1999; 142:343–351. [PubMed: 10229058]
- Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Arch Gen Psychiatry*. 2006; 63:324–331. [PubMed: 16520439]
- Tata, DA.; Raudensky, J.; Yamamoto, BK. Augmentation of methamphetamine-induced toxicity in the rat striatum by Variable stress: contribution of enhanced hyperthermia. 2007.
- Wise RA. Drug-activation of brain reward pathways. *Drug Alcohol Depend*. 1998; 51:13–22. [PubMed: 9716927]
- Wong DL, Tai TC, Wong-Faull DC, Claycomb R, Meloni EG, Myers KM, et al. Epinephrine: A short- and long-term regulator of stress and development of illness. *Cell Mol Neurobiol*. in press.
- Wood DM, Emmett-Oglesby MW. Evidence for dopaminergic involvement in tolerance to the discriminative stimulus properties of cocaine. *European Journal of Pharmacology*. 1987; 138:155–157. [PubMed: 3622606]
- Woods-Kettelberger A, Kongsamut S, Smith CP, Winslow JT, Corbett R. Animal models with potential applications for screening compounds for the treatment of obsessive-compulsive disorder. *Expert Opin Investig Drugs*. 1997; 6:1369–1381.
- Zahniser NR, Sorkin A. Rapid regulation of the dopamine transporter: role in stimulant addiction? *Neuropharmacology*. 2005; 47:80–91. [PubMed: 15464127]

Highlights

We examine behavioral and biochemical responses to two different stressors in rats

- Repeated and variable stress differentially alter the cocaine discriminative stimulus
- Stress-induced alterations in SERT and NET may explain differences in behavior

Figure 1.

Cumulative cocaine dose-effect curves (0–18 mg/kg) for substitution (top panels) and percentage of baseline response rate (bottom panels) after 10 days of Handling, Repeated, and Variable Stress in rats trained to discriminate 10 mg/kg cocaine, IP from saline. Baseline is shown in filled circles, while the Post-Handling/Stress Day 1 is shown as open square and Day 2 as open circle (see Legend). Each data point represents the group mean in the top panels. Bottom panels represent mean $\pm$ SEM.

Figure 2. Mean ($\pm$ SEM) change in body weight as a percentage over 10 days of handling, repeated or variable Stress. *significantly different from Handled and Repeated stress groups; $p < 0.05$

Figure 3. Mean ($\pm$ SEM) corticosterone plasma levels (ng/ml) in Handled, Repeated and Variable stress groups at baseline after 10 days of handling/stress (tail clip; Panel A) and 15 min after IP administration of 10 mg/kg cocaine (trunk blood; Panel B).

Figure 4. Effects of 10 days of repeated or variable stress on dopamine (DAT; A), serotonin (SERT; B) and norepinephrine (NET; C) transporter density in PFC, NAc, CPu, and VTA as a percentage of Handled control levels. *p<0.05 vs. Handled Controls; **p<0.05 vs. Repeated Stress and Handled Controls; #p<0.05 vs. Variable Stress. Shown at top are individual protein bands.

Figure 5.

Effects of 10 days of repeated or variable stress on c-Fos (A) and β -actin (B) levels in PFC, NAc, CPu, and VTA as a percentage of Handled controls. *p<0.05 vs. Handled Controls; **p<0.05 vs. Repeated Stress and Handled Controls; #p<0.05 vs. Repeated Stress. Shown at top are individual protein bands.

Table 1

Variable stress procedure sequence

Day	Time 1	Procedure 1	Duration	Time 2	Procedure 2	Duration
1	12:00 pm	Paired-Housing	50 min	1:00 pm	Swim Stress	4 min
2	11:00 am	Cold Isolation (4°C)	60 min	7:00 pm	Lights On	Overnight
3	12:00 pm	Lights Off	3 h	3:00 pm	Cold Isolation	15 min
4	7:00 pm	Paired-Housing	50 min	7:00 pm	Food/Water Deprivation	Overnight
5	1:00 pm	Swim Stress	3 min	7:00 pm	Cage Tilt (40°)	Overnight
6	11:00 am	Restraint Stress	60 min	3:00 pm	Lights Off	2 h
7	10:00 am	Swim Stress	4 min	4:00 pm	Restraint Stress	60 min
8	7:00 pm	Lights On	Overnight	7:00 pm	Food/Water Deprivation	Overnight
9	10:00 pm	Paired-Housing	20 min	7:00 pm	Food/Water Deprivation	Overnight
10	7:00 pm	Cage Tilt (40°)	Overnight	7:00 pm	Lights On	Overnight