Published in final edited form as:

Biol Psychiatry. 2015 December 15; 78(12): 871–879. doi:10.1016/j.biopsych.2015.04.024.

Anhedonia, reduced cocaine reward, and dopamine dysfunction in a rat model of Posttraumatic Stress Disorder

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Abstract

Background—Posttraumatic stress disorder (PTSD) co-occurs with substance use disorders (SUD) at high rates, but the neurobiological basis of this relationship is largely unknown. PTSD and drug addiction each involve dysregulation of brain reward circuitry, therefore the identification of pathology of the mesolimbic dopamine system may aid in understanding their functional relationship. Dopamine reward dysfunction also may be relevant to the mechanisms underlying the PTSD symptoms of anhedonia and emotional numbing.

Methods—Single-prolonged stress (SPS) was used as a rat model of PTSD, and a series of behavioral and neuropharmacological assays were applied to assess the impact of SPS on reward, cocaine intake, and components of the striatal dopamine system.

Results—Exposure to SPS increased anhedonia-like behaviors and decreased the rewarding properties of cocaine compared to control handling. Altered cocaine intake during extended-access self-administration sessions was observed in rats exposed to SPS, further suggesting a difference in the reinforcing properties of cocaine following severe stress. SPS reduced tissue content of dopamine and its metabolites in the striatum, as well as altered striatal dopamine transporter and D2, but not D1, receptor densities.

Conclusions—These results support a role for altered dopaminergic transmission in reduced reward function in PTSD. Pathology of the dopamine system and the degradation of reward

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FINANCIAL DISCLOSURE

All authors report no biomedical financial interests or potential conflicts of interest.

processes may contribute to PTSD symptomology and have implications for co-occurring psychiatric disorders such as substance abuse or depression.

Keywords

posttraumatic stress disorder; PTSD; reward; cocaine; dopamine; self-administration

INTRODUCTION

Posttraumatic Stress Disorder (PTSD) is a stress-induced psychiatric disorder characterized by perpetuating symptomology and high rates of co-occurring substance use disorders (SUD). The onset of PTSD often precedes and increases the risk for subsequent development of SUD [1, 2]. A functional relationship between PTSD symptom severity and substance use has been demonstrated [1, 3-6], and improved PTSD symptomology predicts improvement of SUD symptoms [4, 7, 8]. Together, these findings suggest that PTSD enhances the susceptibility to substance abuse.

Separate lines of evidence suggest that PTSD and drug addiction each involve dysregulation of brain reward circuitry. Reward and the reinforcing effects of drugs of abuse are mediated by dopaminergic neurotransmission in the mesolimbic pathway (see reviews [9, 10]). Individuals with PTSD exhibit blunted motivation for rewards, and diminished subjective responses when rewards are received [11, 12]. Reduced striatal and midbrain activation in response to rewards and positively valenced stimuli has also been observed in PTSD [13-17]. A recent study supports altered dopamine function, as striatal dopamine transporter (DAT) density is higher in individuals with PTSD than traumatized asymptomatic controls [18]. Deficits in reward functioning are hypothesized to contribute to core PTSD symptoms including the presence of anhedonia and emotional numbing [19, 20]. Approximately two-thirds of PTSD patients report diminished interest in pleasurable activities and endorse the inability to experience positive emotions [21-23]. Individuals with substance use disorders exhibit similar deficiency in reward function and altered dopaminergic neurotransmission including reduced striatal D2 receptor density [24, 25, 26]. Dysregulation of dopaminergic function is hypothesized to contribute to drug-seeking behavior [24-27], and thus may contribute to SUD vulnerability in PTSD.

