Published in final edited form as:

Neuropharmacology. 2013 April ; 67C: 95–103. doi:10.1016/j.neuropharm.2012.09.021.

Perseverative behavior in rats with methamphetamine-induced neurotoxicity

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Abstract

Methamphetamine induces monoamine depletions thought to contribute to cognitive and behavioral dysfunctions. Previously, we reported that methamphetamine-induced neurotoxicity is associated with impaired formation of stimulus-response associations. Additionally, subjective observations suggested that behavioral flexibility might be affected. Thus, the present study examined whether methamphetamine neurotoxicity induces perseverative behavior. Rats were pretreated with (±)-methamphetamine (4×10 mg/kg, 2-hr intervals) or saline. Three weeks later, rats were trained to press a lever on one side of an operant chamber and then retrieve the reinforcer from a magazine on the opposite side until they reached criterion (>50 reinforcers/30-min). After four consecutive sessions performing the task at criterion, rats were sacrificed and brains removed for monoamine determinations. Methamphetamine-pretreated rats had ~50% loss of striatal dopamine and prefrontal serotonin. Methamphetamine- and saline-pretreated rats were not different in the number of sessions required to reach criterion or in the total numbers of lever presses and or head entries made across the four consecutive sessions at criterion-level performance. However, methamphetamine-pretreated rats earned fewer reinforcers, because they made extra lever-presses and head entries when they should have been retrieving the reinforcer or returning to the lever. Latencies for methamphetamine-pretreated rats to switch between the two behaviors also were significantly slower than latencies for controls. Interestingly, the degree of additional lever-presses negatively correlated with serotonin-transporter binding in the prefrontal cortex, even in saline-pretreated controls. These data suggest that methamphetamine-induced partial monoamine toxicity is associated with perseveration and that the degree of perseveration may depend on serotonin innervation of the frontal cortex.

Keywords

Methamphetamine neurotoxicity; Response inhibition; Perseverative behavior; Serotonin (5-HT); Dopamine (DA); Instrumental conditioning

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Conflict of Interest: All authors declare that they have no disclosures or conflict of interest to report.

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1. Introduction

Methamphetamine (METH), a highly addictive psychostimulant drug, is a major public health concern. The long-term consequences of METH abuse include a partial, but persistent, loss of dopamine (DA) and serotonin (5-HT) systems in multiple brain areas, such as striatum, cortex, and hippocampus (Kish et al., 2009; McCann et al., 1998; Morgan and Gibb, 1980; Ricaurte et al., 1980; Seiden et al., 1976; Volkow et al., 2001a; Volkow et al., 2001b). Under such conditions, both humans and rodents exhibit a range of behavioral and cognitive deficits. In chronic METH abusers, the behavioral and cognitive deficits range from learning and memory (Chang et al., 2002; Volkow et al., 2001b) and attentional problems, to deficits in executive function, including inhibitory control and decision-making (Kalechstein et al., 2003; Nordahl et al., 2003; Ornstein et al., 2000; Rogers et al., 1999; Salo et al., 2002; Simon et al., 2000). In studies of laboratory animals, deficits associated with METH-induced neurotoxicity also include impairments in learning and memory (Belcher et al., 2005; Bisagno et al., 2002; Chapman et al., 2001; Daberkow et al., 2005; O'Dell et al., 2012), and behavioral and cognitive flexibility (Izquierdo et al., 2010; Kosheleff et al., 2011; Pastuzyn et al., 2012; Son et al., 2011).

In general, “perseveration” is defined as the continuation or recurrence of experiences, thoughts, or activities without an appropriate stimulus, and is frequently observed in individuals who have experienced neurological events such as strokes, traumatic brain injury, and progressive neurological disorders such as Parkinson’s disease and forms of dementia (Allison, 1966; Helm-Estabrooks, 2004; Sandson and Albert, 1984). Furthermore, it has been proposed that perseveration is not a unitary phenomenon, but rather manifests itself in multiple forms: 1) “recurrent” perseveration – the inappropriate repetition of already complete responses; 2) “stuck-in-set” perseveration – inappropriate maintenance of a category or framework of activity; and 3) “continuous” perseveration – abnormal prolongation or continuation without cessation of a current behavior (Annoni et al., 1998; Sandson and Albert, 1984).

Noticeably, chronic METH-dependent abusers, relative to drug-free subjects, show evidence of such patterns of perseveration on various neuropsychological tests. For example, chronic METH abusers exhibited increased perseverative errors in the Wisconsin card-sorting test (WCST) and the color-word Stroop task (Chung et al., 2007; Han et al., 2008; Kim et al., 2005; Kim et al., 2011; Kim et al., 2009; Nestor et al., 2012). Furthermore, a recent report found that METH-dependent participants in a novel open-field paradigm have elevated perseverative interactions with both novel and already engaged objects relative to control subjects (Henry et al., 2011). Together, these studies suggest that exposure to METH may be associated with perseveration, and that such abnormal behavioral control may contribute to impairments in everyday functions, including communication/planning skills, finance, and medication management observed in chronic METH abusers (Henry et al., 2010; Patterson et al., 1996).

