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Methamphetamine-induced behavioral sensitization in a rodent model of posttraumatic stress disorder

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Abstract

Background—Single prolonged stress (SPS) is a rodent model of posttraumatic stress disorder (PTSD)-like characteristics. Given that PTSD is frequently comorbid with substance abuse and dependence, including methamphetamine (METH), the current study sought to investigate the effects of SPS on METH-induced behavioral sensitization.

Methods—In experiment 1, Sprague-Dawley rats were subject to SPS or control treatment and subsequently tested across four sessions of an escalating METH dosing paradigm. METH was injected (i.p.) in escalating doses (0, 0.032, 0.1, 0.32, 1.0, and 3.2 mg/kg; dissolved in saline) every 15 mins and ambulatory activity was recorded. In experiment 2, SPS and control treated rats were injected (i.p.) with either saline or METH (5 mg/kg) for five consecutive daily sessions and tested for stereotypy as well as ambulatory activity. Two days later, all animals were injected with a challenge dose of METH (2.5 mg/kg) and again tested for activity.

Results—No differences in the acute response to METH were observed between SPS and controls. SPS enhanced METH induced ambulatory activity across sessions, compared to controls. METH-induced stereotypy increased across sessions, indicative of behavioral sensitization; however, SPS attenuated, not enhanced, this effect suggesting that SPS may prevent the development of stereotypy sensitization.

Conclusions—Collectively, results show that SPS increases repeated METH-induced ambulatory activity while preventing the transition across sessions from ambulatory activity to stereotypy. These findings suggest that SPS alters drug-induced neuroplasticity associated with behavioral sensitization to METH, which may reflect an effect on the shared neurocircuitry underlying PTSD and substance dependence.

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Conflict of Interest The authors have no conflicts of interest to disclose.

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Keywords

single prolonged stress; methamphetamine; behavioral sensitization; stereotypy; ambulatory activity; posttraumatic stress disorder

1. INTRODUCTION

Posttraumatic stress disorder (PTSD) is a significant public and military health concern, and its treatment is often confounded by comorbid substance abuse (Chilcoat and Breslau, 1998; Kessler et al., 1995; Swendsen et al., 2010). Abuse of legal substances, such as alcohol and nicotine, are approximately 2–3 times higher in PTSD patients than the normal population (Kessler et al., 1995), and abuse of illegal substances, including the psychostimulants cocaine and methamphetamine, are equally prevalent. For example, methamphetamine (METH) was twice as likely to be used in individuals with PTSD compared to those without PTSD, and individuals with PTSD had longer histories of METH abuse (Smith et al., 2010).

One possible explanation for this observed comorbidity is that trauma associated with the development of PTSD produces a change resulting in sensitization to drugs of abuse, such as METH. Cross-sensitization between stress and psychostimulant effects has been well established in animal models of drug-induced plasticity, including behavioral sensitization (Belujon and Grace, 2011; Steketee and Kalivas, 2011). Behavioral sensitization is frequently observed as enhanced responsivity to a drug, usually a psychostimulant, following repeated administration. With regards to amphetamines, such as METH, sensitization is characterized by changes in ambulatory activity and stereotypy (Fowler et al., 2003; Kuczenski and Segal, 1999; Ridley, 1994). It is believed that behavioral sensitization reflects an increase in responsiveness to drug cues associated with salient incentives (Robinson and Berridge, 1993), shares attributes associated with drug-seeking behaviors (Steketee and Kalivas, 2011), and reflects neural sensitization (Robinson and Berridge, 2008). Research has historically shown that acute and chronic stress enhances the acquisition and expression of behavioral sensitization (Antelman et al., 1980; Robinson and Becker, 1986). However, the effects of a single episode of trauma-like stress that models PTSD on behavioral sensitization are unknown. Given the high incidence of PTSD and substance abuse comorbidity, we examined the impact of a single prolonged episode of severe stressors on behavioral sensitization to METH to determine if exposure to single prolonged stress augments the behavioral response to drugs of abuse.

The current study applied the single prolonged stress (SPS) paradigm, which was originally developed by Liberzon and colleagues (1997), to model traumatic stress-induced changes in behavioral sensitization to METH-induced activity. SPS is a well-validated animal model that produces phenotypes of PTSD including diagnostic behaviors, such as hyperarousal, avoidance, and heightened aversive memories, and enhances negative feedback of the hypothalamic-pituitary-adrenal axis (Pitman et al., 2012; Yamamoto et al., 2009). SPS also decreases glutamate, glutamine, and creatine levels in the medial prefrontal cortex (mPFC) as measured by proton magnetic resonance spectroscopy; an effect thought to reflect cortical hypoactivity (Knox et al., 2010). The mPFC strongly innervates the nucleus accumbens (NAc) and underlies the development and expression of behavioral sensitization (Stephans and Yamamoto, 1995; Tzschentke and Schmidt, 2003). In particular, after sensitization, there is decreased top-down control of mPFC to NAc activity, which may underlie the development and expression of sensitization, as well as drug-seeking behaviors such as self-administration (Kalivas, 2004). Therefore the hypoactivity in cortical glutamatergic tone following trauma-like stress may alter drug-induced behavioral sensitization. Combining SPS with behavioral sensitization provides a unique model to investigate trauma-induced

