Title: Changes in neural circuitry regulating response-reversal learning and *Arc*-mediated consolidation of learning in rats with methamphetamine-induced partial monoamine loss

Running title: METH toxicity alters reversal-learning circuitry

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Abstract

Methamphetamine-induced neurotoxicity results in long-lasting depletions of monoamines and changes in basal ganglia function. We previously reported that rats with methamphetamine-induced neurotoxicity no longer engage dorsomedial striatum during a response reversal-learning task, as their performance is insensitive to acute disruption of dorsomedial striatal function by local infusion of an *N*-methyl-D-aspartate receptor antagonist or an antisense oligonucleotide against the activity-regulated cytoskeleton-associated (*Arc*) gene. However, methamphetamine-pretreated rats perform the task as well as controls. Therefore, we hypothesized that the neural circuitry involved in the learning had changed in methamphetamine-pretreated rats. To test this hypothesis, rats were pretreated with a neurotoxic regimen of methamphetamine or with saline. Three to five weeks later, rats were trained on the reversal-learning task and *in situ* hybridization for *Arc* was performed. A significant correlation between *Arc* expression and performance on the task was found in nucleus accumbens shell of methamphetamine-, but not saline-, pretreated rats. Consistent with the idea that the correlation between *Arc* expression in a brain region and behavioral performance implicates that brain region in the learning, infusion of an antisense oligonucleotide against *Arc* into the shell impaired consolidation of reversal learning in methamphetamine-, but not saline-, pretreated rats. These findings provide novel evidence suggesting that methamphetamine-induced neurotoxicity leads to a shift from dorsal to ventral striatal involvement in the reversal-learning task. Such reorganization of neural circuitry underlying learning and memory processes may contribute to

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impaired cognitive function in individuals with methamphetamine-induced neurotoxicity or others with striatal dopamine loss, such as patients with Parkinson's disease.

Key words: *Arc*, reversal learning, memory consolidation, methamphetamine, striatum, nucleus accumbens

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Introduction

Methamphetamine (METH) abuse continues to have considerable societal impact, with 12 million Americans reporting use in their lifetime (2011 National Survey on Drug Use and Health, SAMHSA). METH abuse in humans causes decreases in the dopamine transporter (DAT) (Wilson *et al*, 1996) and serotonin transporter (SERT) (Sekine *et al*, 2006). Further, recent data indicate that people with a history of hospitalization for METH abuse are at higher risk of developing Parkinson's disease (Callaghan *et al*, 2012).

The monoamine loss resulting from METH abuse in humans can be recapitulated in rodents. METH-induced neurotoxicity causes partial depletions of dopamine (DA) and serotonin (5-HT) (Wagner *et al*, 1980). As in human METH abusers (Dean *et al*, 2013), this partial monoamine loss is associated with cognitive deficits, including impairments in odor and object recognition, attentional set-shifting (Marshall and O'Dell, 2012), sequential motor learning (Chapman *et al*, 2001; Daberkow *et al*, 2005), formation of stimulus-response associations (Son *et al*, 2011), and inhibitory control over behavior (Son *et al*, 2013). The deficits in basal ganglia-mediated behaviors may arise secondary to impaired phasic DA neurotransmission in the partially denervated striatum (Howard *et al*, 2013a; Howard *et al*, 2011).

Arc (activity-regulated cytoskeleton-associated gene) is an effector immediate-early gene involved in synaptic plasticity and memory consolidation (Shepherd and Bear, 2011). Hippocampal *Arc* expression correlates with performance and is necessary for memory consolidation on the spatial version of the Morris water maze (Guzowski *et al*, 2000; Guzowski *et al*, 2001). Similarly, our lab has reported correlations between *Arc*

mRNA in dorsomedial striatum (DMS) and performance on a striatally-mediated response reversal-learning task in normal (Daberkow *et al*, 2007, 2008), but not METH-pretreated (Daberkow *et al*, 2008), rats, suggesting that although METH-pretreated rats perform as well as normal rats on the reversal-learning task, they may rely on different brain circuitry to perform the task (Daberkow *et al*, 2008; Pastuzyn *et al*, 2012). To test this hypothesis, we looked for correlations between *Arc* mRNA expression in different brain regions and reversal learning in METH-pretreated rats relative to controls. We found a significant correlation in the nucleus accumbens (NAc) shell in METH-pretreated rats that did not exist in saline-pretreated rats. Further, disruption of *Arc* signaling in the NAc shell of METH-, but not saline-, pretreated rats impaired consolidation of the reversal learning. Taken together with our previously published observations (Pastuzyn *et al*, 2012), these data suggest that METH-induced neurotoxicity is associated with reorganization of neural circuitry engaged in a learning and memory task typically dependent on DMS, and that correlations between *Arc* mRNA expression in brain regions and behavioral performance may be a viable *ex vivo* approach for mapping neural circuitry engaged in learning and memory tasks.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC, USA; 275-300 g) were singly housed in tub cages on a 12:12 hr light cycle. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Utah and followed the *Guide for the Care and Use of Laboratory Animals*.

Methamphetamine pretreatment

Rats were treated with a neurotoxic regimen of (±)-METH-HCl (4x10 mg/kg, free base, at 2-hr intervals, s.c.; NIDA, Research Triangle Park, NC, USA) over the course of one day as previously described (Daberkow *et al*, 2008; Son *et al*, 2013). The day after treatment, rats were returned to their home cages and given free access to food and water until training began.

Dopamine and serotonin transporter autoradiography

DAT and SERT autoradiography was performed as detailed previously (Barker-Haliski *et al*, 2012a; Boja *et al*, 1992; Pastuzyn *et al*, 2012; Son *et al*, 2013). For striatal sections, the buffer contained fluoxetine (Sigma-Aldrich, St. Louis, MO, USA) to block binding to the SERT, whereas for prefrontal cortical (PFC) sections, fluoxetine was omitted from the buffer, as PFC sections incubated in buffer containing fluoxetine showed no staining (data not shown). Slides were apposed to film (Kodak Biomax MR film; Eastman Kodak, Rochester, NY, USA) for 24 hr.