The basis for the perseveration observed in individuals with a history of METH exposure is not yet clear; however, it has been suggested that corticostriatal circuitry is highly associated with perseveration (Graybiel, 1998, 2000; Haber and Calzavara, 2009). Previously, we have reported that METH-induced neurotoxicity is associated with altered learning and memory processes subserved by corticostriatal circuitry (Pastuzyn et al., 2012; Son et al., 2011). Additionally, anecdotal observations from the previous study suggested that there are alterations in behavioral control; that is, extra lever-presses being made prior to collecting the reinforcer, in METH-pretreated rats engaged in an appetitive instrumental task. Interestingly, Evenden and Robbins (1983) reported a similar phenomenon – rats trained to lever-press for food under three different instrumental conditions continued to press even

after food had been delivered when high doses of amphetamine were given (Evenden and Robbins, 1983). Therefore, the purpose of the present study was to assess whether METH-induced neurotoxicity is associated with perseveration on an appetitive instrumental learning task.

2. Materials and Methods

2.1. Animals and drug treatment

Male Sprague-Dawley rats (300–325 g; Charles River, Wilmington, MA) were housed in hanging wire cages in a room controlled for temperature and lighting (12:12 h) and allowed free access to food and water. All animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee of the University of Utah and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th Ed., National Research Council).

(±)-Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD). Doses were calculated as the free base. The night before drug treatment, rats were weighed and re-housed in plastic tub cages (33×28×17 cm, 6 rats/tub). The next day, rats received 4 injections at 2-hr intervals of either 0.9% saline or METH (10 mg/kg, s.c.). Body temperatures were monitored via a rectal probe every hour during the injections. The rats were removed and put in a cage over ice to decrease hyperthermia when core temperature exceeded 40°C. Twelve hours after the last injection, rats were returned to their home cages and given free access to food and water. Two weeks later, all rats were re-housed singly and food restricted for 1 week (10–15g chow/day) to achieve approximately 80% of free-feeding weight.

2.2. Instrumental training

Behavioral training and testing took place in standard operant chambers, housed within sound- and light-attenuating boxes (Coulbourn Instruments, Whitehall, PA). Each operant chamber had one lever on the wall opposite to that containing a recessed food magazine equipped with a photocell sensor. A 3-W, 24-V house-light, mounted on the top center of the wall opposite the magazine, provided illumination. The lever remained inside the chamber throughout the session. When the rat made a lever press, a pellet dispenser delivered a 45-mg sucrose pellet (fixed ratio 1 schedule (FR1); Research Diets Inc, New Brunswick, NJ) into the magazine. Importantly, additional “extra” lever-presses made prior to collecting the reinforcer and “extra” head-entries (photobeam breaks) before returning to the lever had no scheduled consequences and were considered perseverative responses. Thus, sequential performance of lever press, pellet retrieval, lever press, pellet retrieval, *etc.* was required to obtain the maximum number of reinforcers in a session. A computer equipped with Graphic State 3.0 software (Coulbourn Instruments) controlled the equipment and recorded lever presses.

Rats (saline-pretreated, n=17; METH-pretreated, n=16) were first trained with two, 30-min magazine training sessions per day for two days during which reinforcers (sucrose pellets) were delivered on a random-time, 60-sec schedule with no lever present. The next day, lever-press training began on the FR1 schedule. All rats were given a 30-min session twice per day, with the sessions separated by at least a 2-hr interval. Each training session began with illumination of the house light and insertion of the lever into the chamber and ended with retraction of the lever and turning off the light. Once training began, rats were fed each day after the training sessions (20–30g) and given free access to water. Rats were considered to have learned the instrumental response when they reached the criterion of >50 reinforcers in a 30-min session. Once rats reached that criterion, they completed three more 30-min sessions over the next 1–2 days. All rats performed at or above the criterion over the next

three consecutive sessions. Dependent measures of behavior at criterion levels of responding reported herein were averaged across these four consecutive testing sessions for each animal and then statistically compared across the treatment groups. At the end of the fourth session of criterion-level responding, rats were sacrificed, and the brains were removed for monoamine determinations.

2.3. Tissue preparation

At the end of behavioral training, rats were sacrificed by exposure to CO₂ for 1 min. Brains were removed rapidly and frozen in 2-methylbutane (Mallinckrodt Baker, Phillipsburg, NJ) chilled on dry ice. Brains were stored at -80°C until they were sectioned at 12-μm on a cryostat (Cryocut 1800, Cambridge Instruments, Bayreuth, Germany). Frozen sections of prefrontal cortex (Bregma: +3.70 ~ +1.60) and striatum (Bregma: +1.60 ~ -1.40 mm) (Paxinos and Watson, 1998) were collected onto slides and stored at -20°C.

