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Altered Learning and *Arc*-Regulated Consolidation of Learning in Striatum by Methamphetamine-Induced Neurotoxicity

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Methamphetamine (METH) causes partial depletion of central monoamine systems and cognitive dysfunction in rats and humans. We have previously shown and now further show that the positive correlation between expression of the immediate-early gene *Arc* (activity-regulated, cytoskeleton-associated) in the dorsomedial (DM) striatum and learning on a response reversal task is lost in rats with METH-induced striatal dopamine loss, despite normal behavioral performance and unaltered *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory post-synaptic currents, suggesting intact excitatory transmission. This discrepancy suggests that METH-pretreated rats may no longer be using the dorsal striatum to solve the reversal task. To test this hypothesis, male Sprague–Dawley rats were pretreated with a neurotoxic regimen of METH or saline. Guide cannulae were surgically implanted bilaterally into the DM striatum. Three weeks after METH treatment, rats were trained on a motor response version of a T-maze task, and then underwent reversal training. Before reversal training, the NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP5) or an *Arc* antisense oligonucleotide was infused into the DM striatum. Acute disruption of DM striatal function by infusion of AP5 impaired reversal learning in saline-, but not METH-, pretreated rats. Likewise, acute disruption of *Arc*, which is implicated in consolidation of long-term memory, disrupted retention of reversal learning 24 h later in saline-, but not METH-, pretreated rats. These results highlight the critical importance of *Arc* in the striatum in consolidation of basal ganglia-mediated learning and suggest that long-term toxicity induced by METH alters the cognitive strategies/neural circuits used to solve tasks normally mediated by dorsal striatal function.
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INTRODUCTION

Methamphetamine (METH) abuse is a significant problem worldwide. METH causes partial loss of dopamine (DA) and serotonin systems in the brain (Morgan and Gibb, 1980; Ricaurte *et al*, 1980; Seiden *et al*, 1976; Wagner *et al*, 1980). In humans, METH-induced neurotoxicity is evident as decreases in DA transporter (DAT) binding in the caudate-putamen that can last for up to 11 months (McCann *et al*, 1998; Volkow *et al*, 2001a, b; Wilson *et al*, 1996). METH-induced toxicity is also evident as decreases in serotonin transporter (SERT) binding across multiple brain regions, including the caudate-putamen and the frontal cortex (Kish *et al*, 2009; Sekine *et al*, 2006), as well as loss of glutamatergic neurons in the somatosensory cortex (Eisch *et al*, 1998; Pu *et al*, 1996). Cognitive impairments have also been seen in association with METH-induced neurotoxicity, and include deficits in motor sequence learning (Chapman *et al*, 2001), object recognition (Belcher *et al*, 2005; Herring *et al*, 2008; Schröder *et al*, 2003), visual discrimination and attentional set-shifting (Izquierdo *et al*, 2010), and novel odor recognition (O'Dell *et al*, 2011).

In some tasks, however, behavioral impairments associated with METH-induced neurotoxicity are not apparent. Such tasks include those examining conditioned placed aversion (Achat-Mendes *et al*, 2005), spatial learning on the Morris water maze (Herring *et al*, 2008; Schröder *et al*, 2003), and motor response reversal learning on a T-maze (Daberkow *et al*, 2008). With regard to the response reversal learning task on the T-maze, expression of *Arc* (activity-regulated cytoskeleton-associated gene), an immediate-early gene important in consolidation of learning, in the dorsomedial (DM) striatum is correlated with number of trials to criterion on the reversal learning task in saline-pretreated rats, but not, interestingly, in METH-pretreated rats (Daberkow *et al*, 2007, 2008). Thus, although METH-pretreated rats behaviorally appear to be normal on this task, the relation between *Arc* expression in the striatum and behavior is lost. Guzowski *et al* (2000; 2001)

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previously suggested that the correlation between *Arc* expression and learning reflects the involvement of a brain region in a task. Whether *Arc* in the DM striatum is necessary for consolidation of response reversal learning has not heretofore been examined; furthermore, whether loss of correlation in METH-pretreated rats indicates a change in the brain regions engaged during the task is unknown.

Therefore, the goal of the present studies was to test whether *Arc* in the DM striatum is critical for consolidation of response reversal learning and whether loss of the correlation in METH-pretreated rats reflects a loss of dependence of the reversal learning on DM striatal function. We locally infused an *Arc* antisense oligonucleotide (Guzowski *et al*, 2000; Hearing *et al*, 2010) or the *N*-methyl-D-aspartate (NMDA) receptor antagonist AP5 into the DM striatum prior to rats engaging in motor response reversal learning on the T-maze task. The results indicate that *Arc* signaling in the DM striatum is necessary for consolidation of the reversal learning and that METH-induced neurotoxicity is associated with a change in the neural substrates mediating such reversal learning.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC; 275–300 g) were singly housed in tub cages on a 14 : 10-h light cycle. Animal care and experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Utah.

METH Pretreatment

Rats were treated with a neurotoxic regimen of (±)-METH-HCl (4 × 10 mg/kg free base, s.c.; NIDA, Research Triangle Park, NC) over one day as described previously (Daberkow *et al*, 2008). One hour after the final injection, rats were returned to their home cages and given free access to food and water until behavioral training began (METH-pretreated, $n = 25$; saline-pretreated, $n = 29$).

Surgery

Two weeks after pretreatment, rats were anesthetized with ketamine/xylazine (90/10 mg/kg, i.p.) and placed in a stereotaxic apparatus. A dual, 21-gauge guide cannula (Plastics One, Roanoke, VA) was lowered to end just dorsal to the DM striatum (mm from bregma: AP + 0.2; ML ± 1.9; DV-3.2). The guide was secured with skull screws and dental acrylic, and dummy cannulae were inserted. Subsequent infusions were made through 33-gauge infusion cannulae extending 1.8 mm beyond the guides. The infusion cannulae remained in place for 1 min after infusion before being withdrawn.

Reversal Learning Task

Response reversal learning on the T-maze was conducted as described previously (Daberkow *et al*, 2007). Beginning 1 week after surgery, rats were food-restricted and habituated to the food reward and maze. The turn bias of each rat was determined, followed by acquisition training for 3 days and then reversal learning. During the reversal learning task, rats had to turn in the opposite direction from acquisition to receive the reward. The criterion for learning on both acquisition and reversal tasks was 9/10 correct turns in a row.