changes in responsiveness to METH. Since psychostimulant, *e.g.* METH, abuse is increased in individuals with PTSD, it was hypothesized that SPS-exposed animals would have increased behavioral sensitization to repeated METH administration. Separate experiments were conducted to investigate: 1) SPS effects on sensitization to METH-induced ambulatory activity across a range of METH doses using a within-session increasing dose-response paradigm and 2) SPS effects on acute and repeated high-dose METH on ambulatory and stereotypy sensitization. Doses for METH in the first study were selected based on preliminary evidence and previously published evidence (McGuire et al., 2011; Milesi-Hallé et al., 2005; Seiden et al., 1993) showing the range of the effects of METH on ambulatory activity. Doses for METH in the second study were selected to administer a bolus dose based on the combined dose from multiple administrations in the first study. They were also selected because ambulatory activity in the first study was observed to decrease at the highest dose (3.2 mg/kg), which showed the greatest differences between control and SPS animals. A within-session increasing dose-response paradigm was conducted in the first study to determine whether SPS produced any dose-dependent behavioral change across a range of METH, because this paradigm measures both sensitivity to the acute effects of METH, as well as sensitization (McGuire et al., 2011).

2. METHODS

Guidelines laid out in the Guide for the Care and Use of Laboratory Animals 7th edition (Institute of Laboratory Animal Resources (U.S.), 1996) were adhered to and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Wayne State University prior to being carried out. Wayne State University maintains campus-wide AAALAC-accredited facilities.

2.1. Animals

Male Sprague-Dawley rats (N=46; CD IGS 001; Charles River Laboratories, Portage, MI) weighing approximately 225–250 g upon arrival were allowed to acclimate in group housing (n=3–4 per cage) to the vivarium for 5–7 days before experimentation (during which time the animals were weighed and briefly handled daily). Rats were placed into single housing 4 days prior to experimental procedures in standard microisolator rat (home) polycarbonate cages (45 cm × 26 cm × 21 cm) with bedding. Animals were allowed food and water *ad libitum* in their home cages and housed on a 12 h light/dark cycle with lights on at 0700. Standard temperature (~24°C) and humidity (35–40%) were maintained in the vivarium and behavioral testing laboratory.

2.2. Single prolonged stress (SPS)

The SPS paradigm is a well validated model of PTSD (for reviews see Yamamoto et al., 2009; Pitman et al., 2012). While no psychiatric model is perfect, the SPS paradigm shows strong face validity. It involves an acute extreme/traumatic stress exposure followed by protracted behavioral, neuroendocrine and neurobiological effects 7 or more days later. The effects are not observed 1 day after the acute stressors and require the incubation/sensitization period (Kohda et al., 2007; Liberzon et al., 1999), which is consistent with development of acute stress versus PTSD following a traumatic event. The SPS model also shows strong construct validity. The behavioral effects caused by SPS mirror the construct of PTSD symptomology as defined by the DSM-IV-TR (American Psychiatric Association, 1994). For example, enhanced fear conditioning and startle behavior 7, but not 1, day after the acute stressors models the enhanced fear-response and recurring memories seen in humans with PTSD (Kohda et al., 2007); additionally, enhanced negative feedback of the HPA axis system is observed after SPS (Liberzon et al., 1997) and models the response to the dexamethasone-suppression test in PTSD patients (Yehuda, 2001). The SPS model also

shows strong predictive validity. The primary pharmacotherapeutics used in PTSD are the selective serotonin reuptake inhibitors, and these drugs block the expression of enhanced fear conditioning in the SPS model (Takahashi et al., 2006).

Approximately half of the animals (n=24 SPS, n=22 control) were exposed to the SPS paradigm as previously described (Eagle et al., 2013; Knox et al., 2010; Liberzon et al., 1997). Briefly, rats were restrained for 2 h followed by group forced swim (n = 8 per swim) for 20 min in 24°C water in a 56 cm diameter tub filled to 30 cm depth. Following a 15 min recuperation period after group swim, rats were exposed to ethyl ether anhydrous (EMD Millipore, Darmstadt, Germany) until unconscious. After regaining consciousness, approximately 5–15 min, animals were returned to the vivarium and housed undisturbed for 6–7 days. The other half of the animals (*i.e.* the control animals) were briefly handled and then transported back to the vivarium for undisturbed housing until testing began.