2.4. Dopamine transporter (DAT) and serotonin transporter (SERT) autoradiography

DAT levels in striatum and SERT levels in the prefrontal cortex were determined by [¹²⁵I] RTI-55 binding (PerkinElmer, Waltham, MA), as reported previously (Boja et al., 1992; O'Dell et al., 2012; Pastuzyn et al., 2012). DAT binding in striatum was determined for all animals undergoing behavioral testing; however, SERT binding in prefrontal cortex was determined only in a subset of the animals, as we did not collect sections of frontal cortex from the initial cohort of animals run on the instrumental training task. As [¹²⁵I] RTI-55 binds with high affinity to both DAT and SERT, 100 nM fluoxetine was included in the pre-incubation and incubation media to block radioligand binding to SERT for determination of striatal DAT levels (Boja et al., 1992). Prefrontal cortex slides incubated in assay buffer containing 100 nM fluoxetine showed no binding (data not shown). Therefore, fluoxetine was omitted from the assay for determination of SERT levels in prefrontal cortex. The slide-mounted, fresh-frozen brain sections were pre-incubated in assay buffer (10 mM NaPO₄, 120 mM NaCl, 100 mM sucrose) for 5 min to remove endogenous ligands that could interfere with subsequent radioligand binding. Sections were then incubated in assay buffer containing 25 pM [¹²⁵I] RTI-55 for 2-hr at room temperature. The sections were then rinsed twice for 2-min each in cold (~4°C) assay buffer, then once in cold (~4°C) distilled water. The rinsed slides were then rapidly dried under a stream of low-heated air. The dried slides were apposed to BioMax film (Eastman Kodak, Rochester, NY) for 24 hr and developed.

2.5. Tyrosine hydroxylase (TH) immunohistochemistry

TH immunohistochemistry for determination of DA innervation in prefrontal cortex sections was performed on fresh-frozen sections with a modification of methods previously described (Chapman *et al*, 2001; Daberkow *et al*, 2005). Sections were washed in PBS, fixed in 4% formaldehyde, and rinsed in PBS. Endogenous peroxidase activity was quenched with H₂O₂. Sections were then blocked in normal horse serum and incubated 70 hr in mouse anti-TH primary antibody (1:50 dilution, Immunostar Inc., Hudson, WI) at room temperature. Slides were then washed in PBS and incubated with Avidin-Biotin blocking solution (Vector Laboratories, Burlingame, CA) for 15 min. The sections were then incubated in biotinylated horse anti-mouse IgG (1:200 dilution, Vector Laboratories, Burlingame, CA) for 2-hr at 4°C. Sections were then rinsed and incubated with prepared Avidin-Biotin Complex reagent (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Finally, sections were incubated with 3,3-diaminobenzidine (Vector Laboratories, Burlingame, CA) for 3-min, dried and cover slipped.

2.6. Image analysis

Autoradiographic images were captured and analyzed using the image analysis program, ImageJ (<http://rsbweb.nih.gov/ij>), as previously described (Chapman et al., 2001; Pastuzyn et al., 2012). Images of sections were digitized and saved under constant lighting and camera conditions. Images were coded so that the experimenter was blinded to the treatment groups during subsequent analyses. Basic densitometry analysis yielded average density (arbitrary gray) values over the dorsomedial (DMS), dorsolateral (DLS), ventromedial (VMS), and ventrolateral (VLS) striatum, as well as the prelimbic (PrLC), infralimbic (ILC), orbitofrontal (OFC), and anterior cingulate (ACC) cortex. The average gray value of the white matter was subtracted from these values to correct for background labeling. Four sections of the striatum for DAT and four sections of the prefrontal cortex for SERT (averaged across left and right hemispheres) from each animal were analyzed. For the analysis of TH immunohistochemical staining in the cortex, the average gray values of staining were obtained from the medial prefrontal cortex (combined ILC, PrLC, and ACC) and from the OFC across four sections from each animal. For all measures, the values of DAT and SERT binding in METH-pretreated rats are presented as the percent of average levels in saline-pretreated rats.

2.7. Statistical analysis

All data are presented as means $\pm$ SEM. Analyses comparing dependent measures across the METH- and saline-pretreated rats were completed with Student's t-tests. Analysis of the interactions between types of behavioral responses (lever presses or head entries) and pretreatment or between pretreatment groups across sessions were accomplished by multivariate analysis of variance (MANOVA) using JMP 9.0 (SAS, Cary, NC). As appropriate based on the overall MANOVA, post-hoc analysis of significant interactions was accomplished via t-tests with Bonferroni correction to adjust for multiple comparisons. Overall statistical significance was set at $p = 0.05$.