In the present studies, single-prolonged stress (SPS) was used to model PTSD in rodents. Rats exposed to SPS exhibit a behavioral phenotype resembling PTSD that includes heightened startle behavior [28, 29], enhanced contextual and cued fear conditioning [30, 31], impaired extinction of fear and retention of extinction memories [31-34], cognitive dysfunction [35, 36], and enhanced negative feedback of the hypothalamic-pituitary-adrenal axis [37]. However, the effects of SPS on anhedonia and reward function, which may be related to PTSD symptoms and co-occurring substance abuse, are unclear. The effect of SPS on psychostimulant-induced reinforcement is also under-studied. Therefore, we assessed anhedonia-like behavior and the rewarding effects of cocaine, as well as components of the striatal dopamine system following exposure to SPS. We hypothesize that PTSD results in reduced dopaminergic neurotransmission, which contributes to deficient hedonic capacity and related PTSD symptomology, as well as altered psychostimulant reinforcement.

METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in all studies (see Supplement 1). Animal care and experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Experimental protocols were approved by the Institutional Animal Care and Use Committee of Temple University. Separate cohorts of rats were used for each behavioral test and neurochemical assay unless indicated.

Drugs and Chemicals

(-) Cocaine hydrochloride was generously supplied by the National Institute on Drug Abuse (NIDA Drug Supply Program, Bethesda, MD). Cocaine dissolved in sterile saline was administered by intraperitoneal injections (1 ml/kg body weight) or delivered via intravenous infusion in a volume of 0.25 mL/400 g body weight over 2.5-3.5 seconds. Radioligands were from Perkin Elmer (Waltham, MA), and all other chemical reagents were from commercial vendors (Sigma Aldrich, St. Louis, MO; Fischer Scientific, Pittsburgh, PA).

Single-prolonged stress (SPS)

Rats weighing 225-300 g were exposed to a SPS procedure that included individual restraint for 2 hours in plexiglas cylinders (25 × 7.5 × 7cm; Harvard Apparatus, Holliston, MA) followed by 20 minutes of group forced swimming in a circular tub (42 cm tall × 55 cm diameter) filled to 30 cm with 23-24°C water (5-8 rats simultaneously) [29, 32]. Rats were dried and returned to their home cages for 15 minutes, and then moved to a separate chamber where they were exposed to isoflurane until loss of consciousness (<1 minute). Isoflurane exposure was conducted by placing a cotton ball soaked with isoflurane into an open conical tube and placed into the chamber (2-4 rats simultaneously). Rats were housed in pairs undisturbed for the next 7 days, with the exception of cage changes. All further experimental procedures began after the 7-day isolation period (day 9 of the study). Control animals were weighed daily and housed in pairs.

Jugular catheterization surgery and self-administration apparatus

Rats were exposed to SPS or control handling followed by surgical implantation of indwelling silicone catheters into the right jugular vein on day 9. Self-administration was assessed in sound-attenuated operant chambers equipped with a swivel-tether system and a computer-controlled infusion pump (Med Associates, St. Albans, VT). Detailed descriptions are provided in Supplement 1.

EXPERIMENTAL DESIGNS AND PROCEDURES

Experiment 1: Behavioral assessments of anhedonia and reward following SPS

Separate cohorts of rats exposed to SPS or control conditions underwent a sucrose preference test, behavioral activity monitoring, or cocaine-conditioned place preference to assess anhedonia-like behavior and reward function. Supplement 1 contains additional

details regarding these tests. For sucrose preference, rats (n=7-21/group) were acclimated to drinking from two drip-resistant water bottles for 5 days after SPS or control handling. Acclimation was followed by a choice consumption test (48-hours) during which individual rats had access to two bottles, one containing water and one with a sucrose solution (0.25, 0.5, or 1% sucrose). Anhedonia was defined as a reduction in sucrose intake in the SPS group versus the control group.

Behavioral activity was assessed in a second cohort of rats following SPS or control procedures (n=16/group). Rats were placed individually into automated activity monitors consisting of a homecage-like environment. Rats were habituated to the test environment for 30 minutes, followed by activity monitoring for 24-hours (12 hour dark cycle followed by a 12 hour light cycle). A third cohort of rats (7-11/group) underwent cocaine conditioned place preference following SPS or control conditions. Baseline preference scores were established on day 1, during which rats had free access to the two compartments of the conditioning chamber in a drug-free state. On days 2-5, rats were confined to the conditioning chamber twice daily for 30 minutes, receiving an injection of cocaine (5, 10, or 20 mg/kg, i.p.) in the non-preferred compartment, and saline in the opposite compartment at least three hours later. On day 6, preference was again assessed in a drug-free state. Preference scores were calculated by subtracting the time spent on the cocaine-paired side of the chamber during the pretest (day 1) from the preference test (day 6).

