Published in final edited form as:

Eur J Neurosci. 2010 March ; 31(5): 903–909. doi:10.1111/j.1460-9568.2010.07134.x.

Role of the prefrontal cortex and nucleus accumbens in reinstating methamphetamine seeking

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Abstract

While the involvement of the medial prefrontal cortex projection to the nucleus accumbens in the reinstatement of cocaine seeking has been well studied, it is not known if this projection plays a similar role in the reinstatement of cue- and methamphetamine-induced drug seeking in animals extinguished from methamphetamine self-administration. Accordingly, following extinction from long access methamphetamine self-administration rats were bilaterally microinjected with either a combination of the GABA agonists baclofen/muscimol or aCSF vehicle into the infralimbic or prelimbic subcompartments of the medial prefrontal cortex or into the shell or core subcompartments of the nucleus accumbens. Similar to cocaine seeking, inactivation of either the prelimbic cortex or accumbens core eliminated cue- and methamphetamine-induced reinstatement, and inactivation of neither the infralimbic cortex nor shell subcompartments inhibited methamphetamine-induced drug seeking. However, in contrast to previous reports with cocaine, cue-induced reinstatement of methamphetamine seeking was inhibited by inactivation of the infralimbic cortex. In conclusion, while a primary role in reinstated drug seeking by the prelimbic and the accumbens core is similar between cocaine and methamphetamine, the recruitment of the infralimbic cortex by conditioned cues differs between the two psychostimulant drugs of abuse.

Methamphetamine and cocaine are psychostimulants that reinforce behavioral responding and lead to compulsive drug use and vulnerability to relapse (Seiden *et al.*, 1993; McCann & Ricaurte, 2004; Anderson & Pierce, 2005). Using an animal model of cocaine relapse (Shalev *et al.*, 2002), it has been found that cocaine-seeking behavior is associated with increased activity in the projection from the prefrontal cortex to the nucleus accumbens, specifically in the glutamatergic projection from the prelimbic cortex (PL) to the core subcompartment of the nucleus accumbens (NAcore) (McFarland *et al.*, 2003; Rebec & Sun, 2005). Although both cocaine and methamphetamine share the dopamine transporter and an increase in NAcore extracellular dopamine as a site of action in the brain (Robinson *et al.*, 1988; Kalivas & Duffy, 1990; Seiden *et al.*, 1993), there are distinctions that could alter the circuitry underlying methamphetamine-seeking in the reinstatement animal model of relapse. For example, while cocaine binds to dopamine transporters to inhibit dopamine elimination from the synapse, methamphetamine is a false substrate that is transported into the neuron and ultimately causes depletion of dopamine from synaptic vesicles (Ritz *et al.*, 1987; Sulzer *et al.*, 1995; Anderson & Pierce, 2005). D-amphetamine shares this presynaptic

action with methamphetamine (Sulzer *et al.*, 1995), and it was recently shown that chronic amphetamine does not produce the same profile of the changes in glutamate transmission as cocaine does in the nucleus accumbens. For example, withdrawal from cocaine, but not amphetamine is associated with a redistribution of the AMPA glutamate receptor subunits GluR1 and GluR2 in accumbens tissue slices (Conrad *et al.*, 2008; Nelson *et al.*, 2009), and a blunting of long-term potentiation elicited from the hippocampus (Lodge & Grace, 2008). Also, while some cognitive deficits and psychosis have been reported after chronic cocaine treatment, these impairments may be more profound after chronic methamphetamine (Brady *et al.*, 1991; Sato, 1992; Salo *et al.*, 2007; McCann *et al.*, 2008).

In this report we use the reinstatement animal model of drug seeking to determine if inactivation of the projection from the PL to the NAc core prevents methamphetamine seeking induced by either cues previously associated with methamphetamine infusion or induced by a noncontingent injection of methamphetamine. Inactivation of the parallel projection by microinjecting the GABA agonists baclofen and muscimol (B/M) into the infralimbic prefrontal cortex (IL) or into the shell of the nucleus accumbens (NAshell) was also examined. Inhibition of this pathway was previously shown ineffective at reducing cocaine-induced reinstatement of drug seeking in animals trained to self-administer cocaine (McFarland & Kalivas, 2001), and a recent study found that inactivation of the IL elicited cocaine seeking (Peters *et al.*, 2008). Accordingly, inactivation of the IL or NAshell by B/M was also examined for the capacity to reinstate drug seeking in animals extinguished from methamphetamine self-administration. All animals were trained on a long access (6 hr) daily exposure in order to engender escalating methamphetamine intake (Koob *et al.*, 2004), and to induce long lasting changes in dopamine transporter content in the prefrontal cortex (Schwendt *et al.*, 2009).