Arc *correlations with response-reversal learning*

Reversal-learning task

Response-reversal learning on a T-maze was conducted as previously described (Barker-Haliski *et al*, 2012b; Daberkow *et al*, 2007; Pastuzyn *et al*, 2012). Beginning three weeks after METH pretreatment, rats (METH-pretreated, *n*=9; saline-pretreated, *n*=10) were food restricted and habituated to the food reward and maze. The turn bias

of each rat was determined, followed by acquisition training for three days and then reversal learning. During reversal learning, 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. Five minutes after reaching criterion on reversal, rats were exposed to CO_2 for one minute and then sacrificed by decapitation. Brains were quickly removed, flash frozen in 2-methylbutane (Mallinckrodt Baker, Phillipsburg, NJ, USA) on dry ice, and stored at -80°C until sectioning.

Radioactive in situ *hybridization histochemistry*

Frozen brains were sectioned (12-µm; Cryocut 1800; Leica, Wetzlar, Germany). Sections from PFC (mm from Bregma: +3.7 to +2.2), striatum (+1.6 to -0.92 mm), and dorsal hippocampus (-2.3 to -3.6 mm) were thaw-mounted onto Superfrost Plus slides (VWR, Aurora, CO, USA) and stored at -20°C. Infusion cannula placements were determined by eye at this time and recorded on schematic diagrams from a rat brain atlas (Paxinos and Watson, 1998).

To assess *Arc* mRNA expression, slides containing striatal, PFC, or hippocampal sections were post-fixed and delipidated as previously described (Ganguly and Keefe, 2001). Detection of *Arc* mRNA was accomplished using a full-length ribonucleotide probe (Barker-Haliski *et al*, 2012a; Daberkow *et al*, 2007, 2008; Howard *et al*, 2013b). The plasmid containing the cDNA for *Arc* (Lyford *et al*, 1995) was linearized with *Eco*RI. The antisense ribonucleotide probe was transcribed using ^{35}S-UTP (striatum) or ^{33}P-UTP (PFC and hippocampus) (PerkinElmer, Waltham, MA, USA) and T7 RNA polymerase (Roche, Indianapolis, IN, USA). Radioactive *in situ* hybridization was

performed as previously described, with slightly modified final washing procedures (Ganguly and Keefe, 2001). Slides were apposed to film (Biomax MR) for four-six days.

Image analysis

Densitometric analysis of digitized film images was conducted using NIH ImageJ software, yielding background-subtracted average gray values in several brain regions from one hemisphere of each of the four sections on the slide. The regions analyzed were: cingulate (Cg1), prelimbic (PLC), infralimbic (ILC), ventral orbitofrontal (vOFC), and lateral orbitofrontal (lOFC) cortices; DMS and dorsolateral striatum (DLS) and nucleus accumbens (NAc) core and shell; CA1, CA3, upper and lower blades of dentate gyrus (DG), and hilus of the dorsal hippocampus. For cortical regions, all cortical layers were analyzed.

Effect of* Arc *antisense oligonucleotide infusion in nucleus accumbens shell

Two weeks after saline (*n*=11) or METH (*n*=13) pretreatment, rats were anesthetized with ketamine/xylazine (90/10 mg/kg, i.p.) and placed in a stereotaxic instrument (Stoelting Co., Wood Dale, IL, USA). A dual 26-gauge guide cannula (Plastics One, Roanoke, VA, USA) was lowered to end bilaterally just dorsal to NAc shell (mm from Bregma: +2.2 AP; ±1.0 ML; -6.4 DV) and secured. Bilateral infusions into NAc shell during behavioral experiments were made through 33-gauge infusion cannulae extending 1.1 mm beyond the end of the guides into NAc shell.

The reversal task was the same as described above, except that two hours before undergoing reversal learning, either an *Arc* antisense oligonucleotide, *Arc*

nonsense oligonucleotide, or 0.1M PBS (vehicle) was infused into NAc shell. The *Arc* antisense and nonsense oligonucleotides were prepared and infused (1 µL of oligonucleotide; 1 nmol/µL in 0.1M PBS, pH 7.4 or PBS) at 0.33 µL/min bilaterally into NAc shell, as previously described for DMS (Pastuzyn *et al*, 2012). The design of the oligonucleotides was based on the prior work of Guzowski and colleagues (Guzowski *et al*, 2000). Further, the concentration of oligonucleotide and volumes and rates of infusion were also based on that work, as well as that of other labs showing restricted delivery of the antisense oligonucleotide to specific brain regions, including the nucleus accumbens core, lateral amygdala, and anterior cingulate cortex (Holloway and McIntyre, 2011; Lv *et al*, 2011; Ploski *et al*, 2008). Post-infusion, rats rested in their home cages for two hours, were trained to criterion (9/10 correct trials) on reversal, and were returned to their home cages overnight. “Reversal retention” occurred 24 hr later, during which rats were rewarded for turning in the reversal direction learned the previous day, until criterion (9/10 correct trials) was reached. No further infusions were made on the reversal-retention day.

Statistical analysis

Unpaired *t*-tests were used to compare RTI-55 autoradiographic signals and trials to criterion on the reversal-learning task for the saline and METH pretreatment groups on which *ex vivo* analysis of *Arc* mRNA expression was completed. Trials to criterion on the reversal-learning task were also correlated with *Arc* mRNA expression. A two-factor MANOVA (pretreatment x day) followed by *post hoc* analysis with paired *t*-tests across acquisition days was used to assess any effect of METH pretreatment on trials to

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criterion on the three days of response acquisition. A two-way ANOVA was used to evaluate the effects of pretreatment (saline or METH) and treatment (infusion of *Arc* antisense, *Arc* nonsense, or PBS) on trials to criterion on the reversal-learning and reversal-retention tasks. All statistical tests were run using JMP v.9.0 (SAS Institute Inc., Cary, NC, USA).

Results

DAT and SERT autoradiography

The administration of METH resulted in significant reductions in striatal and NAc DAT binding (Fig. 1a). DAT depletions did not differ between rats used for *ex vivo* analysis of *Arc* mRNA expression and those used to examine the effects of *Arc* antisense oligonucleotide infusion into NAc shell, as two-way ANOVA revealed no main effect of "group" (*ex vivo Arc* or *Arc* antisense group: DMS, p=0.99; DLS, p=0.7; NAc core, p=0.97; NAc shell, p=0.5) and no group x pretreatment (METH or saline) interaction in DMS (p=0.8), DLS (p=0.9), NAc core (p=0.97), or NAc shell (p=0.5). There were main effects of pretreatment in all four striatal regions. Unpaired, one-tailed t-tests revealed a significant decrease in DAT binding in rats pretreated with METH in DMS (t=12.1, p=0.0001), DLS (t=9.2, p=0.0001), NAc core (t=5.0, p=0.0001), and NAc shell (t=3.3, p=0.002).