Experiment 2: Effect of SPS on the acquisition and escalation of cocaine self-administration

Following exposure to SPS or control procedures, the acquisition of cocaine self-administration was assessed during daily 2-hour sessions over 15 consecutive days. Escalating doses of cocaine (0.1875, 0.375, or 0.75 mg/kg/infusion) were available in 3 consecutive phases lasting 5 days each on a fixed-ratio 1 (FR1) schedule of reinforcement (i.e. days 1-5 delivered 0.1875 mg/kg cocaine, days 6-10 delivered 0.375 mg/kg, days 11-15 delivered 0.75 mg/kg). Separate cohorts of rats underwent SPS or control conditions and acquisition of self-administration followed by extended-access to cocaine to facilitate the escalation of self-administration for 14 consecutive days. Cocaine (0.375 mg/kg/infusion) was available for 6-hours daily under a FR1 schedule of reinforcement (described in Supplement 1).

Experiment 3: Effect of SPS on striatal tissue dopamine levels and receptor/transporter densities

Tissue dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assessed in the nucleus accumbens and caudate putamen of brains dissected on day 9 or 16 after SPS or control conditions. Tissues were sonicated in 0.1 M HClO₄/0.1 mM EDTA buffer and centrifuged. Supernatants were collected and sent to Wayne State University for HPLC measurement of dopamine and metabolites. Supplement 1 provides detailed methodologies.

The density of dopamine D1 and D2 receptors and DAT were measured by quantitative *in vitro* receptor autoradiography after SPS or control procedures. Brains were removed on day

9 and rapidly frozen. Coronal sections (16 μM) of striatum were collected and labeled with [^3H]SCH23390 (for D1 receptors), [^3H]raclopride (for D2 receptors), and [^3H]WIN35428 (for DAT). Autoradiograms were generated from radiolabeled sections and quantified by methods described in Supplement 1 and previous publications [38-41].

RESULTS

Experiment 1: Behavioral assessments of anhedonia and reward following SPS

SPS and control rats underwent a sucrose preference test to examine anhedonia-like behavior and sucrose reward (Figure 1A). A two-way ANOVA of sucrose intake indicated significant effects of stress and sucrose concentration [interaction: $F(2,86)=0.8337$, $p>0.05$; stress: $F(1,86)=4.623$, $p<0.05$; sucrose concentration: $F(2,86)=10.92$, $p<0.0001$], in that SPS rats consumed less sucrose than controls. Total fluid intake (sucrose + water) was similar between groups (data not shown). These data suggest an increase in anhedonia-like behavior or a reduction in the rewarding properties of sucrose following SPS versus control handling.

Spontaneous activity was assessed for 24-hours in SPS or control rats. Cumulative activity counts over each 12-hour cycle are shown in Figure 1B. Two-way ANOVA revealed a significant interaction and effects of stress and dark/light cycle [interaction: $F(1,60)=5.699$, $p<0.05$; stress: $F(1,60)=14.23$, $p<0.001$; light/dark cycle: $F(1,60)=251.1$, $p<0.0001$]. Bonferroni post-tests indicated that nocturnal activity was significantly greater in both SPS and control groups compared to activity during the light cycle ($p<0.001$). Analyses also showed significantly lower spontaneous nocturnal activity in the SPS group compared to controls ($###p<0.001$). The time-course of activity is shown in Figure S1.