MATERIALS AND METHODS

Animals and Housing

Procedures were conducted in accordance with the “Guide for the Care and Use of Laboratory Rats” (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and approved by the IACUC of the Medical University of South Carolina. Male Long-Evans rats (n=44; Charles-River; 275–300 g) were individually housed in a temperature- and humidity-controlled vivarium on a 12-h reversed light–dark cycle (6 A.M. lights off).

Animals were allowed a minimum of 5 days to acclimate to the vivarium, were handled on a daily basis before undergoing surgery and allowed a minimum of 5 days for postsurgical recovery. Water was available *ad libitum* and 25g food was placed in home cages following the end of each daily testing session. The night prior to commencement of self-administration animals were maintained on 15–20 g of standard rat chow per day to facilitate the acquisition of lever responding. This was continued for the first 2–3 days of self-administration training. Animals were weighed daily on all test and training days.

Surgical procedures

In preparation for surgery, rats were anesthetized with ketamine HCl (100 mg/kg/ip), xylazine (2 mg/kg/ip), and ketorolac (3mg/kg/ip) and implanted with intravenous catheters and bilateral intracranial microinjection guide cannula. The jugular catheter was assembled using a guide cannula (C313G; 11mm length; Plastics One) bent at a 90 degree angle and attached to Silastic tubing (12 cm; 0.025 inner diameter; 0.047 outer diameter; Dow Corning Corporation, Midland, MI). The cannulae and tubing assembly was attached to ProLite Mesh (Hudson, NH) via a cranioplastic/dental acrylic mold. A silicone ball was constructed 3.0 cm from the end of the catheter that was opposite the cannula. Catheters were checked for leaks immediately prior to surgery. The completed catheter assembly was inserted subcutaneously between the shoulder blades, with the guide cannula externalized through a dermal biopsy hole (3 mm). An incision was made in the right jugular vein and Silastic tubing was threaded through the vein until the silicone ball was at the base of the vein. The catheter was secured to the vein using silk thread ties below and above the silicone ball. Sutures secured the catheter and vein to the underlying muscle tissue. Additional sutures were made subcutaneously on either side of the incision on the neck, before external sutures were made to close the incision site. Postsurgical administration of an antibiotic solution of cefazolin (mixed in 100 U/ml heparinized saline), and an additional dose of the analgesic ketorolac (1mg/kg/iv) were administered to the animals. Animals were placed in an incubation chamber and were closely monitored until muscle tone and mobility recovered.

For intracranial drug administration, 15mm cannulae (26ga; Plastics One, Roanoke, VA) were implanted bilaterally in the NAcore [anteroposterior (AP), +1.6 mm; mediolateral (ML), $\pm$ 1.6 mm; dorsoventral (DV), -5.6 mm; microinjectors projected an additional +1mm DV beyond cannulae] and in the NAsell [AP, +1.8; ML, $\pm$ 0.8mm; DV, 5.8mm; microinjectors projected +1mm DV beyond cannulae]. A single double-barreled cannulae (26 ga; cut 5mm below pedestal; 1.2 mm spacing; Plastics One) was implanted in the PL [AP, +3.0 mm; ML, $\pm$ 0.6mm; DV, -2.8 mm] as previously described (Peters et al., 2008), microinjectors projected an additional 1mm beyond cannulae for dPFC and an additional 3 mm further when microinjections were made into the IL. All coordinates were according to Paxinos & Watson (1986). For cannula implantation, the skull was exposed, and holes were drilled at the target locations. Additional holes were drilled peripheral to the target locations, and jewelers screws were inserted to serve as anchors. The cannulae were lowered to the desired location, and then cannulas and screws were embedded in dental acrylic to fasten them to the skull. Metal obturators (33 gauge; Small Parts) were inserted to extend 0.2 mm beyond the tip of the injection cannulas to prevent their obstruction by debris. Microinjectors were constructed using 33 ga Hypotube connected to PE20 tubing which was attached to 10 μ l Hamilton syringes controlled by an infusion pump. Double-barreled injectors for prefrontal cortex were purchased from Small Parts (Miami, FL).