Pretreatment with the binge regimen of METH also resulted in reductions in SERT binding in PFC (Fig. 1b). As with DAT, SERT depletions were not significantly different between the two groups of rats, so the data were collapsed for the purpose of this analysis (no effect of group or group x pretreatment interaction, respectively, in Cg1

(*p*=0.5; *p*=0.5), PLC (*p*=0.3; *p*=0.3), ILC (*p*=0.4; *p*=0.4), vOFC (*p*=0.6; *p*=0.6), or lOFC (*p*=0.4; *p*=0.4)). Unpaired, one-tailed *t*-tests revealed significant decreases in SERT binding in all regions of PFC examined in METH-pretreated rats: Cg1, *t*=9.1, *p*=0.0001; PLC, *t*=6.1, *p*=0.0001; ILC, *t*=3.96, *p*=0.0003; vOFC, *t*=10.4, *p*=0.0001; lOFC, *t*=8.8, *p*=0.0001.

Effect of METH-induced neurotoxicity on trials to criterion for acquisition and reversal of response learning

As previously reported (Daberkow *et al*, 2008; Pastuzyn *et al*, 2012), there was no effect of METH pretreatment on acquisition of the response-learning task ($F_{(1,17)}$=0.2, *p*=0.6) and no pretreatment x acquisition day interaction ($F_{(2,16)}$=0.3, *p*=0.7). There was a main effect of acquisition day ($F_{(2,16)}$=8.4, *p*=0.03), with the rats overall taking significantly fewer trials to reach criterion on the third day of acquisition relative to both the second (*t*=-3.2, *p*=0.003) and first (*t*=-3.7, *p*=0.0008) days (data not shown). Rats with METH-induced monoamine depletions also did not differ from saline-pretreated controls in the numbers of trials to criterion on the reversal day (*t*=-0.9, *p*=0.4; data not shown).

Arc *mRNA expression*

Analysis of the film autoradiograms revealed no significant differences between the levels of *Arc* mRNA expression in METH- vs. saline-pretreated rats in DMS (*t*=0.2, *p*=0.8), DLS (*t*=0.2, *p*=0.8), NAc core (*t*=0.5, *p*=0.6), or NAc shell (*t*=1.6, *p*=0.1).

As in striatum, there was no significant effect of METH pretreatment on the levels of *Arc* mRNA expression as reflected in the radioactive *in situ* hybridization signal in

Cg1 (*t*=-1.1, *p*=0.3), PLC (*t*=-1.2, *p*=0.2), ILC (*t*=0.2, *p*=0.8), vOFC (*t*=-0.9, *p*=0.4), or lOFC (*t*=-0.8, *p*=0.4). In hippocampal subregions, there were also no significant differences between the intensity of the *Arc* mRNA signals in the METH- vs. the saline-pretreated rats in CA1 (*t*=-1.6, *p*=0.1), upper blade of the DG (*t*=-1.5, *p*=0.1), and lower blade of the DG (*t*=1.1, *p*=0.3). However, the intensity of the *Arc* mRNA *in situ* hybridization signal was significantly greater in METH- vs. saline-pretreated rats in CA3 (*t*=-2.5, *p*=0.02) and hilus (*t*=-4.3, *p*=0.0007).

Previous work suggests that although *Arc* mRNA expression is induced in multiple brain regions in animals learning a particular behavior, the degree of that induction in a given brain area only correlates with measures of learning if that brain area is involved in the learning (Daberkow *et al*, 2007; Guzowski *et al*, 2001). Consequently, we did not include a caged control group in the present studies, because our prior work showed that reversal learning induces *Arc* throughout the brain (Daberkow *et al*, 2007), and we were testing whether there was a correlation between *Arc* in various brain regions and behavior, not whether there simply was an induction of *Arc*. Furthermore, our prior work suggests that rats with METH-induced monoamine depletions no longer rely on “normal” striatal circuitry for response-reversal learning (Daberkow *et al*, 2008; Pastuzyn *et al*, 2012). Therefore, we used *ex vivo* analysis of *Arc* mRNA expression across multiple brain regions that might be involved in reversal learning in an attempt to reveal neural substrates being used by the METH-pretreated rats as they learned the reversal response.

Although PFC has been implicated in reversal learning (for review, see Ragozzino, 2007), we found no significant correlations in either saline- or METH-

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pretreated rats between *Arc* mRNA expression in PFC regions and trials to criterion on the reversal-learning task (Table 1). We further speculated that the METH-pretreated rats might be relying on a spatial strategy to solve the reversal task, and thus looked for correlations between *Arc* mRNA expression in hippocampal subregions and trials to criterion. Again, no significant correlations were found in either saline- or METH-pretreated rats (Table 1).

In contrast to the lack of significant correlations in the PFC and hippocampus, significant correlations were apparent in striatum, and the region in which the correlations were observed varied as a function of METH pretreatment. As previously reported (Daberkow *et al*, 2007, 2008), *Arc* mRNA expression in DMS, but not DLS, was significantly negatively correlated with trials to criterion on the reversal-learning task in saline-pretreated rats (Fig. 2). No such significant correlation (p=0.9) was apparent for DMS of METH-pretreated rats, again consistent with our prior observations (Daberkow *et al*, 2008). However, in NAc shell, there was a significant negative correlation between *Arc* mRNA expression and performance in METH-pretreated rats (R^2=0.44, p=0.0497) that was not apparent in saline-pretreated rats (p=0.2). Thus, prior exposure to a neurotoxic regimen of METH is associated with a change in the brain regions in which *Arc* mRNA expression correlates with behavioral performance, suggesting that the METH-pretreated rats might be relying on NAc shell, rather than DM striatum, in this task.

Effect of Arc *antisense on response-reversal learning and its retention*

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Prior work from our lab (Pastuzyn *et al*, 2012) and others (Czerniawski *et al*, 2011; Guzowski *et al*, 2000; Hearing *et al*, 2011; Holloway and McIntyre, 2011; Maddox and Schafe, 2011; Ploski *et al*, 2008) has shown that disruption of *Arc* in a brain area known to be involved in completion of a particular learning/memory task disrupts consolidation of the memory. Thus, to further examine whether the circuitry mediating response-reversal learning and consolidation of that learning in METH-pretreated rats had shifted to rely on NAc shell, we determined whether local infusion of an *Arc* antisense oligonucleotide into NAc shell had differential effects on retention of the reversal learning in METH- vs. saline-pretreated rats. Figure 3 illustrates the locations of the tips of the infusion cannulae in NAc shell for each rat.