To examine the rewarding properties of cocaine in the SPS group versus controls, rats underwent a conditioned place preference procedure. Data in Figure 1C represent the mean preference scores for the cocaine-paired side of the conditioning chamber in SPS and control rats. Two-way ANOVA revealed significant interaction, stress, and dose effects [interaction: $F(2,45)=5.040$, $p<0.05$; stress: $F(1,45)=11.16$, $p<0.01$; dose: $F(2,45)=18.69$, $p<0.0001$]. Bonferroni post-tests determined that SPS significantly reduced the preference score for the cocaine-paired environment at 10 mg/kg cocaine ($###p<0.001$), which is the dose that produced maximum preference in controls.

Experiment 2: Effect of SPS on cocaine self-administration

Acquisition of cocaine self-administration was assessed using a FR1 schedule of reinforcement over 15 daily sessions under a 2-hour access procedure. Daily cocaine intake was analyzed by two-way repeated measures ANOVA (Figure 2A). A significant effect of dose was revealed, however no interaction or significant difference in cocaine intake between stressed and unstressed rats was detected [interaction: $F(14,378)=0.7749$, $p>0.05$; stress exposure: $F(1,378)=0.8653$, $p>0.05$; session: $F(14,378)=136.0$, $p<0.0001$]. Cocaine intake based on dose and the latency to acquire was also assessed (Figure S2); no significant differences between SPS and control groups were detected.

Rats exposed to SPS or control conditions were given unlimited access to cocaine for 6 hours daily under a FR1 schedule of reinforcement for 14 days to facilitate the escalation of

self-administration. Daily cocaine intake was compared (Figure 2B). Two-way repeated measures ANOVA revealed significant effects of stress and session [interaction: $F(13,104)=0.8220$, $p>0.05$; stress: $F(1,104)=6.590$, $p<0.05$; session: $F(13,104)=12.51$, $p<0.0001$; subjects: $F(8,104)=16.36$, $p<0.0001$]. SPS-exposed rats consumed less cocaine daily under extended-access compared to controls. Further analysis indicated a trend ($p=0.08$) toward a greater increase in cocaine consumption from day 1 to 14 in SPS-exposed rats compared to controls (see results in Supplement 1).

Experiment 3: Effect of SPS on dopamine levels and dopamine receptor and transporter densities

Tissue levels of dopamine and its metabolites DOPAC and HVA were measured in the nucleus accumbens and caudate putamen at two time points after SPS, one day after the 7-day isolation period (day 9) and one week later (day 16) (Figure 3). Two-way ANOVA of dopamine in the nucleus accumbens (Figure 3A) indicates significantly lower levels following stress versus control conditions [interaction: $F(1,50)=1.075$, $p>0.05$; stress: $F(1,50)=4.447$, $p<0.05$; day: $F(1,50)=1.075$, $p>0.05$]. Similarly, accumbal DOPAC levels (Figure 3B) [interaction: $F(1,50)=0.0351$, $p>0.05$; stress: $F(1,50)=6.847$, $p<0.05$; day: $F(1,50)=0.0351$, $p>0.05$] and HVA levels (Figure 3C) were reduced by SPS [interaction: $F(1,50)=0.1081$, $p>0.05$; stress: $F(1,50)=8.713$, $p<0.01$; day: $F(1,50)=0.1081$, $p>0.05$].

Two-way ANOVA of dopamine in the caudate putamen (Figure 3D) revealed significantly lower levels of dopamine following stress exposure [interaction: $F(1,47)=0.5666$, $p>0.05$; stress: $F(1,47)=8.152$, $p<0.01$; day: $F(1,50)=0.5666$, $p>0.05$]. Similarly, analysis of DOPAC (Figure 3E) [interaction: $F(1,49)=0.0787$, $p>0.05$; stress: $F(1,49)=6.801$, $p<0.05$; day: $F(1,49)=0.0787$, $p>0.05$] and HVA (figure 3F) levels revealed a significant effect of stress compared to control [interaction: $F(1,50)=0.0074$, $p>0.05$; stress: $F(1,50)=7.925$, $p<0.01$; day: $F(1,50)=0.0074$, $p>0.05$]. Overall, tissue content of dopamine, DOPAC, and HVA in the nucleus accumbens and the caudate putamen were lower in rats exposed to SPS compared to control handling.