Acute Pharmacological Manipulations

On the day of reversal training, rats were infused through their cannulae with either AP5 (0.5 μl/2 min, 25 nmol in 0.1 M PBS, pH 7.4; Tocris Bioscience, Ellisville, MO) (Palencia and Ragozzino, 2004) or an *Arc* antisense oligonucleotide. The *Arc* antisense oligonucleotide was a chimeric phosphorothioate/phosphodiester oligonucleotide against bases 209–228 of the *Arc* gene (Guzowski *et al*, 2000). The nonsense/control oligonucleotide was composed of the same bases, but in a scrambled sequence. A 1-μl volume of oligonucleotide (1 nmol/μl, 0.1 M PBS, pH 7.4) (Guzowski *et al*, 2000) or PBS vehicle was infused (0.39 μl/min) into each DM striatum. After infusions into DM striata, rats were returned to their home cages for 5 min (AP5) or 2 h (*Arc* antisense) prior to reversal training. Five minutes after reaching criterion, rats infused with AP5 and the corresponding PBS-infused controls were killed, and brains were removed and frozen in isopentane chilled on dry ice. Rats infused with the *Arc* antisense oligonucleotide and the corresponding controls (PBS or *Arc* nonsense oligonucleotide) were returned to their home cages upon reaching criterion. The following day, these rats were tested on reversal retention to determine the number of trials needed to again reach criterion on the reversal direction learned the previous day. Five minutes after reaching criterion, rats were killed and brains were removed and frozen.

DAT and SERT Autoradiography

Fresh-frozen brains were sectioned (12 μm), thaw-mounted onto Superfrost Plus (VWR, Aurora, CO) slides, and then stored at −20 °C. Infusion sites were verified during sectioning (Figure 1). DAT levels in the striatum were determined by [^{125}I]RTI-55 (PerkinElmer, Waltham, MA) binding, as reported previously (Boja *et al*, 1992; O'Dell *et al*, 2011). SERT binding in the prefrontal cortex was similarly performed except that fluoxetine was omitted. Prefrontal cortex slides incubated in buffer containing fluoxetine showed no binding (data not shown). The slides were apposed to film (Biomax MR; Eastman Kodak, Rochester, NY) for 24 h and developed. Images were digitized and densitometric analysis was performed using the NIH ImageJ software, yielding average, background-subtracted gray values in the DM and dorsolateral (DL) striatum and six prefrontal cortical regions. Two rostral and two middle striatal sections, and four prefrontal cortical sections, were analyzed per rat. DAT and SERT binding in METH-pretreated rats were then converted to percent of average levels in saline-pretreated rats.

Error Analysis

The numbers of perseverative and regressive errors made during reversal learning by METH- and saline-pretreated

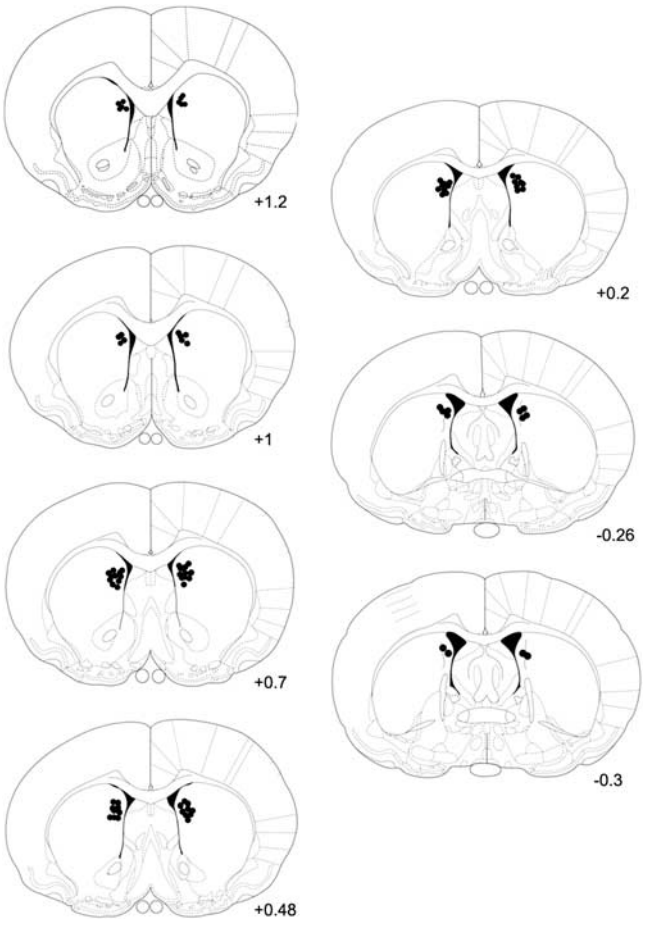

Figure 1 Infusion sites in the DM striatum. The black dots indicate placement of infusion sites in the DM striatum of rats in AP5 and *Arc* experiments. The numbers indicate mm from bregma (Paxinos and Watson, 1998).

rats were calculated as defined by Palencia and Ragozzino (2004), with modification owing to the task differences. Wrong turns were counted as perseverative errors if they occurred before a rat made more than three turns in the reversal direction. Incorrect turns occurring after the rat had made more than three turns in the new correct direction were counted as regressive errors.

In Situ Hybridization Histochemistry

The left hemisphere from animals used for electrophysiological experiments (see below) was frozen, sectioned (12 μm), and processed for *in situ* hybridization histochemical determination of Grin2a NMDA receptor subunit expression as described previously (Ganguly and Keefe, 2001) using a full-length ribonucleotide probe synthesized from the cDNA (gift from Dr Peter Seeburg) using ^{35}S-UTP and T7 RNA polymerase (Roche, Indianapolis, IN). Slides were hybridized overnight in humid chambers at 55 °C, washed, treated with Ribonuclease-A (5 μg/ml), washed, dried, and then apposed to X-ray film for 1 week. Images from films were digitized and densitometric analysis was performed using ImageJ, yielding average, background-subtracted gray values in the DM, DL, and ventromedial striatum.

Determination of Striatal DA Content

DA content was determined in striatal tissue punches collected during sectioning of frozen brain hemispheres for *in situ* hybridization (Chapman *et al*, 2001). A blunt-tip, 18-gauge needle was used to collect 1-mm^3 punches from both the medial and lateral striatum (+ 0.3 mm anterior to bregma). Punches were sonicated in tissue buffer (0.05 M sodium phosphate/0.03 M citric acid buffer, 25% methanol (v/v), pH 2.5) and centrifuged. A 20-μl volume of the supernatant was injected onto a high-pressure liquid chromatography system coupled to an electrochemical detector (EOx = + 0.6 V) for separation and quantification of DA levels. Values were expressed per mg of protein. Protein content was determined by Lowry protein assay.