2.3. Experiment 1: Within-session increasing dose METH ambulatory sensitization testing

One week following SPS exposure, animals (n=7 control; n=8 SPS) underwent testing for ambulatory activity across a within-session increasing dose range of METH. In particular, an increasing paradigm allows for a broad analysis of sensitivity to METH across a range of doses, as previously demonstrated (Tkac et al., 2009), in addition to examining sensitization to repeated administration. Ambulatory activity was tested intermittently for one week for a total of 4 sessions (see Fig. 1A for study design). Animals were placed into polycarbonate cages (45 cm × 26 cm × 21 cm) similar to their home cages except devoid of bedding, and the cages were placed within the monitoring system. An automated monitoring system (Digiscan DMicro, Accuscan Instruments, Columbus, OH) consisting of 16 parallel infrared emitter/detector photocells was used to measure activity. Baseline ambulatory activity was recorded for 20 min prior to injections. After baseline recording, animals were injected intraperitoneally (i.p.), once every 15 min, with increasing whole doses of METH (0, 0.032, 0.1, 0.32, 1.0, and 3.2 mg/kg). Racemic (±) methamphetamine hydrochloride (METH; NIDA Drug Supply Program, Bethesda, MD) was dissolved in normal sterile saline (0.9% NaCl). During each 15 min period, the first 5 min were omitted to remove possible activity due to the injection as opposed to the drug. The last 10 min of activity prior to the next injection were collected and used for analysis (see Fig. 1B for injection design). Ambulatory activity was recorded as total photocell beam breaks during each 10 min period.

2.4. Experiment 2: High dose METH ambulatory and stereotypy sensitization testing

Six days following exposure to the stressors, animals underwent testing for METH-induced behavioral sensitization of ambulatory activity and stereotypy (see Fig. 2 for study design). To test for activity, animals were placed individually into testing boxes made of Plexiglas with a matte black floor and walls (72 cm × 30 cm × 34 cm; Formtech Plastics, Oak Park, MI). Animals were first allowed to acclimate on day 6 post SPS to the testing chamber and injection schedule. For acclimation animals were placed into the testing box for 20 min and activity was recorded. After 20 min, animals were removed, saline was injected (i.p.), and animals were immediately returned to the testing box for 30 min. Animals were then returned to their home cages. After acclimation on day 6, the animals received once daily injections of METH for 5 sessions (days 7–11), then the animals were left drug-free in their homecages on days 12–13, and challenged on day 14. On these test sessions, groups were divided as follows: controls receiving saline injections (CON+SAL, n=6), SPS animals receiving saline (SPS+SAL, n=6), controls receiving METH (CON+METH, n=9), and SPS animals receiving METH (SPS+METH, n=10). During testing, animals in both METH groups received 5 mg/kg METH. Behavioral activity within the testing box was then recorded for 30 min after injection. On the challenge session, animals were tested again for behavioral activity. Procedures were the same on the challenge session as the testing

sessions except all animals, including the groups previously receiving saline were injected with 2.5 mg/kg METH (i.p.).

To observe the effects of SPS on repeated METH-induced activity, both ambulatory activity and stereotypy were recorded in 5 min blocks across the entire 30 min after injection on sessions 1 (day 7), 5 (day 11), and the challenge session (day 14). For ambulatory activity, behavior was recorded with a digital CCD camera that was mounted above the test box and connected to a PC computer installed with an automated tracking software package (Ethovision 6.1, Noldus, Inc., Leesburg, VA). Distance moved (cm) was measured to assess ambulatory activity during the 30 min session immediately following the injection. For stereotypy, recorded digital videos were manually scored for general stereotypy by two observers, one of whom was blinded to the experimental conditions. General stereotypy was scored using procedures modified from Szumlinski et al. (2000). Scoring criteria are shown in Table 1. General stereotypy was assessed during the 30 min session immediately following the injection, and scores were recorded every 15 s. Scoring was rated by two individuals and inter-rater reliability for the stereotypy scores were $r=0.994$ for session 1, $r=0.991$ for session 5, and $r=0.916$ for the challenge session. Scores were then averaged between the two individuals. The average rater scores every 15 s were then averaged across 5 minute time blocks or the entire session.

2.5. Statistical Analysis

All statistical analyses were performed using Graphpad Prism 6 (San Diego, CA) with a 95% confidence interval ($\alpha = 0.05$). In Experiment 1, ambulatory activity (total beam breaks) was recorded during 10 min blocks across a range of increasing whole doses of METH. Ambulatory activity was tested across 4 sessions. Data for each session was first analyzed by applying a nonlinear regression fit of log (METH) vs. total counts with a standard slope (Hill slope = 1.0), and then comparison of maximum, minimum, and logEC₅₀ was conducted using Extra Sum of squares F test. Ambulatory activity produced by each METH dose was also compared across sessions. Data were analyzed by two-way ANOVA followed by Fisher's LSD for post hoc comparisons. ANOVAs were conducted for sensitivity at each session (group across doses) and an ANOVA for sensitization at each dose (group across sessions).