Quantitative autoradiography determined whether dopamine receptor or transporter densities across the rostral-caudal extent of the striatum were altered by SPS (Figure 4). Two-way ANOVA assessed binding of [^3H]raclopride to D2 receptors at mid-caudal levels of the nucleus accumbens (Figure 4A) and caudate putamen (Figure 4B). Results demonstrated significant effects of stress in both regions with stressed rats having lower levels of [^3H]raclopride binding in each [nucleus accumbens: interaction: $F(6,69)=0.2442$, $p>0.05$; stress: $F(1,69)=7.189$, $p<0.01$; distance from bregma: $F(6,69)=10.42$, $p<0.0001$; caudate putamen: interaction: $F(7,77)=0.1309$, $p>0.05$; stress: $F(1,77)=18.90$, $p<0.0001$; distance from bregma: $F(7,77)=7.268$, $p<0.0001$]. No significant difference in [^3H]raclopride binding was detected between SPS or control handled rats in the rostral level of the nucleus accumbens and caudate putamen (data not shown). Representative autoradiograms of striatal [^3H]raclopride binding are shown in Figure 4C.

Binding of [^3H]WIN35428 to DAT was assessed by two-way ANOVA. Results revealed significantly higher binding of [^3H]WIN35428 in the mid-caudal nucleus accumbens of stressed rats compared with controls (Figure 4D) [interaction: $F(6,68)=0.4045$, $p>0.05$;

stress: $F(1,68)=8.806$, $p<0.01$; distance from bregma: $F(6,68)=1.031$, $p>0.05$], whereas no significant effect of stress was detected in the mid-caudal caudate putamen (Figure 4E) [interaction: $F(7,80)=0.5019$, $p>0.05$; stress: $F(1,80)=0.5696$, $p>0.05$; distance from bregma: $F(7,80)=0.2395$, $p>0.05$]. [^3H]WIN35428 binding was not significantly different between SPS or control handled rats in the rostral striatum (data not shown). Representative autoradiograms of [^3H]WIN35428 binding to striatal DAT are provided in Figure 4F.

Binding of [^3H]SCH23390 to D1 receptors in the mid-caudal striatum was measured following SPS or control handling. Two-way ANOVAs indicated no significant effect of stress on [^3H]SCH23390 binding in the nucleus accumbens (Figure 4G) [interaction: $F(6,83)=0.0728$, $p>0.05$; stress: $F(1,83)=2.405$, $p>0.05$; distance from bregma: $F(6,83)=5.894$, $p<0.0001$] or caudate putamen (Figure 4H) [interaction: $F(7,91)=0.4756$, $p>0.05$; stress: $F(1,91)=0.2545$, $p>0.05$; distance from bregma: $F(7,91)=7.809$, $p<0.0001$]. Binding of [^3H]SCH23390 to D1 receptors in the rostral nucleus accumbens and caudate putamen was similar between groups (data not shown). Figure 4I shows representative autoradiographs of [^3H]SCH23390 binding to D1 receptors.

DISCUSSION

The impairment of brain reward processes is hypothesized to contribute to symptoms of anhedonia and restricted positive affect in PTSD, and may also be related to the high comorbidity of PTSD with SUD. The present study investigated hedonic state and reward function in order to gain insight into the potential mechanisms involved in PTSD symptomology and the high susceptibility to cocaine abuse in the presence of PTSD [42-44]. The findings capture the appearance of an anhedonic state and indicate reduced dopaminergic reward function following SPS. The findings of the present study are summarized in Figure S3.