Striatal Slice Preparation

Acute brain slices were obtained, as described previously (Chapman *et al*, 2003), from adult rats (375–460 g) killed 3–5 weeks after pretreatment with saline or METH. Rats were anesthetized (pentobarbital, 50 mg/kg) and decapitated. Brains were removed and placed in ice-cold, oxygenated (95% O_2–5% CO_2) sucrose Ringer solution (pH 7.4) containing (in mM): sucrose (200), KCl (3), NaH_2PO_4 (1.4), $MgSO_4$ (2), $NaHCO_3$ (26), glucose (10), and $CaCl_2$ (2). The brain was divided along the midline and the right hemisphere was glued caudal-side down to a Vibraslicer chuck (Campden Instruments). Coronal sections (300–350 μm) containing striatum were placed in a holding chamber at room temperature containing oxygenated Ringer solution with 126 mM NaCl in place of sucrose (pH 7.37–7.41). The sections remained in the Ringer solution (osmolality 295–305 mOsm) for $\geqslant$1 h before recording.

Patch-Clamp Recordings

Slices were transferred into the recording chamber perfused with fresh, oxygenated, Mg^{2+}-free Ringer solution at room temperature ($\sim$22 °C) by means of a gravity-feed system (4 ml/min). Whole-cell patch clamp was used to record from single striatal neurons, using previously described inclusion criteria and data acquisition (Chapman *et al*, 2003). Borosilicate glass microelectrodes (3–6 MΩ resistance) were pulled using a P-87 micropipette puller (Sutter Instruments). The internal recording solution contained (in mM): K gluconate (130), KCl (10), HEPES (10), EGTA (1), $CaCl_2$ (0.1), ATP (2), GTP (1), and glutathione (1). The external solution was the same as that in the holding chamber.

Excitatory post-synaptic currents (EPSCs) were elicited using local, minimal stimulation to mitigate voltage- and space-clamp errors (Stevens and Wang, 1994; Wilcox *et al*, 1996). A bipolar stimulating electrode was placed near the recording electrode ($<$300 μm). The stimulating electrode was used to deliver current pulses (100-μs duration) of sufficient amplitude to produce the smallest EPSC (25–40 pA) that could be reliably evoked at low frequency (0.1 Hz). To isolate and maximally activate NMDA receptor-mediated EPSCs, the Ringer solution contained 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione, 50 μM picrotoxin,

and 10 μM glycine. Data were acquired with an Axopatch 1D amplifier and the CLAMPEX8 software package interfaced to a Digidata 1200 acquisition board (Axon Instruments). Signals were filtered at 5 kHz and sampled at 10 kHz.

Only recordings not showing substantial changes in holding current or resistance at the electrode tip were used for analysis. All cells required <100 pA to be clamped to -70 mV. Cells with resting membrane potentials above -55 mV were omitted from analysis. The following parameters were determined for averaged NMDA receptor-mediated EPSCs: rise times, peak amplitudes, decay time constants, and weighted τ (τ_w). The decay time constants were fit with a double exponential equation: $I(t) = I_f\exp(-t/\tau_f) + I_s\exp(-t/\tau_s)$, where I_f is the amplitude of the fast component, I_s is the amplitude of the slow component, and τ_f and τ_s are the fast and slow time constants, respectively. Weighted time constants were calculated by using the following equation: $\tau_w = [I_f/(I_f + I_s)]\tau_f + [I_s/(I_f + I_s)]\tau_s$ (Stocca and Vicini, 1998). All data are presented as mean ± SEM.

Statistical Analysis

Dependent measures from animals used in the behavioral studies were compared across pretreatment and treatment groups using two-way ANOVAs and *post hoc* *t*-tests (JMP v.9.0). Dependent measures from animals used for electrophysiological studies were analyzed using unpaired *t*-tests for the striatal region of interest.

RESULTS

DAT and SERT Autoradiography

Pretreatment of rats used for the behavioral studies with a 'binge' regimen of METH resulted in significant decreases in striatal DAT binding. METH-pretreated rats in the AP5 experiment had significantly less DAT binding than saline-pretreated rats in the striatum (Figure 2a; DM striatum: mean ± SEM, 54.6 ± 6.4% of saline, $F_{(1,22)} = 12.8$, $p < 0.01$; DL striatum: 61.1 ± 6.1%, $F_{(1,22)} = 12.2$, $p < 0.01$). A similar decrease in DAT binding was seen in METH-pretreated rats in the *Arc* antisense experiment (graph not shown; DM striatum: 58.4 ± 6.9% of saline, $F_{(1,29)} = 18.4$, $p < 0.001$; DL striatum: 66.4 ± 6.6%, $F_{(1,29)} = 14.2$, $p < 0.001$). METH-pretreated rats also had significantly decreased SERT binding relative to saline-pretreated controls in all prefrontal regions examined (Figure 2b): prelimbic, 57.8 ± 9.5% of saline, $F_{(1,17)} = 14.3$, $p < 0.01$; infralimbic, 73.6 ± 7.3%, $F_{(1,17)} = 14.5$, $p < 0.01$; medial orbitofrontal, 58.9 ± 9.9%, $F_{(1,17)} = 13.8$, $p < 0.01$; ventral orbitofrontal, 47.3 ± 9.2%, $F_{(1,17)} = 25.6$, $p < 0.0001$; lateral orbitofrontal, 49.4 ± 9.4%, $F_{(1,17)} = 20.0$, $p < 0.001$; and cingulate, 44.7 ± 10.9%, $F_{(1,17)} = 19.7$, $p < 0.001$ cortices.

Effects of Acute NMDA Receptor Blockade in the DM Striatum

As reported previously by our lab (Daberkow *et al*, 2008), METH-pretreated rats appear to be behaviorally normal in terms of motor response reversal learning on the T-maze relative to saline-pretreated rats (Figure 3). However, acute disruption of striatal function through bilateral infusion of AP5 into the DM striatum revealed differences in DM striatal involvement in this learning. Analysis revealed a significant overall interaction (pretreatment × infusion; $F_{(1,1)} = 4.6$, $p < 0.05$), as well as significant main effects of pretreatment ($F_{(1,1)} = 5.2$, $p < 0.05$) and infusion ($F_{(1,1)} = 7.6$, $p < 0.05$). Saline-pretreated rats that were infused with AP5 ($n = 5$) required significantly more trials to reach criterion than saline-pretreated, PBS-infused rats (Figure 3; $n = 8$; $t_{(23)} = -3.4$, $p < 0.01$). METH-pretreated rats ($n = 5$), on the other hand, were unaffected by infusion of AP5, and thus were significantly different from saline-pretreated, AP5-infused rats ($t_{(23)} = 2.8$, $p < 0.05$), but not METH-pretreated, PBS-infused ($n = 9$; $t_{(23)} = -0.4$, $p = 0.7$) or saline-pretreated, PBS-infused ($t_{(23)} = -0.3$, $p = 0.7$) rats.