In Experiment 2, behavioral activity (both ambulatory activity and stereotypy) was measured across sessions 1, 5, and the challenge session. Data were analyzed using two-way, mixed-design repeated measures ANOVA for comparisons of the effects of group (CON+SAL, SPS+SAL, CON+METH, and SPS+METH) and time (5 min blocks) on behavioral activity for analysis among groups and within each group across sessions. In addition, two-way ANOVAs were conducted for the effects of METH and SPS on behavioral activity for the entirety of each 30 min session (session 1, 5, and challenge session). In both cases, Tukey Honestly Significant Differences (HSD) post hoc comparisons were conducted for significant ANOVAs to determine group differences at each 5 min block or at each session.

3. RESULTS

3.1. Experiment 1

3.1.1. METH-induced ambulatory activity is dose-dependently enhanced by SPS exposure—A two way mixed-design ANOVA for the effects of 0.32 mg/kg across sessions and between SPS groups revealed a significant interaction on ambulatory activity, $F(3,39)=3.74$, $p<0.05$ (Fig. 3B). Across sessions, injection of 0.32 mg/kg METH increased ambulatory activity in rats exposed to SPS but not in controls. Specifically, 0.32 mg/kg

METH increased activity in SPS rats at session 4 compared to controls, $p < 0.05$. At the 1.0 mg/kg METH dose, no significant differences were found in ambulatory activity; two way ANOVA showed a non-significant interaction of session and SPS, $F(3,39) = 0.24$, $p > 0.05$ (Fig. 3C). After 3.2 mg/kg METH, SPS-treated rats had significantly greater ambulatory activity across sessions compared to controls; two way ANOVA revealed a significant interaction of session and SPS, $F(3,39) = 3.15$, $p < 0.05$ (Fig. 3D). The differences between groups were significant at sessions 3 and 4, $p < 0.05$ for both comparisons. The 0.0 (Fig 3A), 0.032, and 0.1 mg/kg METH doses (data not shown) did not produce a significant effect of dose across sessions or group, $p > 0.05$.

Dose-response curves were sigmoidal and revealed that the parameters of the curve (maximum, minimum, and $\log EC_{50}$) did not differ between groups on session 1, $p > 0.05$ (Fig. 3E). However, at session 4 (Fig. 3F), analyses revealed that these parameters were significantly different between control and SPS groups, $p < 0.01$. Post hoc comparisons indicated that while minimum and $\log EC_{50}$ did not differ between control and SPS, $p > 0.05$, the maximum (top) of the curve which reflects the maximal effect of METH on ambulatory activity, was significantly greater for SPS compared to control, $p < 0.01$. Ambulatory activity at each testing session was also compared across the doses using ANOVA. During session 1, ANOVA analysis revealed a dose-dependent increase, $F(4,52) = 52.04$, $p < 0.001$ (Fig. 3E), however no differences were found between groups. During session 4, analysis revealed a significant effect of both dose, $F(4,52) = 38.25$, $p < 0.001$, and group, $F(1,13) = 5.67$, $p < 0.05$, and in particular, SPS had greater activity than controls at 0.32 mg/kg and 3.2 mg/kg METH (Fig. 3F).

3.2. Experiment 2

3.2.1. METH-induced ambulatory activity to single daily high dose administration is enhanced in SPS exposed rats—

Separate two-way mixed-design ANOVAs were conducted on the effects of group (CON+SAL, SPS+SAL, CON+METH, and SPS+METH) and time block (5 min intervals) on ambulatory activity. Analyses for each session revealed three main findings. First, in session 1 acute METH relative to saline-only controls (...+SAL), produced a locomotor stimulating effect across blocks of ambulatory activity, with the two way ANOVA revealing a significant interaction of block and group, $F(3,27) = 4.823$, $p < 0.01$ (Fig. 4A). Specifically, CON+METH had increased ambulatory activity compared to CON+SAL at the 10 min block, $p < 0.05$, while SPS+METH had increased ambulatory activity at the 10, 20, and 25 min blocks, relative to CON+SAL, $p < 0.05$ for all comparisons at session 1. No differences were observed, however, between CON+METH and SPS+METH, $p > 0.05$. Second, by session 5 ambulatory activity was significantly enhanced in SPS+METH across blocks compared to CON+METH, with the two way ANOVA revealing a significant interaction of block and group $F(3,26) = 3.848$, $p < 0.05$, however ambulatory activity was not increased in CON+METH compared with CON+SAL (Fig. 4B). SPS+METH had greater ambulatory activity at the 25 and 30 min blocks, compared to SPS+SAL, and at the 20 min block compared to CON+METH, $p < 0.05$ for all comparisons at session 5. Third, during the challenge session no group differences were found across blocks, regardless of METH or SPS group, $F(3,27) = 1.52$, $p > 0.05$ (Fig. 4C). No differences in ambulatory activity were observed between CON+SAL and CON+METH across 5 min blocks, $p > 0.05$, and no SPS-induced differences were observed at the challenge session.