Prior behavioral and endocrinological investigations indicate that SPS is a relevant rodent model of PTSD [37, 45, 46]. The behavioral features induced by SPS include heightened fear [30, 31], disrupted extinction learning [31-34], cognitive dysfunction [35, 36], and exaggerated startle responses [28, 29], which may be relevant to clinical symptoms of intrusion, avoidance, and hyperarousal seen in persons with PTSD [20]. Characterizing the expression of reduced hedonic tone is relevant to the requirements for PTSD diagnosis [20], and also because of the high prevalence of anhedonia symptoms and their association with adverse outcomes in PTSD patients (see review [23]). This study is the first to utilize SPS to assess anhedonia-like behaviors and diminished positive affect. By recapitulating anhedonia and reward dysfunction, our findings extend the validity of the SPS procedure as a rodent model of PTSD. SPS-exposed rats in the present study exhibited lower sucrose intake and spontaneous nocturnal activity when compared to controls. Consumption of palatable solutions is used as a measure of hedonic state in rodents following stressors [47-50]. Reduced activity may be indicative of an anhedonic state, as physical activity is inversely related to anhedonia in humans [51, 52]. Published studies form the basis for the interpretation of our behavioral findings as indicative of an anhedonic state following SPS [47-53].

The examination of drug reinforcement in rodent models of PTSD may provide further insight into the mechanisms underlying hedonic deficits in PTSD, and we hypothesize that drug reward is impacted by traumatic stress. The psychostimulant properties of cocaine depend heavily on dopaminergic reinforcement mechanisms [54, 55]. Despite the high clinical co-occurrence of cocaine abuse with PTSD [42-44], cocaine reinforcement is understudied in rodent models of PTSD. The results of our study demonstrate a reduction in cocaine-conditioned reward following exposure to SPS versus control handling. This finding is consistent with the induction of anhedonia, and was further supported by an attenuation of cocaine intake in the extended drug access condition. Previous studies have also shown alterations in psychostimulant-induced hyperactivity and behavioral sensitization following SPS [56-59]. Overall, the effects of SPS on reward function are consistent with human studies which demonstrate blunted subjective reward and motivation to obtain rewards [11, 12]. Our results are also consistent with the clinical hypothesis that persons with PTSD may require more intense positive stimulation to experience pleasure and express appetitive behaviors [60]. Our findings of reduced psychostimulant reward may have implications for PTSD symptomology, as they further support the presence of reduced hedonic capacity in PTSD.

Our findings of reduced reward function following SPS are consistent with impairment of dopaminergic reinforcement mechanisms in PTSD. Results of the present studies show reduced tissue content of dopamine, DOPAC, and HVA in the nucleus accumbens and caudate putamen of SPS rats, thereby indicating a reduction in dopaminergic tone in the mesolimbic and nigrostriatal pathways. Extracellular dopamine levels were not examined herein, however it is possible that reduced tissue content is associated with deficient basal levels of extracellular dopamine and lower accumulation upon cocaine administration. This potential mechanism is supported by other studies of severe stressors. For example, chronic subordination stress reduces dopaminergic tone, attenuates cocaine-induced extracellular dopamine levels, and reduces saccharin preference, exploratory activity, and cocaine reinforcement in rats [49]. We also observed a decrease in dopamine D2 receptor density in the caudate putamen and nucleus accumbens of SPS rats compared to controls, as well as an increase in DAT in the nucleus accumbens. Increased levels of DAT are consistent with a recent study in non-substance abusing humans with PTSD [18]. The neurochemical findings herein are consistent with the hypothesis that dopaminergic pathology contributes to the attenuation of reward function and the PTSD symptom of anhedonia. Although not examined, our results may also have implications for avoidance symptoms in PTSD [20], as the mesolimbic dopamine system is implicated in the encoding of aversion and its successful avoidance [61]. Future studies are necessary to determine the neurobiological events leading to dysregulation of the dopamine system after SPS. Disruption of reward circuitry in the nucleus accumbens may be related to dysfunction of other brain regions in PTSD. For instance, increased amygdalar reactivity in response to risk occurs concurrently with reduced activity of the nucleus accumbens in response to reward [17]. Prior investigations utilizing the SPS model have shown alterations in stress-reactive systems within the hippocampus, amygdala, prefrontal cortex, and hypothalamus at time points similar to our neurochemical examinations [45, 46]. The effects of SPS on these brain structures and their role in PTSD pathology has been thoroughly reviewed (see [45, 46]). Future avenues for

investigation might include the contribution of glucocorticoid, corticotropin-releasing factor, norepinephrine, glutamate, or serotonin systems in the dysregulation of mesolimbic dopamine function in the SPS model [45, 46].