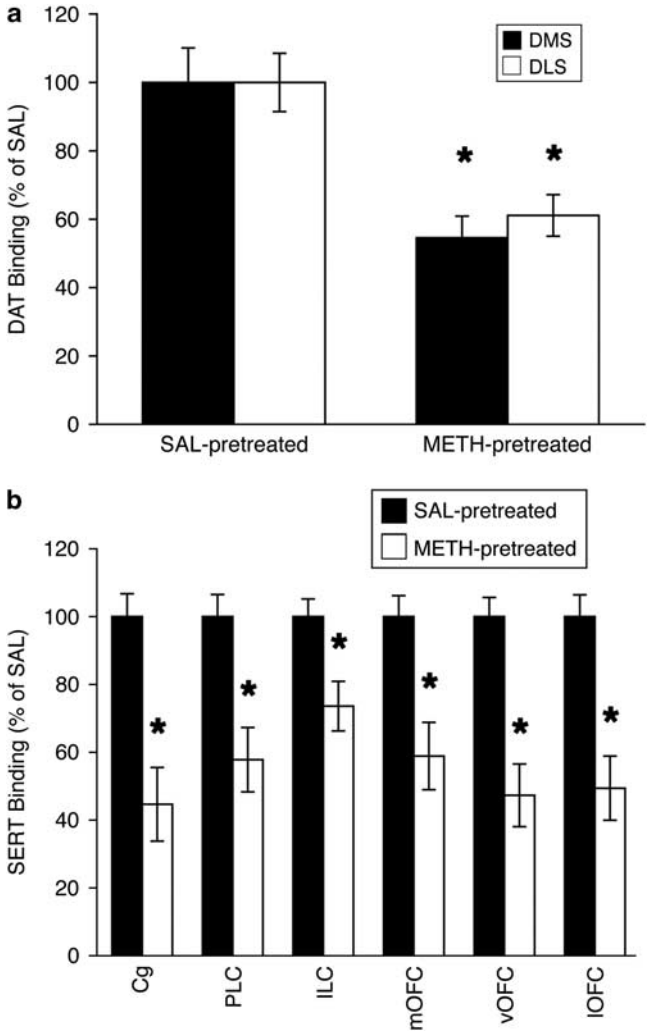

Figure 2 DAT and SERT binding. (a) DAT decreases (mean ± SEM), expressed as percent of average values in saline-pretreated controls, in rats pretreated with (±)-METH (4 × 10 mg/kg, 2-h intervals; $n = 25$) or saline (SAL; $n = 29$) approximately 7 weeks prior to being killed. (b) SERT decreases (mean ± SEM), expressed as percent of average values in saline-pretreated controls, in rats pretreated with METH ($n = 12$) or saline ($n = 11$) approximately 7 weeks after METH pretreatment. *Significantly different from SAL-pretreated values for the same brain region ($p < 0.01$). Cg, cingulate cortex; PLC, prelimbic cortex; ILC, infralimbic cortex; mOFC, medial orbitofrontal cortex; vOFC, ventral OFC; lOFC, lateral OFC.

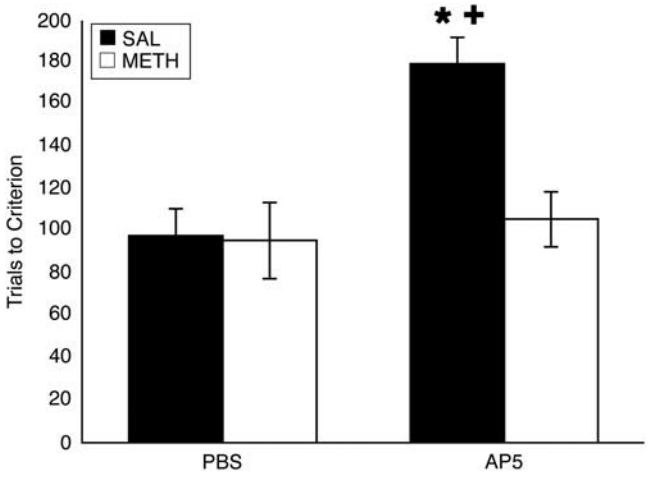

Figure 3 Effects of acute NMDA receptor blockade in the DM striatum. Mean trials to criterion (9/10 correct consecutive trials; ± SEM) on a motor response reversal task. Rats were given bilateral infusions of AP5 or PBS 5 min prior to the beginning of reversal learning. *Significantly different from SAL-pretreated, PBS-infused rats ($p<0.01$). [+]Significantly different from METH-pretreated, AP5-infused rats ($p<0.05$).

Effects of Acute Arc Disruption in the DM Striatum

Consistent with prior reports making use of *Arc* antisense in different brain regions and in different learning and memory paradigms (Guzowski *et al*, 2000; Hearing *et al*, 2010), we observed no effects of *Arc* antisense infusion into the DM striatum on initial reversal learning in either saline- or METH-pretreated rats (Figure 4a). Two-way ANOVA on trials to criterion on the reversal learning task revealed no main effects of pretreatment ($F_{(1,30)}=0.01$, $p=0.9$) or infusion ($F_{(1,30)}=0.69$, $p=0.5$), and no interaction ($F_{(1,2)}=0.09$, $p=0.9$). However, again consistent with prior reports (Guzowski *et al*, 2000; Hearing *et al*, 2010), analysis of retention of the reversal learning 24 h after the initial reversal learning task revealed a significant overall interaction ($F_{(1,2)}=4.07$, $p<0.05$) but no main effects of pretreatment ($F_{(1,30)}=0.9$, $p=0.3$) or infusion ($F_{(1,30)}=1.22$, $p=0.3$). Saline-pretreated rats infused with *Arc* antisense ($n=13$) took significantly more trials to reach criterion on the retention test (Figure 4b) than controls (saline-pretreated, *Arc* nonsense-infused: $n=4$, $t_{(30)}=-1.8$, $p<0.05$; saline-pretreated, PBS-infused: $n=4$, $t_{(30)}=-2.8$, $p<0.01$) and METH-pretreated, *Arc* antisense-infused rats ($n=8$, $t_{(30)}=3.7$, $p<0.001$). METH-pretreated, *Arc* antisense-infused rats, however, were not significantly different from the control groups (METH-pretreated, *Arc* nonsense-infused: $n=4$, $t_{(30)}=1.6$, $p=0.1$; METH-pretreated, PBS-infused: $n=3$, $t_{(30)}=0.4$, $p=0.7$). These results indicate that *Arc* in the DM striatum is necessary for consolidation of response reversal learning under normal conditions, but not in rats with METH-induced neurotoxicity.