Ambulatory activity during the entire session (sum of all 5 min blocks) was also examined. Separate two-way ANOVAs were conducted on the effects of SPS (CON vs SPS) and METH (SAL vs. METH) for each entire summed session of ambulatory activity. Analyses revealed that METH on session 1 produced a locomotor stimulating effect on ambulatory

activity, $F(1,27)=13.5$, $p<0.001$ (Fig. 4D, Session 1), with METH increasing activity in both the CON+METH compared with CON+SAL, $p<0.05$, and SPS+METH compared with SPS+SAL, $p<0.05$. No SPS-induced differences were observed, though, $F(1,27)=0.815$, $p>0.05$. However on session 5, SPS+METH had greater ambulatory activity over the entire session compared to CON+METH, $F(1,27)=4.529$, $p<0.05$ for METH and $F(1,27)=4.707$, $p<0.05$ for SPS. No differences were observed between CON+METH and CON+SAL (Fig. 4D, Session 5). Post hoc analysis revealed that SPS+METH had greater ambulatory activity than CON+SAL, SPS+SAL, and CON+METH ($p<0.05$ for all comparisons). Although SPS+METH enhanced ambulatory activity in comparison to CON+METH, no differences were observed within SPS+METH between session 1 and session 5, $p>0.05$, indicating a lack of METH-induced ambulatory sensitization (Fig. 4D, Session 1 and Session 5). In addition, no differences were observed within CON+METH from session 1 to session 5. On the challenge day when all animals received a lower dose (2.5 mg/kg) of METH, ambulatory activity increased compared to session 1 within CON+SAL (now receiving METH), with no effect of SPS, $F(1,27)=60.91$, $p<0.001$ (Fig. 4D, Challenge). No differences were found among any group at challenge regardless of prior drug treatment or trauma exposure, $F(1,27)=2.34$, $p>0.05$ for drug and $F(1,27)=2.073$, $p>0.05$ for stress (Fig. 4D, Challenge). Furthermore, no differences in ambulatory activity over the entire challenge session were observed between CON+SAL and CON+METH, $p>0.05$.

3.2.2. SPS attenuates sensitization to METH-induced stereotypy—Separate two-way mixed-design ANOVAs were conducted for each session across 5 min blocks (block) and between each group (group) on stereotypy. Acute METH (session 1) produced an increase in general stereotypy across 5 min blocks in CON+METH compared to CON+SAL, with no significant effect of SPS, with the ANOVA revealing a significant interaction, $F(3,27)=97.84$, $p<0.001$ (Fig. 5A). Acute METH did not increase stereotypy in the first 5 min after injection, $p>0.05$, however, stereotypy was significantly increased at all subsequent blocks, $p<0.001$ for all comparisons. In session 5, METH significantly increased stereotypy in both CON+METH relative to CON+SAL and SPS+METH relative to SPS+SAL, with the ANOVA revealing a significant interaction, $F(3,26)=117.2$, $p<0.05$ (Fig. 5B). These differences were observed across all time blocks except for the 5 min block, $p<0.01$. However, METH-induced stereotypy was significantly attenuated in SPS+METH compared to CON+METH at the 10, 15, 20, and 25 min blocks, $p<0.05$ for all comparisons. On the challenge day when all animals were administered a lower dose (2.5 mg/kg) of METH, both CON+SAL and SPS+SAL which had previously received saline engaged in mild stereotypy behavior, $F(6,54)=19.5$, $p<0.001$ for the interaction, $p<0.001$ for multiple comparisons to session 1 (Fig. 5D). However, the low stereotypy rating scores of animals previously receiving saline, approximately 1.56 ± 0.12 for CON+SAL and 1.59 ± 0.14 for SPS+SAL (Fig. 5D), are more indicative of ambulatory activity, since scores of 1 and 2 in general stereotypy indicate normal and hyperlocomotion, respectively (see Table 1). Furthermore, CON+METH had enhanced stereotypy across blocks following the 2.5 mg/kg METH injection compared to CON+SAL, $F(3, 27)=16.17$, $p<0.001$ for group, $F(15,135)=1.99$, $p<0.05$ for the interaction (Fig. 5C). This enhancement was observed at the 10, 15, 20, 25, and 30 min blocks, $p<0.001$ for all comparisons. SPS+METH also produced significantly greater stereotypy than SPS+SAL at all time blocks except the 5 min block, $p<0.001$ for all comparisons. Interestingly, SPS+METH had significantly attenuated METH-induced stereotypy compared to CON+METH at blocks 10, 15, 20, 25, and 30, $p<0.05$ for all comparisons.