3. Results

3.1. METH-induced monoamine depletions

Pretreatment of rats with METH resulted in significant decreases in DA and 5-HT innervation of the striatum and prefrontal cortex, respectively, as assessed by [125 I] RTI-55 monoamine transporter binding (Fig. 1). Consistent with other studies (O'Dell et al., 2012; Pastuzyn et al., 2012), DAT binding decreased in DMS by $59 \pm 3.5\%$ ($t=9.6$; $p<0.0001$), DLS by $41 \pm 4.0\%$ ($t=5.7$; $p<0.0001$), VMS by $65 \pm 2.8\%$ ($t=11.2$; $p<0.0001$), and VLS by $58 \pm 4.0\%$ ($t=7.9$; $p<0.0001$), respectively (Fig. 1A & B). Likewise, METH decreased SERT binding in the OFC by $59 \pm 5.7\%$, ($t=8.9$; $p<0.0001$), ILC by $30 \pm 7.5\%$ ($t=3.5$; $p<0.008$), PrLC by $62 \pm 8.7\%$ ($t=5.5$; $p<0.0003$), and ACC by $74 \pm 5.1\%$ ($t=11.9$; $p<0.0001$), respectively (Fig. 1C & D). Together with previous studies, the present data indicate that our METH regimen induced persistent 40–70% monoamine depletions (DAT in striatum and SERT in prefrontal cortex) assessed by [125 I] RTI-55 binding approximately four weeks after the METH exposure.

We also measured DA innervation of the PFC via TH immunohistochemistry. Sesack and others have suggested that TH immunoreactive staining in the deep layers of the PFC mostly arises from the dopamine innervation of PFC (Sesack et al., 1998; Sesack et al., 1995). Scattered, dense TH immunohistochemical staining was apparent in the deep layers of the PrLC, ILC, and ACC, and more diffuse staining was observed in the OFC (Fig. 2). There were 20–25% reductions in TH positive-staining in the prefrontal cortex (PrLC, IL, ACC combined; $t=3.1$, $p<0.02$) and OFC ($t=2.4$, $p<0.05$) of METH-pretreated rats relative to saline-pretreated controls (Fig. 2C).

3.2. Effect of methamphetamine on behavioral responses

To evaluate the effect of METH-induced neurotoxicity on behavioral control, saline- (n=17) and METH-pretreated rats (n=16) were trained on a lever-pressing task in which animals were required to press a lever on one side of an operant chamber and then to retrieve a reinforcer (sucrose pellet) from a magazine on the opposite side (see Section 2.2). In agreement with previous findings (Son et al., 2011), there was no apparent effect of METH-pretreatment on the acquisition of instrumental behavior; that is, saline- and METH-pretreated animals required similar numbers of sessions to reach the criterion (50 reinforcers/30-min session) (saline-pretreated, 4.2 ± 0.7 sessions; METH-pretreated rats, 3.4 ± 0.7 sessions, $t=0.8$; $p=0.42$, Fig. 3A). Additionally, analysis of total lever presses across the four sessions at which the animals performed at or above this criterion level (Fig. 3B) revealed a main effect of session ($F_{3,29}=3.1$, $p=0.04$), but no main effect of pretreatment ($F_{1,31}=1.1$, $p=0.3$) and no session x pretreatment interaction ($F_{3,29}=1.5$, $p=0.2$). Likewise, analysis of total head entries into the food magazine across the four sessions at which the animals performed at or above this criterion level (Fig. 3C) revealed a main effect of session ($F_{3,29}=6.0$, $p=0.003$), but no main effect of pretreatment ($F_{1,31}=0.009$, $p=0.9$) and no session x pretreatment interaction ($F_{3,29}=0.2$, $p=0.9$).

Two-tailed, Student's t-tests of the average performance of the animals across the four consecutive sessions at which the animals performed at or above criterion (>50 reinforcers/30 min session) showed that METH-pretreated rats made a similar number of average total lever-presses/30-min session relative to controls (saline-pretreated, 124 ± 7.3 ; METH-pretreated, 133 ± 9.2 ; $t=-0.77$; $p=0.45$; Fig. 4A), as well as a similar number of average total head-entries into the food magazine/30-min session (saline-pretreated, 244 ± 19.0 ; METH-pretreated, 245 ± 9.5 ; $t=-0.016$; $p=0.99$; Fig. 4B). However, although the behavioral responses were grossly similar between saline- and METH-pretreated rats, a two-tailed Student's t-test revealed that METH-pretreated rats earned fewer reinforcers on average across these sessions (85 ± 3.6) relative to saline-pretreated rats (101 ± 6.0 ; $t=2.25$; $p=0.03$; Fig. 4C). These data suggest that METH-pretreated rats do not execute behaviors in the same way as normal controls do, but rather must exhibit different patterns of behavior.