Error Analysis

As demonstrated previously (Daberkow *et al*, 2008) and again in this study (Figure 3), METH-pretreated rats perform as well as normal rats on response reversal learning on the T-maze. We also analyzed the types of errors

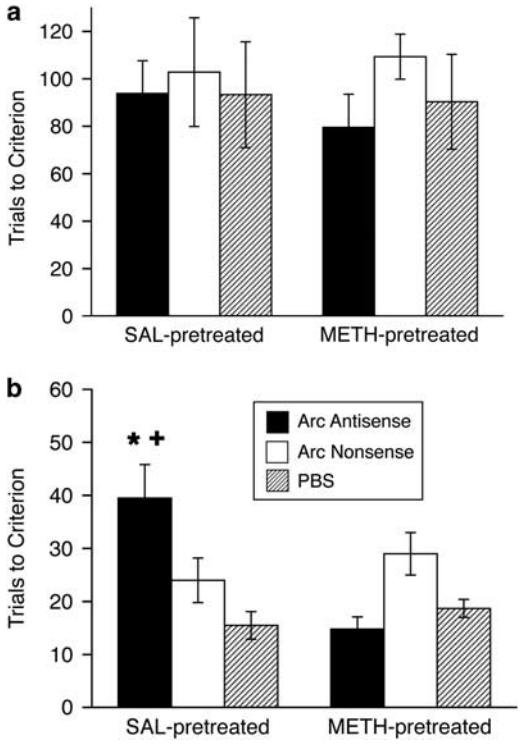

Figure 4 Effects of acute *Arc* disruption in the DM striatum. Mean trials to criterion (± SEM) on the motor response reversal task (a) and the reversal retention task (b). (a) Rats were given bilateral infusions of an *Arc* antisense oligonucleotide, an *Arc* nonsense oligonucleotide, or PBS 2 h prior to beginning reversal training. No significant interactions or main effects of pretreatment or infusion were found. (b) Rats were tested on retention of the previous day's reversal learning. No further infusions were made. *Significantly different from SAL-pretreated, *Arc* nonsense oligonucleotide and PBS controls ($p<0.05$). [+]Significantly different from METH-pretreated, *Arc* antisense-infused rats ($p<0.05$).

(Palencia and Ragozzino, 2004) made by METH- or saline-pretreated, PBS-infused rats to determine whether the METH-induced monoamine depletions altered behavioral flexibility. Consistent with the lack of effect on trials to criterion, we found no differences between METH- and saline-pretreated rats in numbers of perseverative (saline-pretreated, 25.5 ± 7.4; METH-pretreated, 28.7 ± 6.5; $t_{(15)}=-0.3$, $p=0.8$) or regressive (saline-pretreated, 35.9 ± 8.8; METH-pretreated, 45.7 ± 11.0; $t_{(15)}=-0.7$, $p=0.5$) errors.

In Situ Hybridization Histochemistry for Striatal Grin2a Subunit

The pharmacological properties of NMDA receptors are determined to a large extent by the subunit composition of the receptors, with the NR2 subunits being of critical importance in this regard. In particular, prior work has shown that NR2a subunit incorporation yields NMDA receptors with higher affinity for competitive antagonists such as AP5 (Buller *et al*, 1994). Therefore, to assess the possibility that the lack of effect of AP5 infusion into the DM striatum reflects a change in the pharmacological properties of NMDA receptors in the DM striatum in METH-pretreated rats, we examined the expression of the NMDA receptor NR2a subunit in the striatum of saline- and

METH-pretreated rats. In this experiment, the METH-pretreated rats had significant depletions of striatal DA (Table 1). These depletions, as determined by HPLC-ECD analysis of tissue DA content in the striatum, are slightly larger than those observed in the cohorts of rats used for the behavioral experiments described above. Other work in our laboratory (unpublished observations) indicates that the magnitude of the DA depletions estimated by DAT binding is typically less than the magnitude measured through determination of DA tissue content, although the two measures are very highly and significantly correlated (r^2 values of 0.8–0.9). Thus, although the magnitude of the depletions in this cohort of animals used for determination of NMDA receptor expression and function after METH treatment appears to be greater, we think that they are roughly equivalent degrees of depletion and that any difference simply reflects subtle differences in the actual magnitude of depletion induced in different cohorts of animals treated with METH at different times and by different investigators.

Analysis of film autoradiograms for Grin2a mRNA expression in striatal sections (+0.7 mm from bregma) from these saline- and METH-pretreated rats revealed a main effect of region ($F_{(2,39)}=6.74$, $p<0.01$), but no main effect of pretreatment ($F_{(1,39)}=0.2$, $p=0.7$) and no significant interaction ($F_{(1,2)}=0.05$, $p=0.95$) (Figure 5g). *Post-hoc* analysis confirmed previous reports (Buller *et al*, 1994; Ganguly and Keefe, 2001; Standaert *et al*, 1999) of greater Grin2a mRNA expression in both the DM ($t_{(39)}=-2.1$, $p<0.05$) and DL ($t_{(39)}=-3.7$, $p<0.001$) striatum relative to the VM striatum.

Electrophysiological Properties of NMDA Receptor-Mediated EPSCs

To further assess whether there might be changes in the properties of NMDA receptors in striatal efferent neurons induced by METH exposure and whether this might underlie the differential sensitivity of the METH-pretreated rats to AP5 and *Arc* antisense oligonucleotide infusion, we compared NMDA receptor-mediated EPSCs from both the DL and VM aspects of the striatum of both saline- and METH-pretreated rats, as there are regional differences in NMDA receptor function in the adult striatum (Chapman *et al*, 2003). As we have reported previously (Chapman *et al*, 2003), the kinetics of the NMDA receptor-mediated EPSCs were faster in the DL than the VM striatum; however, prior exposure to a neurotoxic regimen of METH did not change the kinetics (Figures 5 and 6). That is, the rise times (Figure 6a; main effect of region, $F_{(1,72)}=9.64$, $p<0.01$), τ_f (Figures 5 and 6b; main effect of region, $F_{(1,78)}=11.27$, $p=0.001$), and τ_w (Figures 5 and 6d; main effect of region, $F_{(1,78)}=72.66$, $p<0.0001$) were significantly faster in the DL striatum, consistent with the greater expression of the NMDA receptor Grin2a subunit in that region of the striatum. There was also a trend for τ_s to be faster in the DL striatum, although the main effect of region was not statistically significant ($F_{(1,78)}=1.13$, $p=0.3$). However, for none of these kinetic parameters was there a significant main effect of pretreatment (rise times, $F_{(1,72)}=0.0001$, $p=0.99$; τ_f, $F_{(1,78)}=0.03$, $p=0.9$; τ_s, $F_{(1,78)}=0.02$, $p=0.9$; τ_w, $F_{(1,78)}=0.2$, $p=0.7$) or a significant interaction (rise times, $F_{(1,72)}=1.2$, $p=0.3$; τ_f, $F_{(1,78)}=0.4$, $p=0.5$; τ_s, $F_{(1,78)}=2.1$, $p=0.2$; τ_w, $F_{(1,78)}=1.0$, $p=0.3$), indicating that METH-induced neurotoxicity was not associated with changes in the fundamental subunit composition or electrophysiological characteristics of NMDA receptors in the dorsal striatum.