Across the entire session 1 (i.e. the sum stereotypy of the entire session), acute METH produced general stereotypy, $F(1,27)=289.6$, $p<0.001$ (Fig. 5D, Session 1). Specifically, both CON+METH compared with CON+SAL and SPS+METH compared with SPS+SAL produced robust stereotypy, $p<0.001$ for both comparisons. As observed with ambulatory

activity at session 1, there were no SPS-induced differences in the acute response to METH, $F(1,27)=1.279$, $p>0.05$. In session 5, ANOVA revealed that METH significantly increased stereotypy in both CON+METH and SPS+METH, compared to CON+SAL with a significant effect of METH, $F(1, 27)=344.1$, $p<0.001$, and the interaction of SPS and METH, $F(1, 27)=4.71$, $p<0.05$. In addition, CON+METH had significantly greater stereotypy at session 5 compared to session 1, $p<0.05$, indicating METH-induced sensitization to stereotypy. However, the sensitization to METH-induced stereotypy was significantly attenuated in SPS+METH compared to CON+METH, $p<0.05$. On the challenge day, METH-induced stereotypy was enhanced in SPS+METH compared to SPS+SAL, $F(1, 27)=37.45$, $p<0.001$ for METH, $F(1,27)=5.25$, $p<0.05$ for the interaction of SPS and METH. As in session 5, SPS+METH displayed attenuated METH-induced stereotypy during the challenge session, $p<0.01$ for multiple comparisons.

4. DISCUSSION

The current study found that SPS augmented repeated METH-induced *ambulatory* activity while attenuating repeated METH-induced *stereotypy*. Interestingly, SPS significantly impacted repeated, but not acute, METH-induced ambulatory activity or stereotypy. Collectively, these findings suggest that the severe stress exposure of SPS does not produce cross-sensitization to an acute METH response but does produce a complex cross-sensitization to repeated METH administration. This is in line with previous findings of cross-sensitization between stress and psychostimulants (Belujon and Grace, 2011; Steketee and Kalivas, 2011). Reports have specifically indicated that both acute and chronic stress can enhance the acquisition and/or expression of behavioral sensitization (Antelman et al., 1980; Robinson and Becker, 1986). The findings in the current study, however, report that a single prolonged episode of trauma-like stress produces changes in sensitization that may be more complex than simple enhancement of sensitization.

The current findings also demonstrate the lack of a simple pharmacological mechanism and support a more complex interaction with processes and circuitry involved that are germane to behavioral sensitization and severe (traumatic-like) stress. Interestingly, it was found that control animals did not sensitize to repeated METH-induced ambulatory activity. The lack of ambulatory sensitization in control animals (CON-SAL relative to CON+Meth in Fig. 4B) could be considered a result of tolerance; however, this is unlikely given that sensitization occurs during development of drug taking, while tolerance occurs in response to high doses taken chronically (Schenk and Partridge, 1997). However, as demonstrated in experiment 2, repeated METH injections in control animals resulted in an increase in stereotypy; in fact, controls increased stereotypy from day 1 to day 5, which persisted after a 2-day drug free period. The dramatic increase in stereotypic behavior may account for the loss of METH-induced ambulatory activity in controls over consecutive days. A dose-dependent shift from ambulatory to stereotypy during repeated administration of amphetamine-like psychostimulants has been documented (Fog, 1969; Fowler et al., 2003; Kuczenski and Segal, 1999) and it has been hypothesized that a greater response to stereotypy acutely predicts sensitization (Battisti et al., 1999). Behavioral sensitization and the shift to stereotypy are driven by changes in *neural* sensitization (Robinson and Berridge, 2008), namely increased striatal dopamine release (Creese and Iversen, 1974; Ridley, 1994; Seiden et al., 1993), sensitized dopamine receptor mechanisms (Kuczenski and Segal, 1999), and increases in both prefrontal glutamate efflux (Stephans and Yamamoto, 1995) and prefrontal glutamate release into the NAc (Tzschentke and Schmidt, 2003), along with possible mediation from other regions, such as the hippocampus (Steketee and Kalivas, 2011). Any of these potential mechanisms may have produced the enhancement of stereotypy across repeated METH sessions. Regardless of the mechanism, however, METH-induced focused stereotypies interfere with ambulation (Seiden et al., 1993), which likely

contributed to the decrease or lack of change in ambulation in control animals across testing observed in the present study.

In this study ambulatory activity (i.e., behavioral sensitization) was increased in SPS compared to non-SPS controls; therefore, it is tempting to speculate that the enhanced ambulatory activity reflects enhanced *neural* sensitization. However, this most parsimonious interpretation does not explain all of the study observations. First, the increase in ambulatory activity was greater compared to controls, but did not increase in comparison to the first session of METH. While this indicates some possible neuroplasticity to account for the SPS-induced behavioral difference to controls, the lack of increase in ambulatory activity across sessions may indicate a lack of METH-induced ambulatory sensitization in both SPS and control animals. Second, METH produced robust stereotypy in all treated animals, which increased across sessions; however, those animals exposed to SPS-induced trauma, while also showing an increase in stereotypy across sessions, exhibited significantly less stereotypic behavior in comparison to controls. When examining ambulatory activity, this effect was reversed, with SPS showing significantly more ambulation than controls. While many studies tend to focus on only ambulatory activity, neural sensitization underlies many different aspects of psychomotor sensitization, including both ambulatory activity and stereotypy (Robinson and Becker, 1986; Saal et al., 2003). As previously noted, complex stereotypic behavior interferes with ambulation. Therefore, the decreased stereotypy observed in the SPS animals supports the increase in METH-induced ambulatory activity after SPS. Cross-sensitization between the trauma-like SPS exposure and the psychomotor effects of METH is complex, but it appears that SPS interferes with the development of *neural* sensitization, manifesting as a lack of shift from ambulatory activity to stereotypy during *behavioral* sensitization. While these data do not provide enough evidence to infer a neurobiological mechanism, the decrease in (stereotypy) behavioral sensitization suggests that SPS may attenuate, not augment, accumbal dopamine neurotransmission. Additionally, since METH also targets the serotonin and norepinephrine transporters, the contribution of these neurotransmitter systems to this behavioral observation cannot currently be ruled in or out (Han and Gu, 2006).