Therefore, to assess the nature of behavioral control in the METH-pretreated animals, a more stringent analysis was undertaken of the total number of lever-presses made. That is, we re-classified lever-presses as those that were the "first" lever-press on each FR1 schedule that triggered delivery of reinforcer into the magazine *vs.* those that were "extra" lever presses that had no scheduled consequences because the reinforcer earned via the first lever press had not yet been retrieved (i.e., the photobeam attached to the magazine had not yet been broken). The repeated-measures MANOVA (lever-press type x pretreatment) revealed a significant main effect of lever-press type ($F_{1,31} = 82.1$, $p<0.0001$) and a significant lever-press type x pretreatment interaction ($F_{1,31} = 10.1$, $p<0.005$), but no main effect of pretreatment ($F_{1,31} = 0.59$, $p=0.45$). Post-hoc analysis revealed that saline-pretreated rats made more "first" lever-presses triggering delivery of reinforcer (100 ± 6.0) than did METH-pretreated rats (85 ± 3.6 ; $t=2.2$; $p=0.02$). Conversely, METH-pretreated rats made more "extra" lever-presses (48 ± 9.5) than did saline-pretreated rats (23 ± 3.7 ; $t=-2.4$, $p=0.01$) (Fig. 5A). Thus, the fewer reinforcers earned in METH-pretreated rats appear to be due to the fact that METH-pretreated rats make fewer lever presses resulting in reward delivery and more lever presses during the time they should be retrieving the earned reinforcer.

Similarly, head entries into the food magazine were re-classified as those that were the "first" head entry to obtain a reinforcer that had been delivered after a lever press *vs.* those that were "extra" head-entries into the magazine after the earned reinforcer had already been obtained (Fig. 5B) and the animal should have been returning to the lever on the opposite

side of the chamber. The repeated-measures MANOVA on the head-entry data revealed a significant main effect of head-entry type ($F_{1,31}=30.9$, $p<0.0001$; Fig. 5B), but no main effect of pretreatment ($F_{1,31}=0.0002$, $p>0.05$). There also was no statistically significant head-entry type $\times$ pretreatment interaction, although there was a trend for the results to be similar to those for the lever presses ($F_{1,31}=2.7$, $p=0.1$). Thus, although both METH- and saline-pretreated rats both made more “extra” head-entries, METH-pretreated rats tended to make more “extra” head entries whereas saline-pretreated rats tended to make more “first” head entries.

To further evaluate these behavioral differences, we evaluated the latencies between the two responses rats had to make on each FR1 schedule; that is, the “First lever-press to first head-entry” latency and the “First head-entry to first lever-press” latencies. As might be expected given the greater number of “extra” responses made by the METH-pretreated rats, the “First lever-press to head-entry” latency was significantly greater in the METH-pretreated rats (METH-pretreated, 4.6 ± 0.5 sec) than in the saline-pretreated rats (3.1 ± 0.2 sec; $t=-2.9$; $p<0.009$; Fig. 5C). Likewise, the “First head-entry to first lever-press” latency was also greater for the METH-pretreated rats (METH-pretreated, 20.5 ± 1.6 sec; saline-pretreated, 16.0 ± 1.4 sec; $t=-2.1$; $p<0.05$; Fig. 5D). Taken together, these data suggest that METH-pretreated rats exhibited perseverative behavior (i.e., inappropriately repeated lever presses and head entries) relative to saline-pretreated rats.

3.3. Relation of SERT and DAT binding to perseverative lever pressing

The role that specific monoamine depletions induced by METH play in the perseverative behavior was assessed by examining the relation between the levels of monoamine depletions in PFC and striatum and the number of “extra” lever presses (Fig. 6). Significant negative correlations between the levels of SERT binding and the number of “extra” lever presses across both saline- and METH-pretreated rats combined were found in the OFC ($r^2=0.62$, $p<0.002$), ILC ($r^2=0.76$, $p<0.0001$), PrLC ($r^2=0.68$, $p<0.001$), and ACC ($r^2=0.58$, $p<0.003$). Interestingly, these significant negative correlations were still apparent when the saline-pretreated controls alone were analyzed (OFC $r^2=0.74$, $p<0.05$ (Fig. 6A); ILC $r^2=0.78$, $p<0.01$ (Fig. 6B); PrLC $r^2=0.71$ (Fig. 6C); trend in ACC $r^2=0.46$, $p=0.09$ (Fig. 6D)). Additionally, trends toward (OFC, ILC, PrLC) or statistically significant (ACC) correlations were apparent when the METH-pretreated rats alone were analyzed (OFC $r^2=0.55$, $p=0.09$ (Fig. 6A'); ILC $r^2=0.55$, $p=0.09$ (Fig. 6B'); PrLC $r^2=0.48$, $p=0.13$ (Fig. 6C'); ACC $r^2=0.71$, $p<0.05$ (Fig. 6D')). There were no significant correlations between SERT binding in these regions of prefrontal cortex and first lever presses (r^2 values range 0.004–0.03). Likewise, there were no significant correlations between SERT binding in prefrontal cortex and “first” or “extra” head entries (r^2 values range 0.004–0.13).