Table 1 Striatal DA Tissue Content 3 Weeks after a Neurotoxic Regimen of METH

Treatment	Striatal DA tissue content
	Dorsolateral
Saline ($n=8$)	314 ± 26
METH ($n=8$)	98 ± 15[a]
	Ventromedial
Saline ($n=8$)	282 ± 26
METH ($n=8$)	94 ± 26[a]

Values are average (± SEM) DA content (ng DA/mg protein) in striatal tissue determined by HPLC-ECD analysis of 1-mm^3 tissue punches from the dorsolateral or ventromedial striatum. Values are ng DA/mg protein.
[a]Significantly different from saline ($p<0.05$).

DISCUSSION

This study confirms previous observations that the DM striatum is involved in motor response reversal learning (Palencia and Ragozzino, 2004) and that METH-pretreated rats appear behaviorally normal on this task (Daberkow *et al*, 2008). However, the present results extend these prior observations in three important ways. First, the present results establish a critical role for *Arc* in the DM striatum in consolidation of reversal learning in normal rats. Second, they provide additional support, in a novel brain area, for the hypothesis put forth by Guzowski *et al* (2001) that the correlation between *Arc* mRNA in a brain region and behavioral performance reflects task-relevant encoding processes occurring in that brain area. Finally, the present results provide the first direct evidence that METH-induced neurotoxicity is associated with a change in the neural substrates engaged to solve a behavioral task normally dependent on the DM striatum. These results therefore highlight the critical importance of striatal *Arc* for consolidation of basal ganglia-mediated learning and suggest that long-term toxicity induced by METH alters neural circuits and/or cognitive strategies used to solve tasks normally mediated by the dorsal striatum.

The present data provide the first direct evidence that *Arc* is a critical mediator of consolidation of reversal learning mediated by the DM striatum. This brain region has previously been implicated in cognitive flexibility, including that required for motor response reversal learning. In particular, Ragozzino *et al* (2002) have established previously that acute blockade of cholinergic muscarinic or glutamatergic NMDA receptors in the DM striatum impairs response reversal learning (Palencia and Ragozzino, 2004; Ragozzino *et al*, 2002). Additionally, depletion of DA, but not serotonin, in the DM striatum impairs reversal learning

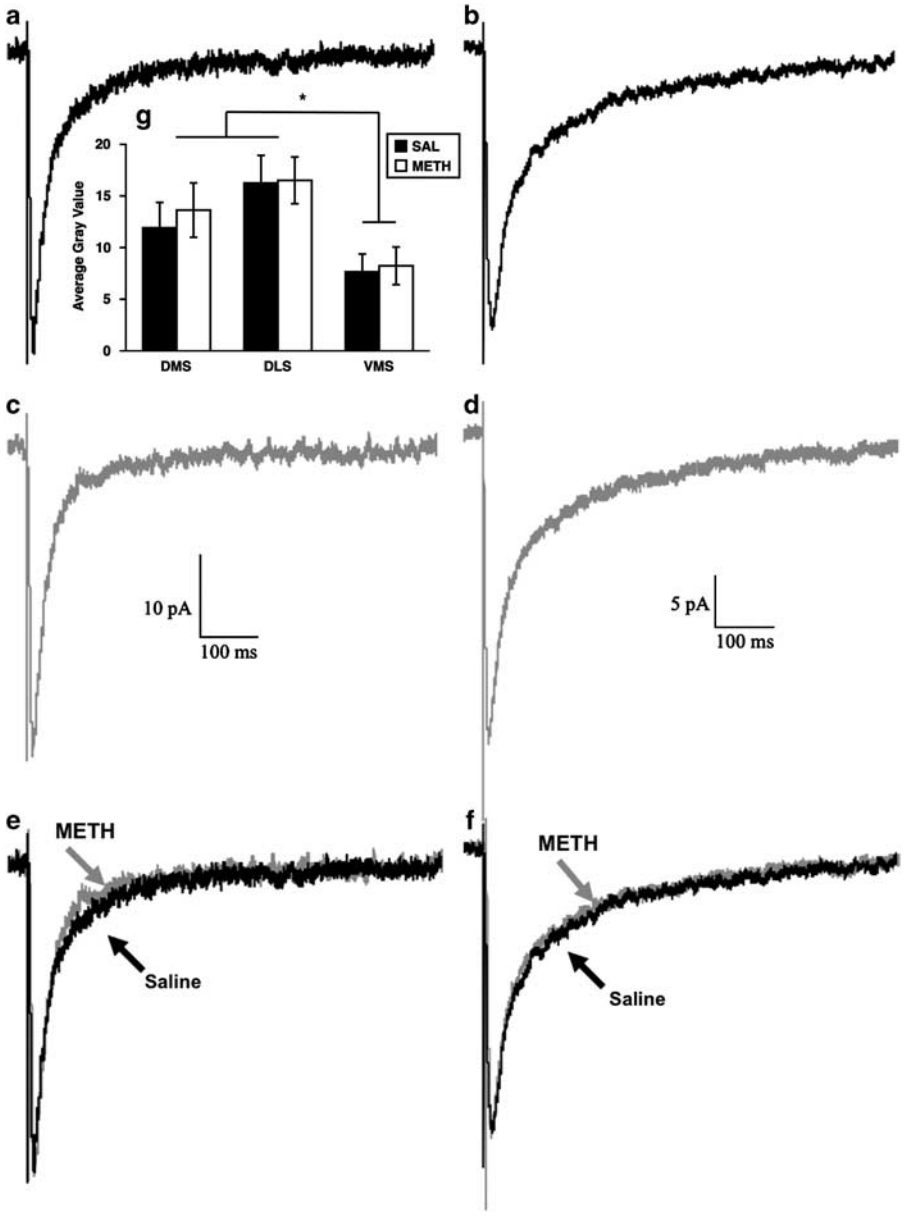

Figure 5 Grin2a mRNA expression and decay kinetics of NMDA receptor-mediated EPSCs. (a–f) Local, minimal stimulation of the striatum in proximity (<300 μm) to the recorded cell elicits a long-lasting, NMDA receptor-mediated EPSC in the striatum. The average of 35 EPSCs evoked at 0.1 Hz is shown. Representative traces showing the decay-time kinetics of NMDA receptor-mediated EPSCs in the dorsolateral (a, c, e) and ventromedial (b, d, f) striatum of saline- (a, b) and METH- (c, d) pretreated rats are shown, as are normalized, superimposed traces from the DL (e) and VM (f) striatum. (g) Grin2a mRNA expression in the DL, DM, and VM striatum from the hemisphere opposite to that used for electrophysiological recordings. Data are average gray values (±SEM) from densitometric analysis of film autoradiograms. *Both the DM and DL striatum are significantly different from the VM striatum ($p<0.01$).