Consistent with our prior observations (Daberkow *et al*, 2008; Pastuzyn *et al*, 2012), METH- and saline-pretreated rats did not differ in trials to criterion during the acquisition days (data not shown). Infusion of an *Arc* antisense oligonucleotide did not alter performance on the day of reversal learning (Fig. 4a), as a two-way ANOVA on pretreatment (saline, METH) x treatment (*Arc* antisense, *Arc* nonsense, PBS) for trials to reach criterion on the reversal task revealed no significant main effect of pretreatment ($F_{(1,1)}$=0.05, p=0.8) or infusion ($F_{(2,2)}$=1.0, p=0.4) and no significant interaction ($F_{(2,2)}$=0.1, p=0.9).

Rats were tested for retention of the reversal learning 24 hr later. Two-way ANOVA on pretreatment x treatment for trials needed to reach criterion on the reversal-retention test revealed a main effect of pretreatment (Fig. 4b; $F_{(1,18)}$=5.29, p=0.03), a trend towards an effect of treatment ($F_{(2,18)}$=2.81, p=0.09), and a significant pretreatment x treatment interaction ($F_{(2,2)}$=6.2, p=0.009). Tukey HSD *post hoc* analysis of the

significant interaction revealed that infusion of *Arc* antisense into NAc shell during the reversal learning did not impair retention of the reversal learning in the saline-pretreated rats, as the trials to criterion on the retention day were not different from those in the saline-pretreated rats infused with a nonsense oligonucleotide (p=0.97) or PBS (p=0.995). Conversely, infusion of the *Arc* antisense oligonucleotide into NAc shell did impair retention of reversal learning in the METH-pretreated rats. METH-pretreated rats infused with the *Arc* antisense oligonucleotide during reversal learning took significantly more trials to reach criterion on the retention test the following day relative to METH-pretreated rats infused with the nonsense oligonucleotide (p=0.01) or PBS (p=0.03), as well as relative to the saline-pretreated rats infused with the *Arc* antisense oligonucleotide (p=0.003). Taken together with our prior results showing that infusion of *Arc* antisense into DMS impairs retention of reversal learning in saline-, but not METH-, pretreated rats (Pastuzyn *et al*, 2012), the accumulating evidence suggests that the neural circuitry in which consolidation of reversal learning occurs is altered as a consequence of METH-induced neurotoxicity.

Discussion

Previous results suggest that the correlation between *Arc* mRNA expression in a brain region and behavioral performance on a task, rather than the simple presence of gene expression, reflects the necessity of synaptic modifications in that brain region for learning and its consolidation (Daberkow *et al*, 2007; Guzowski *et al*, 2001; Hearing *et al*, 2011; Pastuzyn *et al*, 2012). The present findings provide additional support for this view by showing again that disruption of *Arc* in a brain region impairs consolidation of

learning only if a significant correlation between *Arc* expression in that brain region and behavioral performance was observed. The present work also confirms earlier results showing a loss of the normal correlation between *Arc* mRNA expression in DMS and response-reversal learning in rats with METH-induced neurotoxicity (Daberkow *et al*, 2008). The present study extends those findings by demonstrating the appearance of a novel correlation between *Arc* expression in NAc shell and behavioral performance as a consequence of prior METH exposure and subsequent sensitivity of reversal-learning consolidation to infusion of an *Arc* antisense oligonucleotide into NAc shell. These findings suggest that prior neural injury—in this case, METH-induced neurotoxicity—leads to alterations in the neural circuitry engaged when an animal performs a learning and memory task, and that this change in circuitry can be monitored by evaluating the correlation between *Arc* mRNA expression in various brain regions and behavioral performance. This approach may therefore serve as an *ex vivo* imaging approach to interrogate neural circuits engaged in learning and memory tasks and how those circuits are affected by CNS insult.

The reversal learning examined in the present study is typically dependent on the functional integrity of DMS, as infusion of an NMDA receptor antagonist or an antisense oligonucleotide against *Arc* into DMS in normal animals disrupts learning and consolidation of that learning, respectively (Palencia and Ragozzino, 2004; Pastuzyn *et al*, 2012). Despite this apparent specific role of DMS in response-reversal learning, *in situ* hybridization histochemical staining revealed expression of *Arc* mRNA throughout the brain. Thus, as previously suggested (Daberkow *et al*, 2007, 2008; Guzowski *et al*, 2001), evidence is accumulating that it is not simply the presence of *Arc* mRNA

induction in a brain region that implicates plasticity processes in that region in the learning/memory formation; rather, it appears to be the *correlation* between *Arc* and the measure of learning that is the hallmark implicating synaptic plasticity processes in a brain region as being critical for the particular learning being examined and its consolidation.

Evidence that the correlation between *Arc* mRNA expression in a brain region and behavioral performance is the critical dependent measure for using *Arc* mRNA expression to identify brain regions involved in the learning/memory being examined comes from studies using site-specific infusions of antisense oligonucleotides to disrupt *Arc* function. For example, Guzowski and colleagues (2001) reported a significant inverse correlation between hippocampal *Arc* expression and latency to escape in a spatial, but not cued, version of the Morris water maze. Antisense-mediated knockdown of *Arc* in hippocampus during learning on the spatial task impaired memory consolidation, as evidenced by impaired retention of the previously learned spatial location (Guzowski *et al*, 2000). Likewise, prior work by Hearing and colleagues revealed a significant correlation between *Arc* mRNA expression in DLS and context-induced lever pressing (cocaine-seeking) during a one-hour extinction test (2008). Infusion of an *Arc* antisense oligonucleotide during that one-hour extinction session impaired consolidation, as evidenced by greater lever pressing in the antisense-infused rats when assessed 24 and 48 hr later (Hearing *et al*, 2011). We also previously reported that normal rats show a significant inverse correlation between *Arc* mRNA in DMS and trials to criterion on the reversal-learning task (as reported herein and Daberkow *et al*, 2007, 2008) and that infusion of an *Arc* antisense oligonucleotide into

DMS impairs consolidation of reversal learning in those normal animals (Pastuzyn *et al*, 2012). Importantly, in METH-pretreated rats the correlation between *Arc* mRNA expression in DMS and reversal learning is lost (as reported herein and Daberkow *et al*, 2007, 2008), and infusion of an *Arc* antisense oligonucleotide into the DMS does not impair consolidation of the reversal learning in these METH-pretreated rats (Pastuzyn *et al*, 2012). Similarly, in the present study, infusion of the *Arc* antisense oligonucleotide into NAc shell of normal animals—a brain region in which *Arc* mRNA expression does not correlate with reversal learning—does not impair retention of the learned reversal, even though there is *Arc* expression in this region. Taken together, these findings suggest the interpretation that the correlation between *Arc* in a brain region and the index of learning can be used to map, *ex vivo*, the neural circuitry engaged in the particular learning and memory task.