It appears that trauma-like SPS exposure modifies drug-induced neuroplasticity leading to greater behavioral activity (i.e., behavioral sensitization) to the ambulatory effects of METH. Indeed, numerous studies have found that other types of stressors, both acute and chronic, can enhance psychostimulant sensitization (Kikusui et al., 2005; Nikulina et al., 1998; Prasad et al., 1995; Sapolsky, 1996). Given that behavioral sensitization is hypothesized to reflect neuroplasticity associated with later drug seeking (Robinson and Berridge, 1993, 2008), it likely reflects the development of drug-seeking behaviors. Therefore, it is tempting to speculate that SPS may increase drug seeking, which is in line with the clinical finding that trauma exposed PTSD patients have increased METH use (Smith et al., 2010). However, it is more likely, as previously mentioned, that SPS attenuated the shift from ambulation to stereotypy. This would not support a hypothesis that SPS would enhance drug-seeking behavior. Therefore, further studies are necessary to support speculation of increased drug-seeking after SPS. Regardless of the direction of behavioral sensitization, the finding that SPS decreases the shift from ambulatory activity to stereotypy suggests neuroplastic changes in the circuits involved in behavioral sensitization, and furthermore, the effect of stress on these regions.

There are a number of potential neurocircuits that may have mediated the SPS-induced attenuation of stereotypy (which, in turn, increased ambulatory activity). For example, the hippocampus regulates accumbal dopaminergic activity and contributes to behavioral sensitization (Steketee and Kalivas, 2011). This is particularly relevant here because SPS has previously been implicated to alter hippocampal function evidenced by enhanced

glucocorticoid receptor expression (Liberzon et al., 1999), impaired hippocampal long-term potentiation (Kohda et al., 2007), and increased apoptotic markers (Li et al., 2010). SPS also impairs hippocampal-dependent behaviors, such as spatial learning (Kohda et al., 2007; Peng et al., 2010). Finally, SPS increases the concentration of dopamine, serotonin, and norepinephrine within the hippocampus (Harvey et al., 2006). These neurochemicals are also known to be affected by METH in other regions (Berger et al., 1992; Löwenberg et al., 2008). However, it remains to be determined whether SPS-induced alterations in hippocampal function affect hippocampal-dependent control of sensitization associated pathways.

Another neurocircuitry potentially playing a key role in the effects that SPS exposure has on behavioral sensitization to repeated METH is the top-down control from the mPFC to the NAc regulated by glutamate. Glutamate neurotransmission to the NAc is known to be altered in cocaine addiction (Kalivas, 2004). Prefrontal glutamatergic control over NAc sensitization circuitry (Tzschenke and Schmidt, 2003) and PFC glutamate efflux is also known to be disrupted after repeated METH administration (Stephans and Yamamoto, 1995). Therefore, it is reasonable to speculate that alterations in the mPFC-NAc circuit may be underlying the difference observed in SPS animals exposed to repeated METH.

Our own studies have found that mPFC glutamate homeostasis is altered after SPS (Knox et al., 2010), suggesting that SPS may alter mPFC modulation of NAc neurotransmission. Topiramate, an AMPA receptor antagonist, attenuates an SPS-induced exaggerated startle response (Khan and Liberzon, 2004). SPS also increases hippocampal NMDA receptor mRNA levels and impairs fear extinction learning, both of which are blocked by D-cycloserine, a partial agonist of the NMDA receptor (Yamamoto et al., 2008). These findings collectively suggest that SPS alters glutamatergic function in many circuits involved in neural sensitization. PTSD may similarly produce alterations in drug-induced neuroplasticity through modulation of mPFC and/or hippocampal pathways that regulate NAc activity and drug responsiveness.

In conclusion, the finding that SPS decreased METH-induced stereotypy likely reflects decreased sensitization, since the response to stereotypy in normal animals predicts sensitization (Battisti et al., 1999) and is associated with greater accumbal dopamine release (Seiden et al., 1993). Furthermore, the attenuation of stereotypy by SPS likely contributed to the increased ambulatory activity compared to controls. This may be indicative of less responsiveness to *neural* sensitization produced by psychostimulants such as METH. Further understanding of the mechanisms elucidated in these models may provide insights into the SPS-induced effects observed in the current study, and ultimately comorbid PTSD and substance abuse.

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Figure 1.