(Clarke *et al*, 2011; O'Neill and Brown, 2007). Furthermore, we have demonstrated previously that, in normal animals, there is a correlation between *Arc* mRNA in the DM, but not DL, striatum and trials to criterion on a response reversal learning task (Daberkow *et al*, 2007). Guzowski *et al* (2001) initially reported such a correlation between *Arc* expression in the hippocampus and spatial learning on the Morris water maze, leading them to speculate that such correlations reflect the involvement of the encoding processes in that particular brain region in the consolidation of spatial learning. Therefore, we proposed (Daberkow *et al*, 2007) that the correlation between *Arc* mRNA in the DM striatum and trials to criterion on the reversal learning task reflected the fact that this reversal is normally dependent on DM striatal function, and that *Arc* must be a critical mediator of plasticity in the DM striatum underlying consolidation of reversal learning. The present results support this hypothesis, as infusion of an *Arc* antisense oligonucleotide, but not a scrambled oligonucleotide or a vehicle, into the DM striatum impaired performance in normal rats on a reversal retention test administered 24 h later. Taken together with prior results showing that *Arc* antisense oligonucleotide infusions into the DL striatum disrupt consolidation of extinction learning occurring during context-induced

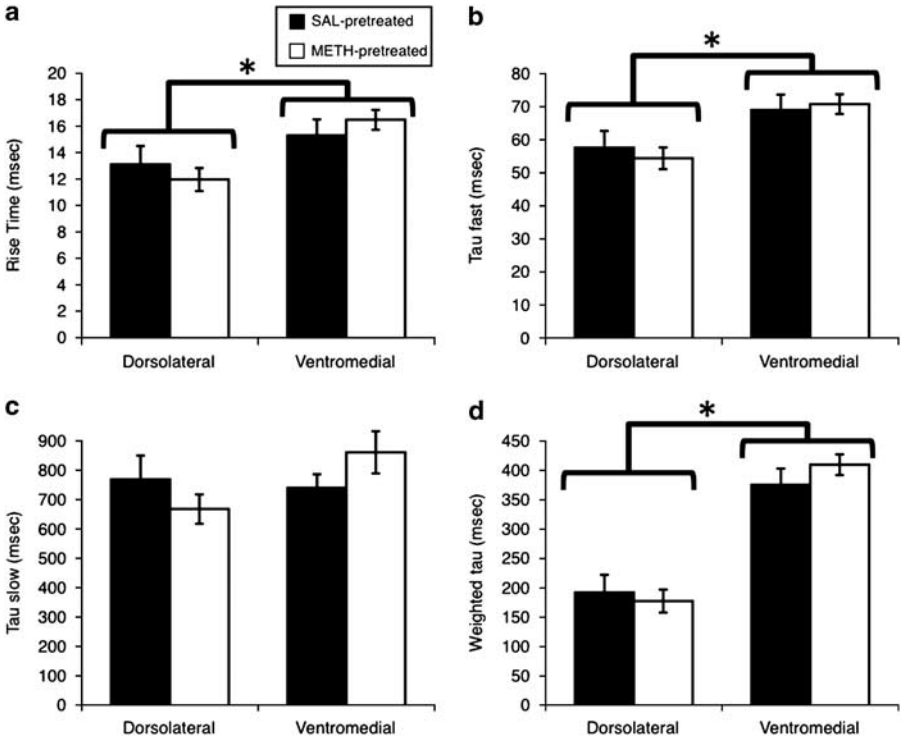

Figure 6 Kinetic properties of striatal NMDA receptor-mediated EPSCs in saline- and METH-pretreated rats. The values are average kinetic parameters (±SEM) calculated from whole-cell, patch-clamp recordings of NMDA receptor-mediated EPSCs in the dorsolateral and ventromedial striata of rats pretreated with saline (SAL-pretreated; $n = 10$ for DL, $n = 12$ for VM) or a neurotoxic regimen of METH (METH-pretreated; $n = 25$ for DL, $n = 19$ for VM). (a) 10–90% rise time. The decay of the EPSCs was fit with a double exponential equation, $I(t) = I_f\exp(-t/\tau_f) + I_s\exp(-t/\tau_s)$, yielding fast (b; τ fast) and slow (c; τ slow) time constants. Weighted time constants (d; weighted τ) were calculated by using the following equation: $\tau_w = [I_f/(I_f + I_s)]\tau_f + [I_s/(I_f + I_s)]\tau_s$ (Stocca and Vicini, 1998). *Significant main effect of region ($p < 0.05$).

reinstatement of cocaine-seeking behavior (Hearing *et al*, 2010), the data strongly implicate *Arc* as a general, critical mediator of encoding processes underlying striatally based learning and memory functions.

Our previous studies of rats with METH-induced neurotoxicity have shown that, although these rats appear to be behaviorally normal with respect to response reversal learning, *Arc* induction in the DM striatum is attenuated and no longer correlates with trials to criterion, leading us to hypothesize that METH-induced neurotoxicity promotes a shift in the neural substrates mediating this behavior (Daberkow *et al*, 2008). The present findings support this hypothesis: in rats with METH-induced neurotoxicity, acute disruption of DM striatal function by infusion of the NMDA receptor antagonist AP5 or an *Arc* antisense oligonucleotide fails to alter response reversal learning or its retention. Thus, although rats with METH-induced neurotoxicity appear to be normal on the surface, the neural substrates mediating the behavior have changed. These findings are similar to those reported, for example, in Parkinson's disease patients, in which behavior appears unimpaired relative to controls, but functional imaging reveals a change in the brain regions engaged during the task (Moody *et al*, 2004). These findings highlight the need for studies assessing the impact of neurotoxicity on learning and memory to examine not simply behavioral measures of the learning, but also the processes and brain regions mediating the behavior, before concluding that there is a lack of effect of such toxicity on a particular behavior.