We also examined the relation between DA depletions in prefrontal cortex and striatum and behavioral performance. There were no significant correlations between TH staining in any of the PFC areas and the number of extra lever-presses (combined PrLC, ILC, and ACC, $r^2=0.21$, $p=0.11$; OFC, $r^2=0.01$, $p=0.65$). There were, however, significant positive correlations between the number of “first” lever-presses and DAT binding across all animals (saline- plus METH-pretreated combined) in the DMS ($r^2=0.19$, $p<0.02$), DLS ($r^2=0.21$, $p<0.007$), VMS ($r^2=0.19$, $p<0.02$), and VLS ($r^2=0.19$, $p<0.02$), as well as significant negative correlations between the number of “extra” lever-presses and DAT binding across all animals in the DMS ($r^2=0.14$, $p<0.04$), DLS ($r^2=0.11$, $p=0.06$), VMS ($r^2=0.16$, $p<0.03$), and VLS ($r^2=0.12$, $p<0.05$); however, these correlations generally did not persist when either just the saline-pretreated (r^2 value range 0.001–0.15) or METH-pretreated (r^2 value range 0.003–0.17) rats were analyzed, with the exception of significant correlations between the number of “first” lever-presses and DAT binding in DMS ($r^2=0.27$, $p<0.04$) and DLS ($r^2=0.33$, $p<0.03$) of METH-pretreated rats. Taken together, these analyses suggest a general

relation between levels of SERT binding in the PFC and inhibitory control over instrumental behavior under both normal and pathological conditions.

4. Discussion

Consistent with previous findings (Son et al., 2011), the present findings indicate that although rats with METH-induced neurotoxicity appear to perform similarly to controls during the learning of an appetitive instrumental task, their criterion-level performance is not the same. Rather, the present results show that METH-pretreated rats earned fewer reinforcers. Such discrepancy likely reflects differences observed in the behavior; that is, the METH-pretreated rats engaged in more responding that had no reward contingency and showed longer latencies when moving from one response (e.g. lever press) to another (e.g. reward retrieval). For both the METH- and the saline-pretreated animals, the number of “extra” responses made were significantly and negatively correlated with the degree of SERT binding in areas of the PFC. The data therefore suggest that serotonin regulation of the PFC in general may play a role in the control of perseverative behavior and that METH-induced damage to the serotonin innervation of the PFC may underlie increased perseverative responding in METH-pretreated rats.

The behavioral paradigm used in the present study is a not a standard task for assessing perseverative behavior in rodents. Rather, in animal models, perseverative tendency historically has been examined in instrumental extinction and discrimination reversal tasks related to food-rewarded responses (Butter, 1969; Evenden and Robbins, 1983; Izquierdo and Jentsch, 2012). Although the present experimental paradigm was not exactly matched to the tasks mentioned above, the “extra” responses observed herein are similar to what is observed in the Evenden and Robbins’ study (1983), in which rats exhibited increased numbers of “extra” lever-presses made prior to the collection of food in three different instrumental paradigms, supporting that the behaviors observed herein can be interpreted as perseverative behavior.

Although we can not presently exclude the possibility that the METH-treated rats learned a different association between numbers of lever presses and delivery of the reinforce, several pieces of evidence suggest that the METH-pretreated rats exhibit perseverative behavior, rather than altered learning of the instrumental response. First, there was no significant difference in the number of training sessions required for animals to reach the first day of criterion-level responding (>50 reinforcers/30-min session). Second, there were no significant differences between saline- and METH-pretreated rats in the overall numbers of responses (lever presses or head entries) made across the four days of criterion-level responding. Finally, our prior work has demonstrated normal rates of appetitive instrumental learning in METH-pretreated rats, although the associations (action-outcome vs. stimulus-response) driving the instrumental behaviors are different (Son et al., 2011). Taken together, these data suggest that appetitive instrumental learning is intact in rats with METH-induced neurotoxicity, and that the aberrant characteristics of the behavior reflect perseveration; however, future studies in which the METH-induced neurotoxicity is induced after the instrumental response is learned will be necessary to conclusively establish that perseveration vs. differential learning is, in fact, the basis for the behavioral differences reported in the present work.

The basis for the perseverative behaviors observed herein is unclear. It is interesting that the perseveration is only statistically significant when one examines lever presses, and not when head entries are examined, although the pattern for fewer “first” and more “extra” head entries is evident, and the head entry to lever press latency was significantly elevated in the METH-pretreated rats as well. This greater apparent impact of perseveration on lever