Although a goal of the present study was to examine susceptibility to SUD in PTSD, our behavioral findings are inconsistent with the clinical relationship between traumatic stress and cocaine abuse [42-44]. Stressed rats demonstrated similar levels of cocaine consumption and latencies to acquire self-administration when compared to controls under limited-access conditions, a finding that is consistent with a previous report [57]. Additional analysis of escalated cocaine intake indicates a trend toward a greater percent increase in drug consumption over time in SPS-exposed rats than controls. It is tempting to speculate that stressed rats compensated for a deficit in cocaine reward by escalating their drug consumption to a greater extent than controls. However, our data overall suggest a reduction in cocaine reward that does not directly support increased vulnerability to SUD. The utilization of progressive-ratio schedules would be useful in determining the effect of SPS on motivation to obtain cocaine, while binge and extended-access procedures would provide more information regarding the transition to drug dependence. Additional studies on drug reinforcement might utilize more thorough dose-response curves to fully elucidate whether the effects of SPS are dose-dependent. The use of reinstatement with stressors or trauma-related cues could provide valuable insight into other potential mechanisms triggering drug-seeking in the context of PTSD. Drug choice may differ across individuals with PTSD as human studies show that depressants such as alcohol, benzodiazepines, marijuana, and heroin ease hyperarousal and intrusion symptoms in patients with PTSD, while symptoms of emotional numbing and avoidance are highly prevalent in patients with comorbid cocaine dependence [1, 44]. It is important to note that the impact of PTSD on subjective drug reward has never been examined in humans. Interestingly, our results support similarities in dopamine neuropathology in PTSD and chronic drug exposure. Decreased striatal D2 receptor density is also observed in human cocaine addicts [62], as well as in rodents following escalating binge cocaine administration [63]. Increased striatal DAT is found in post-mortem brains of cocaine users, *in vivo* during withdrawal, and in primates following chronic cocaine exposure [64-68]. Dopaminergic dysfunction is hypothesized to contribute to drug-seeking and taking behavior [24-27], however whether the pathological similarities identified herein contribute to vulnerability to cocaine abuse in PTSD remains unclear.

Stressful life events are also a risk factor for major depressive disorder, and dopaminergic reward dysfunction is apparent in individuals with this disorder [69]. As PTSD is highly comorbid with depression and the two illnesses share anhedonia as a common symptom [20, 70], it is difficult to say with certainty that our findings are PTSD-specific. It should be noted however, that anhedonia symptoms are present in PTSD patients without depression [22], and studies show deficient reward function in PTSD even after correcting for comorbid depression [11, 12]. While behavioral despair has been reported in a study utilizing the SPS procedure [71], the literature maintaining SPS as a rodent PTSD model provides support that our findings are related to PTSD [46].

Results of the present study provide evidence for altered hedonic capacity and dopaminergic neurotransmission in a rodent model of PTSD. Pathology of the dopamine system and

reduced reward function may contribute to PTSD symptomology including anhedonia and reduced positive affect. Similarities in neuroadaptations reported for SPS and following chronic cocaine exposure in humans and rodent models provide evidence for shared pathology of PTSD and SUD. The results of our study support the hypothesis that SPS produces anhedonia and reduced cocaine-reward through dysfunction of the mesolimbic dopamine system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by NIH R01 DA018326 (EMU), P30 DA13429 (EMU) and T32 DA07237 (EMU/NE). The authors would like to thank Robert J. Kohler, Dr. Yong Zhang, and Mary McCafferty for their technical contributions.