Experiment 1 Study Paradigm, including (A) Testing Design and (B) Within-Session Increasing Dose Paradigm. A) Rats were subjected to single prolonged stress (SPS) and tested 1 week later intermittently in four separate methamphetamine (METH)-induced activity sessions. B) During each METH testing session, after a 20 min baseline, rats were administered increasing doses of METH every 15 min. Ambulatory activity was measured from the 10 min preceding the administration of each subsequent injection.

Figure 2.

Experiment 2 Study Paradigm. Rats were subjected to single prolonged stress (SPS) and acclimated to a behavioral chamber 6 days later. After acclimation, five consecutive daily testing sessions were conducted for methamphetamine (METH)-induced activity. The testing was followed by a 2-day drug free period and then rats were challenged for METH-induced activity at a lower dose.

Figure 3.

Single prolonged stress (SPS) increases repeated methamphetamine (METH)-induced ambulatory activity in an increasing dose-response paradigm. Rats received increasing log dose increases in METH (0.0, 0.032, 0.1, 0.32, 1.0, & 3.2 mg/kg) every 15 min within each 110 min session. Both control (n=7; closed circles/closed lines) and SPS (n=8; open circles/broken lines) groups received every dose of METH. Mean ($\pm$ SEM) total beam break counts in a home cage-like box was measured across 4 intermittent sessions after i.p. injection of METH at (A) 0.0 mg/kg (saline), (B) 0.32 mg/kg, (C) 1.0 mg/kg, or (D) 3.2 mg/kg. Mean ($\pm$ SEM) total beam break counts were also measured across all doses within (E) session 1

and (F) session 4, and a nonlinear sigmoidal fit of the data are shown. $*p<0.05$ for Tukey's HSD comparisons.

Figure 4.

Single prolonged stress (SPS) enhances repeated methamphetamine (METH)-induced ambulatory activity. Mean ($\pm$ SEM) distance moved (cm) in a novel box was measured for either 5 min blocks across the 30 min session (A–C) or during the entire 30 min session (D) after a METH (5 mg/kg) or saline (SAL) injection (i.p.) on the (A) 1st daily testing session and (B) the 5th session. (C) After a 2 day drug-free period, *all* animals were tested in a challenge session for distance moved after METH injection (2.5 mg/kg). Groups were as follows: controls receiving SAL during testing and acute METH at challenge (n=6; CON+SAL), SPS animals receiving SAL during testing and acute METH at challenge (n=6; SPS+SAL), controls receiving repeated METH (n=9; CON+METH), and SPS animals receiving repeated METH (n=10; SPS+METH). Panels A–C: * p <0.05 SPS+METH compared to CON+METH, $^{\dagger}p$ <0.05 CON+METH compared to CON+SAL, $^{\ddagger}p$ <0.05 SPS+METH compared to SPS+SAL and CON+METH. Panel D: * p <0.05, ** p <0.01, and *** p <0.001 for Tukey's HSD comparisons.

Figure 5.

Single prolonged stress (SPS) decreases methamphetamine (METH)-induced stereotypy. Mean ($\pm$ SEM) stereotypy was measured for either 5 min blocks across the 30 min session (A–C) or during entire 30 min session (D) after a METH (5 mg/kg) or saline (SAL) injection (i.p.) on the (A) 1st daily testing session and (B) the 5th session. (C) After a 2 day drug-free period, *all* animals were tested in a challenge session after a lower dose METH injection (2.5 mg/kg; i.p.). Stereotypy scoring (see Table 1) was rated by 2 different individuals. Groups were as follows: controls receiving SAL during testing and acute METH at challenge ($n=6$; CON+SAL), SPS animals receiving SAL during testing and acute METH at challenge ($n=6$; SPS+SAL), controls receiving repeated METH ($n=9$; CON+METH), and SPS animals receiving repeated METH ($n=10$; SPS+METH). Panels A–C: $*p<0.05$ SPS+METH compared to CON+METH, CON+SAL, and SPS+SAL, $^{\dagger}p<0.05$ CON+METH compared to CON+SAL, Panel D: $*p<0.05$, $**p<0.01$, and $***p<0.001$ for Tukey's HSD comparisons.

Table 1**General Stereotypy Rating Scale**

Score	Description
0	Asleep, still, (no locomotion) with normal grooming
1	Normal locomotion and exploration with normal pattern of sniffing and grooming
2	Hyper-locomotion with repetitive exploratory behaviors, rearing, or increased rate of sniffing and grooming
3	Discontinuous sniffing and grooming with periodic locomotion and/or rearing
4	Continuous compulsive sniffing, rearing, and/or grooming without locomotion

Stereotypy scores were made every 15 s during the entire 30 min session immediately following drug administration. Scoring procedure for stereotypy modified from Szumlinski et al., (Szumlinski et al., 2000). Note that scores of 3–4 represent stereotypy whereas scores of 1 and 2 represent normal ambulatory activity and hyperlocomotor ambulatory activity, respectively.