presses may be a consequence of differences in task-specific demands, as perseveration can specifically affect different behaviors within the same behavioral paradigm (Agnoli and Carli, 2012). Attentional load may be another aspect of task demand that influences the degree of perseveration observed. Conceivably, the noise of a reinforcer (45-mg sucrose pellet) dropping into the magazine acts as a cue for animals to switch behavioral performance from the lever press to the head entry. Disrupted attention to this cue could result in continued lever pressing. Studies of chronic or abstinent METH abusers reveal that such individuals show attentional deficits (Salo et al., 2011; Salo et al., 2007). In animal studies, rats in withdrawal states after self-administering METH also show such attentional deficits, demonstrating reduced accuracy on a sustained visual attention task as well as an attentional set-shifting task, respectively (Dalley et al., 2007; Parsegian et al., 2011). However, another study employing a binge-regimen model of METH-induced neurotoxicity did not demonstrate deficits in attentional set shifting, suggesting that attentional deficits may not underlie the perseverative responding observed in the present study (Izquierdo et al., 2010). Finally, Agnoli and Carli (2012) have suggested that perseveration may be greater when the behavioral task requires the “selection and integration of an adequate response in a long sequence leading to reinforcement.” Clearly, additional studies are thus needed to determine how characteristics of a task or particular behavior influence the degree to which it is impacted by perseveration, as the design of therapeutic interventions to address various aspects of METH addiction and long-term consequences of METH abuse may need to differentially consider the impact of perseverative responding on specific behaviors.

Interestingly, perseverative behavior has been reported in chronic METH abusers. For example, Henry et al. (2011) recently reported that chronic METH abusers exhibited increased perseverative interactions with both novel and already-engaged objects in a novel open-field paradigm. Likewise, other studies have reported that METH-dependent individuals make more perseverative errors than controls on the Wisconsin Card Sorting Task and that the greater number of errors is associated with hypometabolism in the prefrontal cortex of those individuals (Chung et al., 2007; Kim et al., 2005; Kim et al., 2009). The present results in an animal model raise the possibility that such perseverative behavior arises as a consequence of METH-induced neurotoxicity, particularly to the serotonin innervation of PFC.

The aspect of METH-induced neurotoxicity that underlies the perseveration observed in the present study has not been clearly established, but the data suggest a role for disrupted 5-HT signaling in corticostriatal circuits. Excitotoxic lesions of the medial PFC and contralateral striatum increase perseveration as assessed with a 5-choice serial reaction time task (5-CSRTT) (Christakou et al., 2001, 2004). Additionally, bilateral lesions of the PrLC increase perseverative responding in the 5-CSRTT (Chudasama et al., 2003). Also, increased perseverative lever-presses on a progressive-ratio task and a signal-attenuation instrumental conditioning task have been observed after lesions of dorsal striatum and OFC (Eagle et al., 1999; Joel et al., 2005; Schilman et al., 2010). In agreement with those studies, our present findings show that METH-induced neurotoxicity, manifested as 40–70% decreases in cortical SERT and striatal DAT binding, is associated with perseveration. Furthermore, the present data show significant inverse correlations between perseverative behavior and the binding of [¹²⁵I] RTI-55 to the SERT in all frontal cortex regions, even when the saline- and METH-pretreated rats are examined separately. The present results thus suggest that the increased perseveration observed herein in METH-pretreated rats may be related to the loss of 5-HT innervation in frontal cortical regions. Furthermore the data suggest that the degree of 5-HT innervation of the PFC, even in normal animals, may be a critical determinant of the degree of perseverative behavior, as SERT binding in the PFC inversely correlated with the number of perseverative responses not only in the METH- and saline-pretreated animals combined, but also in each of cohort of animals alone. Conversely, although the binding of

[¹²⁵I] RTI-55 to the DAT in dorsal striatum also correlated significantly with perseverative (“extra”) lever presses, as well as “first” lever presses, these correlations generally did not persist when the METH- and saline-pretreated groups were separately analyzed, suggesting that those overall correlations likely reflect the concurrent segregation of the two treatment groups on both of these dependent variables. These data thus suggest that the 5-HT innervation of the PFC may be an important regulator of perseverative behavior, consistent with prior data implicating 5-HT systems in inhibitory control underlying operant responding (Harrison et al., 1997; Winstanley et al., 2003). Although we did not measure 5-HT tissue content in the PFC, prior work from our lab (Johnson-Davis et al., 2003) showed that a binge regimen of METH, such as that used herein, resulted in a 40–50% loss of 5-HT content in the PFC, suggesting that the levels of SERT ligand binding in the present studies may reflect the degree of serotonin innervation of the PFC. These data thus suggest that the degree of serotonin innervation of the PFC may be a critical determinant of perseverative tendencies even under “normal” conditions. The data further suggest that manipulations the 5-HT system may be effective in managing consequences of METH exposure on perseverative behavior.

In summary, despite apparent similarity of overall responding on the present appetitive instrumental task, rats with METH-induced neurotoxicity do not execute behaviors the same way as do control rats. Rather, rats with METH-induced neurotoxicity show impaired response inhibition in the form of perseverative responding. The data further suggest a critical role of the 5-HT innervation of frontal cortical areas in this impairment. Furthermore, natural variations in the 5-HT innervation of the frontal cortex as assessed by SERT binding may contribute to “normal” differences in such control over behavior. The present study adds to a growing body of evidence for the loss of cognitive and behavioral control associated with exposure to METH, and raises the possibility that such behavioral abnormality can be a consequence of such exposure. Such consequences may contribute to the decreased everyday life-skills and increased drug-seeking behavior, aggression, and risky sexual activities associated with METH abuse.