We have previously reported that the correlation between *Arc* in DMS and reversal learning normally observed in intact rats is lacking in rats with METH-induced neurotoxicity, despite the fact that they have apparently normal response-reversal learning (Daberkow *et al*, 2008). Therefore, in the present work, we performed a broader evaluation of *Arc* mRNA in different brain regions and reversal learning in METH-pretreated rats. We discovered a novel correlation in METH-pretreated rats between *Arc* in NAc shell and reversal learning. In this case, infusion of *Arc* antisense into NAc shell during the reversal learning impaired consolidation of that learning. Although we did not directly verify that the antisense oligonucleotide-mediated knockdown of *Arc* remained confined to the NAc shell, two lines of evidence suggest that the effect of the antisense oligonucleotide observed in the METH-pretreated rats is

likely due to loss of Arc function in the NAc shell. First, several prior studies have infused biotinylated *Arc* antisense oligonucleotides into specific brain regions similar in size to the NAc shell at concentrations, volumes, and rates of infusion similar to those used here and have reported that the infused oligonucleotide remains restricted to the region in which it was infused (Holloway and McIntyre, 2011; Lv *et al*, 2011; Ploski *et al*, 2008). Second, although restriction of infused *Arc* antisense to the NAc shell has not been directly examined, the prior work by Lv and colleagues (Lv *et al*, 2011) reported dissociable effects of *Arc* antisense infusion into the NAc shell vs. core on morphine-induced conditioned place preference. Taken together, these data suggest that infusion of *Arc* antisense oligonucleotide in the present study likely specifically disrupted Arc function in the NAc shell, thereby disrupting reversal learning in the METH-pretreated rats.

The fact that METH-pretreated rats appear to rely on different striatal circuitry to perform the reversal task relative to intact controls is consistent with literature showing differences in neural circuitry activated during learning paradigms between normal individuals and individuals with CNS injury/disease, such as Parkinson's disease (*e.g.*, Beauchamp *et al*, 2008; Moody *et al*, 2004; Rieckmann *et al*, 2010). For example, previous fMRI analysis of Parkinson's disease patients who performed as well as controls on a probabilistic weather prediction task revealed that they activated medial temporal lobe during the task, whereas controls showed normal activation of basal ganglia circuitry (Moody *et al*, 2004). It is therefore critical to assess not just behavioral performance, but also the neural circuitry underlying behavioral performance, in order to

fully appreciate the impact of CNS insult, as the neural circuitry mediating the behavior may be altered even if gross behavioral performance appears intact.

In the case of the present studies, the basis for this reorganization of task-related processing is unknown, but may be secondary to the METH-induced DA depletions. As confirmed in the present work, exposure to high doses of METH results in partial DA loss (Wagner *et al*, 1980). This loss is associated with impairment of phasic DA signaling (Howard *et al*, 2013a; Howard *et al*, 2011), along with loss of transcriptional activation and normal subcellular distribution of *Arc* mRNA in dorsal striatum (Barker-Haliski *et al*, 2012a), both of which are critical for synaptic plasticity underlying basal ganglia-mediated learning and memory processes (Calabresi *et al*, 2007; Schultz, 2007). As previously reported (*e.g.*, Haughey *et al*, 1999; Johnson-Davis *et al*, 2002; Ricaurte *et al*, 1980; Wallace *et al*, 1999), in the present study, METH-induced DA loss in the NAc, particularly in the shell, was less extensive. Based on previous evidence from our lab, there appears to be a threshold (~40% depletion) necessary for behavioral impairments to be evident (Daberkow *et al*, 2005). Perhaps the ~25% depletion in NAc shell observed in the present study was insufficient to prevent this brain region from being used by METH-pretreated rats in the behavioral task. While we have observed disruption of DA transients in the NAc core of METH-pretreated rats (Howard *et al*, 2013a), whether there is less significant disruption of phasic DA signaling in NAc shell at these levels of METH-induced DA loss remains to be determined.

Both DMS and NAc shell are motor outputs for the basal ganglia, and NAc shell is also often touted as being involved in motivated and goal-directed behavior, (Humphries and Prescott, 2010; Ikemoto, 2007). Therefore, DA-mediated plasticity may

be relatively preserved in NAc shell compared to DMS after METH-induced neurotoxicity, allowing DA-mediated synaptic modifications there to subserve consolidation of response-reversal learning. Furthermore, studies have shown that there are differences in how rostral and caudal NAc shell modulate behavior (Reynolds and Berridge, 2001). Our infusions targeted the rostral NAc shell, and given the differences in behavioral output of rostral and caudal NAc shell, as well as the anatomical inputs/outputs to the two regions (*e.g.*, Groenewegen *et al*, 1999; Usuda *et al*, 1998), it will be interesting in future studies to examine the relative contributions of the rostral *vs*. caudal NAc shell to reversal learning in normal and METH-pretreated rats.

As noted above, the lack of effect of METH pretreatment on the levels of *Arc* mRNA expression in dorsal striatum in the present study appears to be at odds with our prior work showing decreased *Arc* mRNA in such animals (Barker-Haliski *et al*, 2012a; Daberkow *et al*, 2008). This apparent difference likely arises from the approaches and, more so, the dependent measures, used in the different studies. In our former studies, we used fluorescent *in situ* hybridization (FISH) and determined the numbers of striatonigral *vs.* striatopallidal neurons with *Arc* mRNA signal in different subcellular compartments. In the present study, the radioactive *in situ* hybridization signal gives us a broad determination of *Arc* mRNA expression across both populations of striatal efferent neurons and in all subcellular compartments. Our work with FISH has shown that basal *Arc* mRNA transcription is increased in both striatal efferent neuron populations in rats with METH-induced neurotoxicity, but that the animals do not further induce *Arc* mRNA in response to behavioral activation (Barker-Haliski *et al*, 2012a; Daberkow *et al*, 2008). Further, METH-induced DA loss is associated with loss of *Arc*

mRNA specifically in the cytoplasm of striatonigral efferent neurons (Barker-Haliski *et al*, 2012a; Daberkow *et al*, 2008). It is this latter effect that is apparent in our prior work (Barker-Haliski *et al*, 2012a; Daberkow *et al*, 2008), as the dependent measure reported is the number of neurons with *Arc* mRNA signal in the cytoplasm. In the present study, because the radioactive *in situ* hybridization approach incorporates signal in both populations of neurons and in all subcellular compartments, the METH-induced loss of cytoplasmic *Arc* mRNA signal in one subpopulation of neurons is not apparent. That said, what the FISH and radioactive *in situ* approaches have in common is that both reveal the correlation between *Arc* mRNA expression and behavior (findings herein and in Daberkow *et al*, 2007, 2008).