It is conceivable that the lack of effect of acute disruption of NMDA receptor and *Arc* function in the DM striatum on reversal learning and its consolidation reflects a decrease in sensitivity of the DM striatum to these manipulations, rather than a reorganization of the neural circuitry mediating the behavior. However, we think that this former possibility is unlikely, as *in situ* hybridization histochemical analysis of Grin2a mRNA expression and electrophysiological determination of the biophysical properties of striatal NMDA receptors failed to reveal any METH-induced changes in these NMDA receptor subunits or properties. The pharmacology of NMDA receptors is heavily influenced by Grin2 subunit incorporation into the receptor (Buller *et al*, 1994; Traynelis *et al*, 2010), as are the rise time and decay kinetics of the NMDA receptor-mediated current, with Grin2a-containing receptors showing the fastest kinetics (Dingledine *et al*, 1999). Striatal efferent neurons, which are the striatal neurons in which *Arc* is expressed (Vazdarjanova *et al*, 2006), express the Grin2a and Grin2B subunits (Standaert *et al*, 1999). The present results confirm our prior observations and those of others that there is greater expression of Grin2a subunits in the DL than VM striatum (Buller *et al*, 1994; Ganguly and Keefe, 2001; Standaert *et al*, 1999), and that the rise times and decay kinetics of these currents are correspondingly faster in the DL than in VM striatum (Chapman *et al*, 2003). These results illustrate our ability to detect differences in the subunit composition of the NMDA receptor using this electrophysiological approach. Importantly, METH-induced

neurotoxicity was not associated with changes in Grin2a subunit mRNA expression or in the biophysical properties of the NMDA receptors in the dorsal striatum, strongly suggesting that METH-induced neurotoxicity is not associated with changes in the subunit composition, and thus the pharmacology, of striatal NMDA receptors. It therefore seems unlikely that a change in the sensitivity of NMDA receptors in METH-pretreated rats to AP5 or endogenous glutamate underlies the lack of efficacy of acute AP5 infusion or *Arc* antisense infusion in those animals in the present studies. Rather, the data suggest that the lack of effect of these agents more likely reflects a change in the neural circuitry engaged in the reversal learning task.

The consequences of METH exposure that lead to this apparent shift in behavioral control are currently unknown; however, the METH-induced partial loss of DA in the DM striatum may be the basis. As is typical (Chapman *et al*, 2001; Hanson *et al*, 2009), the binge regimen of METH resulted in an approximately 40% loss of DA tissue content, as measured by DAT levels, in the DM striatum at the end of the behavioral training. Although METH also induces a loss of serotonin in the DM striatum (Haughey *et al*, 1999), as noted above, DA, not serotonin, neurotransmission in the DM striatum appears to mediate reversal learning (Clarke *et al*, 2011; Darvas and Palmiter, 2011; O'Neill and Brown, 2007). Thus, one strong possibility is that it is the partial loss of DA in the DM striatum that results in the change in sensitivity of response reversal learning to acute manipulations of DM striatal function in METH-pretreated rats.

An alternative possibility is that METH-induced damage to extra-striatal serotonin systems disrupts the function of afferents to the DM striatum or other neural substrates necessary for reversal learning, thereby altering the circuitry engaged during the reversal learning. The neurotoxic regimen of METH used in the present study also induces a loss of serotonin in the prefrontal cortex (Hotchkiss and Gibb, 1980; Ricaurte *et al*, 1980), including an approximately 50% loss of SERT binding in the orbitofrontal cortex (OFC) reported here. Serotonin function in the OFC is known to be critical for reversal learning (Clarke *et al*, 2005, 2007; Robbins and Arnsten, 2009). Thus, changes in the function of the OFC as a consequence of METH-induced neurotoxicity to that region may contribute to the changes in reversal learning observed in the present study. However, the OFC tends to provide afferent innervation to the central and lateral aspects of the dorsal striatum, as well as the nucleus accumbens, and largely does not provide afferents to the DM striatum (Schilman *et al*, 2008). On the other hand, the prelimbic cortex does project strongly into the DM striatum (Lévesque and Parent, 1998; Vertes, 2006). As presented here, a neurotoxic regimen of METH results in about a 40% loss of SERT in the prelimbic cortex. Furthermore, the prelimbic cortex has a role in reversal learning, although the role is more in controlling complex, higher-order set-shifting tasks, rather than simple one-dimensional reversal learning such as the T-maze task used in this study (Birrell and Brown, 2000; Ragozzino, 2003; Ragozzino *et al*, 1999). Finally, the centromedian and paracentral nuclei of the thalamus provide excitatory innervation to the DM striatum (Van der Werf *et al*, 2002). These thalamic nuclei receive relatively dense serotonergic innervation (Vertes *et al*, 2010), and data obtained from abstinent human METH abusers suggest decreased SERT binding in the thalamus (Sekine *et al*, 2006). Thus, METH-induced alterations in the function of excitatory afferents from intralaminar cell groups to the DM striatum might also have a role in the disruption of DM striatal control over reversal learning observed in the present studies. However, the extent to which neurotoxic regimens of METH damage the intralaminar nuclei of the thalamus in rodents has not heretofore been reported. Clearly, further studies examining the effects of selective DA depletions induced by substituted amphetamines *vs* the effects of combined DA/serotonin depletions will be necessary to conclusively rule out a contribution of serotonin loss to the changes in behavioral control observed in the METH-pretreated rats.

An interesting aspect of the present findings is that METH-pretreated rats appear to be behaviorally normal, both in terms of trials to criterion and in the types of errors made during reversal learning. The neural substrates capable of supporting apparently normal reversal learning despite altered DM striatal function remain to be determined. One possibility for an alternate neural substrate is the nucleus accumbens core, which has been implicated in behavioral flexibility (Darvas and Palmiter, 2011; Goto and Grace, 2005; Haluk and Floresco, 2009). The 'binge' regimen of METH exposure often does not induce as much monoamine loss in the nucleus accumbens as in the dorsal striatum (Eisch *et al*, 1992; Haughey *et al*, 1999), and DA signaling in the accumbens has a role in simple reversal learning (Darvas and Palmiter, 2011; Haluk and Floresco, 2009). Future studies thus will be necessary to determine the role of the nucleus accumbens in reversal learning in METH-pretreated rats, the circumstances under which DM striatal *vs* nucleus accumbens DA signaling normally supports behavioral flexibility, and the cognitive cost associated with loss of DM striatal control over behavioral flexibility.

In summary, the present study provides the first evidence that *Arc* in the DM striatum is a critical mediator underlying consolidation of motor response reversal learning, thereby further validating its importance as a molecular substrate of learning and memory function. Furthermore, the present results are the first to show that METH-induced neurotoxicity is associated with a change in the neural substrates underlying basal ganglia-mediated learning and memory, despite the fact that behavioral indices of that learning appear to be normal. These findings suggest that METH-induced neurotoxicity may have important ramifications for the ability of individuals with a history of METH abuse to engage in cognitive behavioral therapies for management of drug addiction, as well as the extent to which they can function optimally in tasks related to their employment and personal lives. Further studies are therefore needed to fully understand the molecular, cellular, and systems level substrates mediating learning and memory processes in corticostriatal circuits that are compromised by METH-induced monoamine loss, and to design approaches to mitigate such effects.

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DISCLOSURE

The authors declare no conflict of interest.

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