Acknowledgments

Special thanks to Drs. John Marshall, Steve O’Dell & Ashley Fricks-Gleason for providing assistance with [¹²⁵I] RTI55 binding assay. This research is supported by NIH Grant DA024036 (KAK) and an ASPET Summer Fellowship (JK).

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HIGHLIGHTS

Methamphetamine (METH) neurotoxicity decreases striatal dopamine innervation.

METH neurotoxicity decreases prefrontal serotonin innervation.

Rats with METH-induced toxicity show perseverative responding in an operant task.

Perseverative responding is inversely correlated with prefrontal SERT binding.

Figure 1.

METH-induced partial loss of monoamines in striatum and prefrontal cortex as assessed by [¹²⁵I] RTI-55 autoradiographic binding assays. Photomicrographs of dopamine transporter (DAT) binding in dorsomedial (DMS), dorsolateral (DLS), ventromedial (VMS), and ventrolateral (VLS) striatum are shown in A, and the magnitude of the loss of striatal DAT binding is shown in B (mean gray values (arbitrary units) ± SEM expressed as % of saline-pretreated rats, n=17; METH-pretreated rats, n=16). Photomicrographs of serotonin transporter (SERT) binding in orbitofrontal cortex (OFC), infralimbic cortex (ILC), prelimbic cortex (PrLC), and cingulate cortex (ACC) are shown in C, and the magnitude of the depletion of SERT binding is shown in D (mean gray values (arbitrary units) ± SEM expressed as % of saline-pretreated rats, n=7; METH-pretreated rats, n=6). ** Significantly different from corresponding brain region in saline-pretreated rats, p<0.01. Scale bars in A and C = 1mm.

Figure 2.

METH induced a partial loss of tyrosine hydroxylase (TH) in the prefrontal cortex (PFC), as assessed with immunohistochemistry. Photomicrographs of TH immunohistochemical staining in A) saline-pretreated animals (n=7) and B) METH-pretreated rats (n=6). Scale bar = 1mm. The magnitude of the loss of TH in the PFC (ILC, PrLC, and ACC combined and OFC) is shown in C) (mean gray values (arbitrary units) $\pm$ SEM expressed as % of saline-pretreated rats, n=7; METH-pretreated rats, n=6). * Significantly different from corresponding brain region in saline-pretreated rats, $p < 0.05$.

Figure 3.

Instrumental learning and performance by session in saline- and METH-pretreated rats. The average number of training sessions required to reach criterion (>50 reinforcers/30-min session) on the instrumental learning task and the average number of total lever presses/30-min session ($\pm$ SEM), and average number of total head entries/30-min session ($\pm$ SEM) over the four, consecutive sessions of criterion-level performance are shown for saline-pretreated ($n=17$) and METH-pretreated ($n=16$) rats are shown in A, B, and C, respectively.

* Significant main effect of session, $p<0.05$.

Figure 4.

Effect of METH-pretreatment on average numbers of behavioral responses made and reinforcers earned over four consecutive sessions of criterion-level performance. The average total number of lever presses/30-min session ($\pm$ SEM), average total number of head entries/30-min session ($\pm$ SEM), and average total number of reinforcers earned for both saline-pretreated ($n=17$) and METH-pretreated ($n=16$) are shown in A, B, and C, respectively. * Significantly different from saline-pretreated rats, $p<0.05$.

Figure 5.

Analysis of types of operant responses made by METH- and saline-pretreated rats in the appetitive instrumental behavior task. A) The mean number of “first” lever-presses (*i.e.* those presses triggering delivery of a reinforcer; $\pm$ SEM; $n=17$ saline, $n=16$ METH) and the mean number of “extra” lever-presses (*i.e.* those occurring after the delivery of the reinforcer but before its retrieval and thus triggering no programmed consequences; $\pm$ SEM) made by saline- and METH-pretreated rats on an FR1 schedule. B) The mean number of “first” head entries (*i.e.* those obtaining delivered a reinforcer; $\pm$ SEM) and the mean number of “extra” head entries (*i.e.* those made after obtaining the reinforcer but before the next lever press; $\pm$ SEM). C) Mean latencies from “First” lever press to “First” head entry ($\pm$ SEM). D) Mean latencies from “First” head entry to “First” lever press ($\pm$ SEM). *

Significantly different from same measure in saline-pretreated rats, $p < 0.05$. ** Significant main effect of different lever-press and head-entry type, $p < 0.01$.

Figure 6.

Correlations between [I^{125}] RTI-55 binding to the SERT, measured as arbitrary gray values from densitometric analysis of film autoradiograms, in orbitofrontal cortex (OFC), infralimbic cortex (ILC), prelimbic cortex (PrLC), and anterior cingulate cortex (ACC) and the mean number of "extra" (*i.e.* perseverative) lever-presses/30 min session made by saline-pretreated (open circles; A, B, C, D) and METH-pretreated (closed squares; A', B', C', D') rats.