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Figure 1. SPS increased anhedonia-like behavior and decreased cocaine-conditioned reward

(A) Exposure to SPS significantly reduced intake of sucrose solution (0.25, 0.5, or 1%) compared to controls (significant main effect of SPS exposure compared to control handling, $*p < 0.05$; $n = 7$ SPS and 15 controls for 0.25%, 15 SPS and 17 controls for 0.5%, 14 SPS and 21 controls for 1%). (B) Behavioral activity counts were significantly lower in SPS-exposed rats versus controls during the dark cycle (significant main effect of stress $***p < 0.001$, significant post-test $###p < 0.001$; $n = 16$ /group). (C) SPS rats conditioned with cocaine (5-20 mg/kg, i.p.) had lower preference scores than controls (significant main effect of SPS $**p < 0.01$). Significant differences were seen following conditioning with 10 mg/kg of cocaine (significant post-test $###p < 0.001$; $n = 7-11$ /group). Data were analyzed by two-way ANOVA and Bonferroni post-hoc analyses. Data points represent the mean $\pm$ SEM.

Figure 2. SPS altered daily cocaine intake upon extended-access, but not limited-access
(A) Rats exposed to SPS or control handling were allowed to self-administer cocaine under a FR1 schedule of reinforcement for 15 daily 2-hr sessions at 3 increasing doses (0.1875, 0.375, or 0.75 mg/kg). Cocaine intake progressively increased over the 15 allotted sessions. Daily cocaine consumption was similar in SPS and control handled rats ($n=13-16/\text{group}$).
(B) Rats exposed to SPS or control handling were allowed to self-administer cocaine (0.375 mg/kg/infusion) during daily 6-hour sessions for 14 consecutive days to facilitate the escalation of cocaine intake. SPS significantly attenuated daily cocaine intake compared to

control handling (significant main effect of stress exposure versus control handling $*p<0.05$; $n=5/\text{group}$). Data were analyzed by two-way repeated measured ANOVAs. Data points represent the mean $\pm$ SEM.

Figure 3. SPS reduced striatal tissue dopamine and metabolite levels

HPLC was used to assess SPS-induced alterations in dopamine levels and the metabolites DOPAC and HVA. (A-C) SPS significantly reduced tissue dopamine, DOPAC, and HVA content in the nucleus accumbens compared to control handling (significant main effect of stress versus control handling $*p<0.05$ and $**p<0.01$). (D-F) In the caudate putamen, a significant effect of SPS on dopamine levels was detected between groups ($**p<0.01$), and reduced dopamine levels were observed in SPS rats on day 16 compared to controls (significant post-hoc comparison $\#p<0.05$). SPS also significantly reduced tissue DOPAC

and HVA content in the caudate putamen compared to control handling (* $p < 0.05$ and ** $p < 0.01$). Data were analyzed by two-way ANOVA and Bonferroni post-hoc analysis. Data points represent the mean $\pm$ SEM (n=11-14/group).

Figure 4. Reduced D2 receptor and elevated DAT levels in the striatum following SPS
 Quantitative receptor autoradiography was used to measure dopamine receptor and dopamine transporter (DAT) densities in SPS and control handled rats. Radioligand binding is plotted as a function of distance from bregma corresponding to medial-caudal levels of the nucleus accumbens and caudate putamen. **(A-B)** Analysis of [³H]raclopride binding to D2 receptors indicated significantly lower D2 receptor density in the nucleus accumbens and caudate putamen of rats exposed to SPS compared to controls (significant main effect of stress compared to control handling ***p*<0.01 and ****p*<0.001; *n*=4-7/group). **(D-E)** Analysis of [³H]WIN35428 binding to DAT indicated significantly higher DAT density in the nucleus accumbens of SPS rats compared to controls, whereas no significant difference was detected between groups in the caudate putamen (significant main effect of SPS versus control handling ***p*<0.01; *n*=4-7/group). **(G-H)** Binding of [³H]SCH23390 to D1 receptors was similar in the nucleus accumbens and caudate putamen of rats exposed to SPS and control handling (*n*=5-8/group). Representative autoradiograms of [³H]raclopride, [³H]WIN35428, and [³H]SCH23390 binding in striatal tissue sections obtained from SPS

and control handled rats are shown in **C, F, I**, respectively. Data were analyzed by two-way ANOVA and data points represent the mean $\pm$ SEM.

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