The results of this study suggest that there is a change, following METH-induced neurotoxicity, in the brain circuitry used in a behavioral task. Further, they provide additional support for the proposition that a correlation between *Arc* mRNA expression in a brain region and the measure of learning on a task implicates synaptic plasticity processes in that brain region as being critical for learning and its consolidation. Since humans with a history of METH abuse also can have partial monoamine loss, they may be forced to rely on potentially “less ideal” neural circuits to perform particular cognitive tasks, and this lack of normal cognitive processes may contribute to cognitive deficits seen (Dean *et al*, 2013), especially in more complicated tasks that may require engagement of several brain regions at once. Better understanding the impact of neurotoxicity on synaptic plasticity mechanisms should allow for the development of targeted therapies to address impaired cognitive function in individuals with METH-

induced neurotoxicity or others with striatal dopamine loss, such as patients with Parkinson's disease.

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Figure Legends

Figure 1. METH neurotoxicity results in decreases in DAT and SERT binding. Graphs showing METH-induced decreases in (**A**) striatal DAT and (**B**) prefrontal cortical SERT as revealed by [^{125}I]RTI-55 binding. Since DAT and SERT binding between rats in the *Arc* correlation experiment and *Arc* antisense infusion experiment were not significantly different, binding values from both sets of rats are combined into one graph. Saline (SAL)-pretreated, n=21; METH-pretreated, n=22. *Significantly different from SAL, p<0.001.

Figure 2. Correlations between *Arc* mRNA in striatal subregions and trials to criterion on the response reversal-learning task. *Arc* mRNA expression was determined by densitometric analysis of film autoradiograms using ImageJ and is expressed as background-subtracted average gray values (arbitrary units). Significant correlations (as indicated by box around R^2 and p values) were in DM striatum of saline (SAL)-pretreated rats (R^2=0.56, p=0.013) and NAc shell of methamphetamine (METH)-pretreated rats (R^2=0.44, p=0.0497). METH-pretreated rats were given a neurotoxic regimen of (±)-METH•HCl (4 x 10 mg/kg free base, s.c., at 2-hr intervals) approximately seven weeks prior to reversal learning. DMS, dorsomedial striatum; DLS, dorsolateral striatum; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell.

Figure 3. Diagrams (Paxinos and Watson, 1998) showing placement of infusion sites (black dots) in the nucleus accumbens shell of rats infused with an *Arc* antisense or *Arc* nonsense oligonucleotide or with PBS. Numbers indicate mm from Bregma.

Figure 4. Knockdown of *Arc* impairs consolidation of reversal learning in METH-, but not saline-, pretreated rats. Rats were infused with an *Arc* antisense oligonucleotide, *Arc* nonsense oligonucleotide, or PBS into NAc shell two hours prior to response-reversal learning on a T-maze. (**A**) None of the compounds had any effect on reversal learning in saline- or METH-pretreated rats. (**B**) Rats were tested on reversal retention 24 hr after reversal learning. Knockdown of *Arc* mRNA in NAc shell via an *Arc* antisense oligonucleotide impaired reversal retention in METH-, but not saline-, pretreated rats. Values are average trials to criterion (9/10 correct consecutive trials; ±SEM, *n*=3-6/group) on the reversal-learning task (**A**) or on the reversal-retention test 24 hr later (**B**). *Significantly different from all other groups, all *p* values<0.05.

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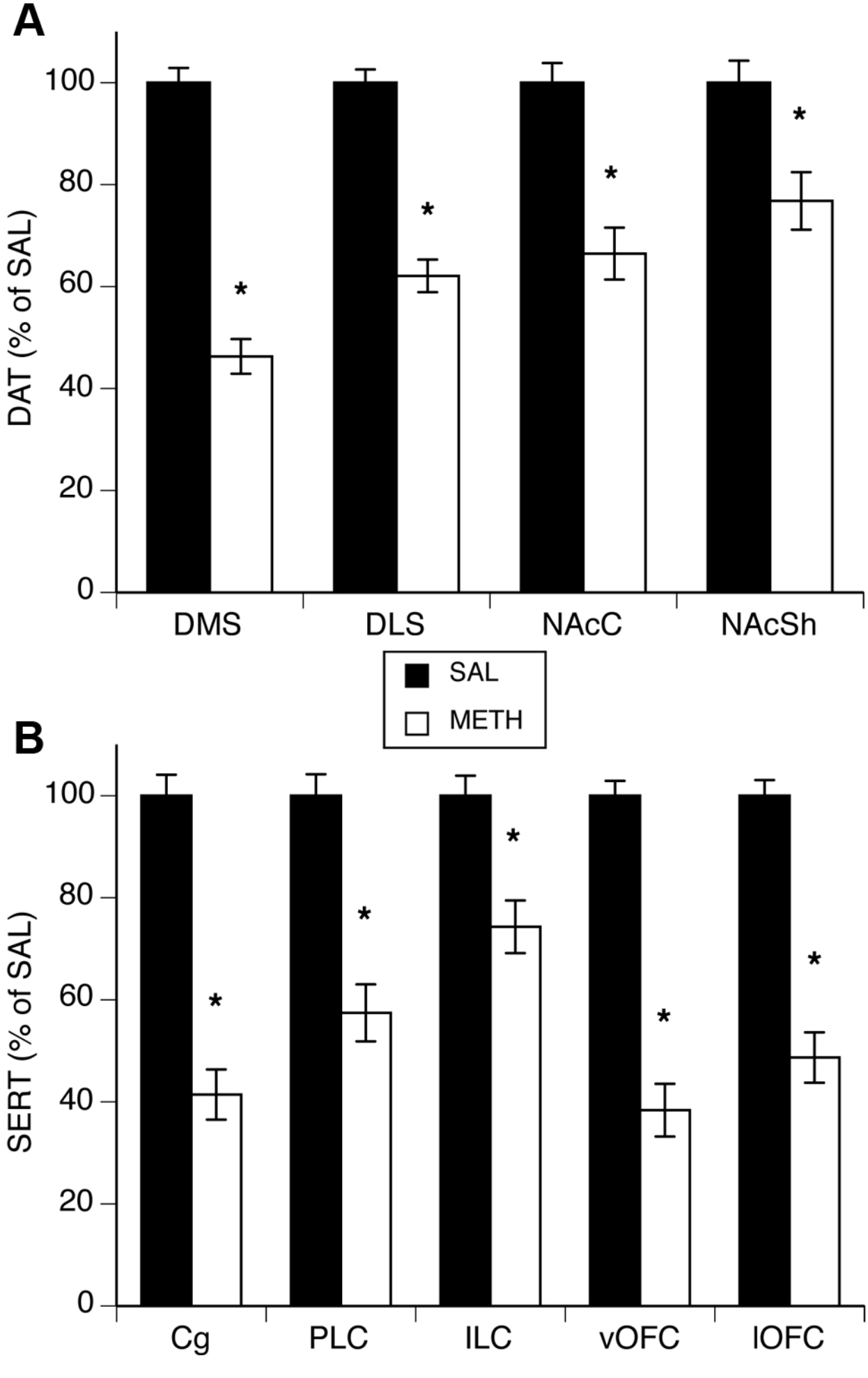
Tables

Table 1. Correlations between *Arc* mRNA expression in subregions of prefrontal cortex and hippocampus and trials to criterion on the response reversal-learning task.

Brain Region	Saline-pretreated		METH-pretreated	
	R^2	*p*-value	R^2	*p*-value
Prefrontal Cortex				
Cg	0.03	0.7	0.03	0.6
PLC	0.08	0.4	0.0002	0.97
ILC	0.2	0.1	0.003	0.9
vOFC	0.01	0.8	0.001	0.9
lOFC	0.001	0.9	0.05	0.6
Hippocampus				
CA1	0.08	0.4	0.01	0.8
CA3	0.03	0.6	0.4	0.07
Upper DG	0.002	0.9	0.07	0.5
Lower DG	0.001	0.9	0.1	0.3
Hilus	0.06	0.5	0.2	0.3

Values are R^2 and respective *p* values obtained from multivariate analysis of the relation between *in situ* hybridization histochemical staining for *Arc* mRNA in each brain region and trials to criterion on a response reversal-learning task for rats pretreated with saline (4 x 1 mL/kg at 2-hr intervals, *n*=10) or a neurotoxic regimen of (±)-METH (4 x 10 mg/kg, at 2-hr intervals, *n*=9) at least seven weeks prior to testing and sacrifice. Cg, cingulate cortex (area Cg1); PLC, prelimbic cortex; ILC, infralimbic cortex; vOFC, ventral orbitofrontal cortex; lOFC, lateral orbitofrontal cortex; CA1, *Cornu Ammonis* 1; CA3, *Cornu Ammonis* 3; upper DG, upper blade of dentate gyrus; lower DG, lower blade of dentate gyrus.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NAcSh
SAL
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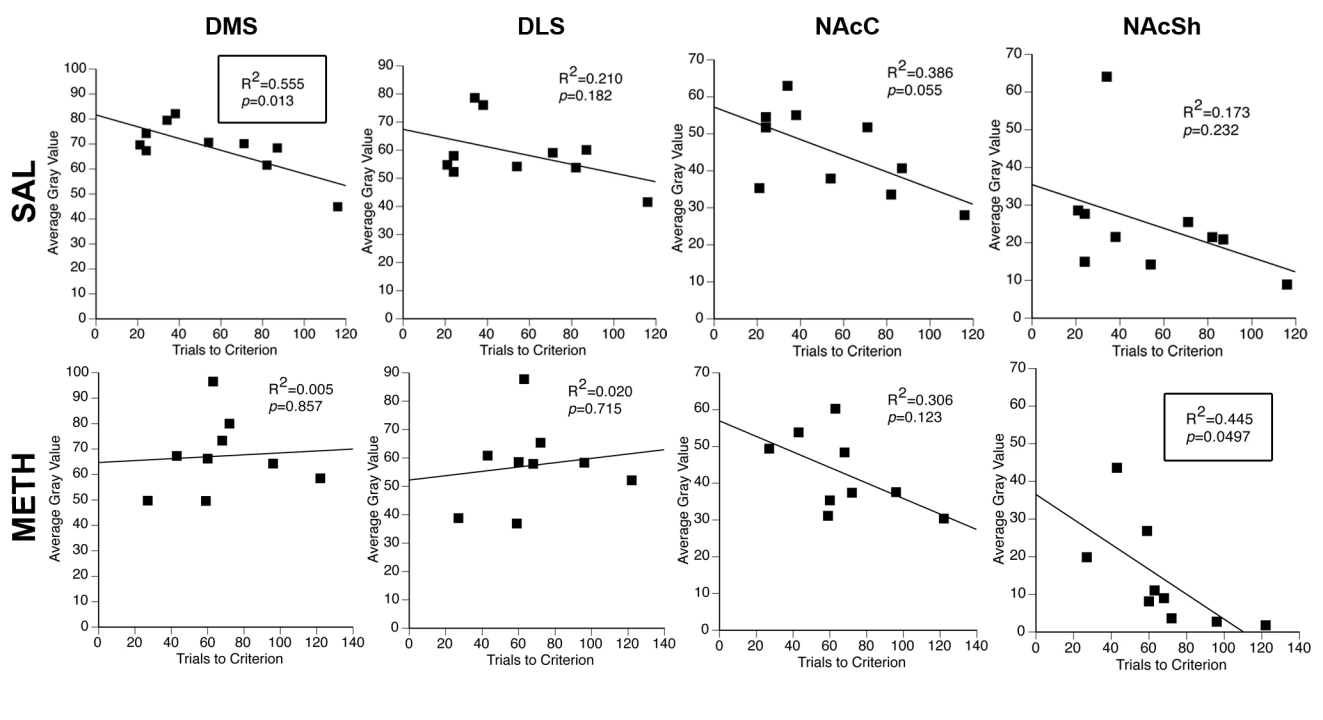
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R2=0.555
p=0.013
R2=0.210
p=0.182
R2=0.386
p=0.055
R2=0.173
p=0.232
R2=0.005
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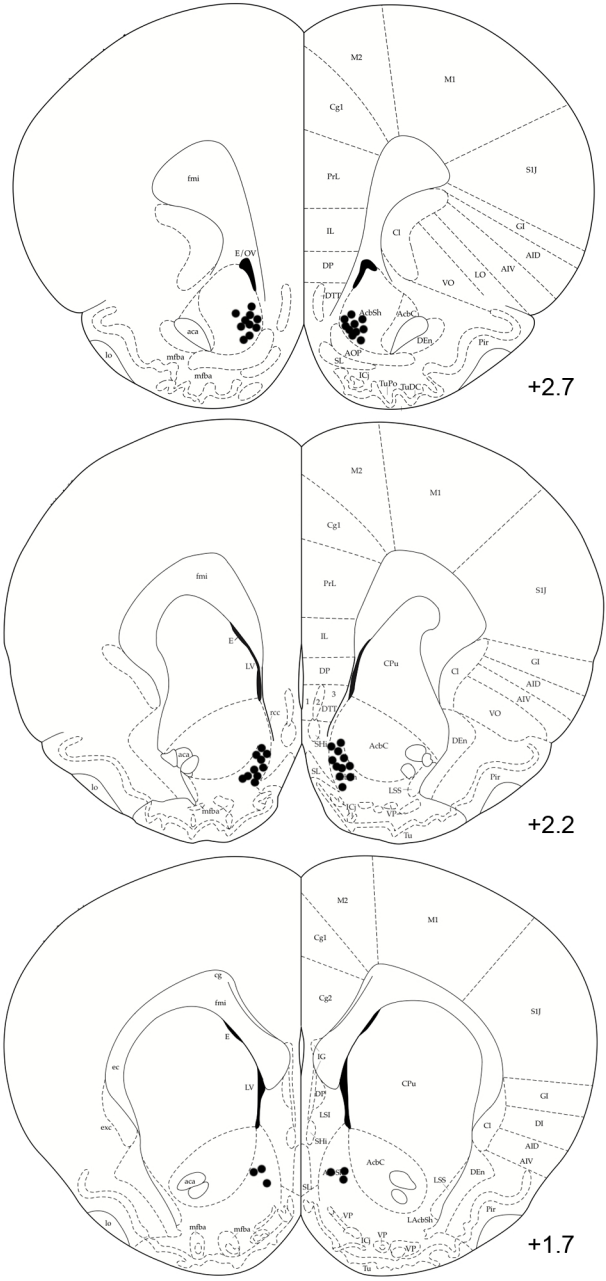
p=0.715
R2=0.306
p=0.123
R2=0.445
p=0.0497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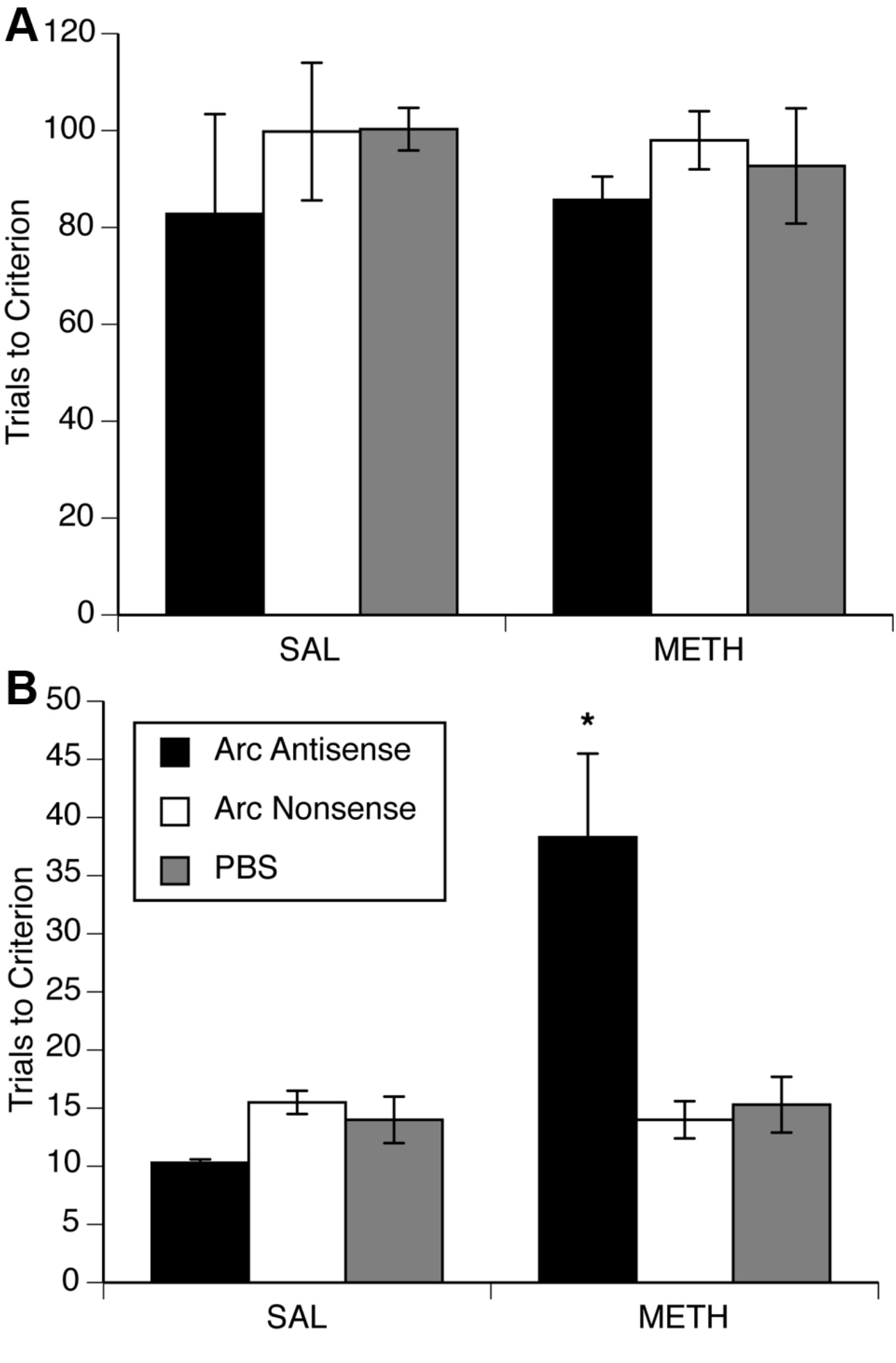
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