Behavioral Apparatus and Methamphetamine Self-administration

Testing was conducted in sound-attenuated self-administration chambers (30 $\times$ 20 $\times$ 20 cm, Med Associates) linked to a computerized data collection program (MED PC). Each chamber was equipped with tone delivery, house light, two retractable levers and a stimulus light above each lever. Each of the levers was located on the right and left side of the same

wall. The right (active) lever was paired with methamphetamine infusions, whereas the left (inactive) lever did not produce any programmed consequences. Infusion pumps (Razel Scientific Instruments; Stamford, CT) controlled drug delivery to each of the boxes. The system was interfaced with 2 computers, each controlling drug delivery and recording data from 8 chambers.

Drug delivery was facilitated by Tygon tubing connecting a drug syringe on an automated pump to the external cannula portion of the catheter. The external portion of the catheter was anchored to the back of the animal via a backplate assembly which consisted of mesh glued to a cranioplast mold. The Silastic tubing from the catheter was threaded subcutaneously into the jugular vein. A wire coil leash protected the tubing and was attached to a base structure with a weighted swivel apparatus above each chamber that provided the leash freedom of movement. The catheter was flushed daily prior to and following daily testing sessions with heparinized saline (0.2 ml of 100 IU) to help maintain catheter patency and cefazolin antibiotic (0.2 ml of 0.1 gm/ml) to help protect against infection.

Methamphetamine hydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA) was mixed in sterile saline and filtered (0.45 μ m) prior to i.v. administration (0.02 mg/50 μ l bolus). This dose is on the ascending limb of the dose effect curve and as such is reinforcing without producing motoric impairments (Roth & Carroll, 2004). All of the animals included in the analysis passed catheter checks performed 1-2 days prior to acquisition testing, following the first day of maintenance testing and after the first day of extinction testing. Patency of catheters was verified using 0.10 ml of methohexital sodium (10.0 mg/ml i.v.), a short-acting barbiturate that produces a rapid loss of muscle tone.

Five days after surgery, rats began acquisition of self-administration on an FR-1 schedule of reinforcement during daily 1-h sessions for 10 days. During this short access phase of training, each response on the active lever resulted in an infusion of methamphetamine (National Institutes of Health, Bethesda, MD) dissolved in sterile 0.9% saline (0.2 mg in 0.05 ml for 10 days). A tone (78 dB, 4.5 kHz) sounded and a stimulus light above the active lever was illuminated for 5 sec at the onset of the 2-sec infusion. A timeout period of 20s followed each i.v. infusion to help prevent overdose. Responses on the active lever during the timeout period and responses on the inactive lever throughout the study were recorded but had no programmed consequences. All of the animals in the study met the criterion for acquisition of methamphetamine self-administration that was a minimum of 10 infusions per day over 2 consecutive daily self-administration sessions. In order to maintain catheter patency during acquisition training, catheters were flushed twice daily with 0.20 ml of a heparinized saline solution; once prior to and once following each daily testing session. Following the 10 days of short access to methamphetamine, all rats received an additional 12 days of training where methamphetamine was available for 6 hrs (long access phase).

After the last day of long access self-administration, rats received daily 1-h extinction sessions. An active lever press no longer elicited a drug infusion or the presentation of conditioned stimuli (i.e. light or tone). Animals continued under extinction conditions for a minimum of 10 days and until <25 active lever presses were made per session for two consecutive days.

Reinstatement and Intracranial Microinjection

Reinstatement testing began for each animal after the extinction criterion was achieved. Immediately prior to reinstatement testing animals received a bilateral microinjection of either aCSF or a mixture of the GABA_B agonist baclofen and the GABA_A agonist muscimol (B/M; 0.1-0.01 mM, respectively). All microinjections were made in a volume of 0.3µl/site over 60sec, and the injectors were left in place 60sec to permit diffusion from the injection site. A minimum of 2 days of extinction training was allowed between each test or until active lever responding was <25 presses for 2 consecutive extinction training days. Each rat received a maximum of four reinstatement tests with a minimum of 2 days of extinction between tests consisting of a B/M and aCSF pretreatment prior to cue- or methamphetamine-induced reinstatement. In the first set of experiments animals were only tested for methamphetamine-induced reinstatement. However, for the majority of experiments, when animals were tested for both cue- and methamphetamine-induced reinstatement, the cue-induced reinstatement trials were conducted first, and for both reinstatement modalities the order of B/M and aCSF microinjection was randomized.

During the conditioned cued reinstatement test, rats were placed into the chambers for 1 h on an FR-1 reinforcement schedule where each active lever press resulted in a 5-s conditioned stimulus (e.g. light and tone) presentation in the absence of drug reinforcement. aCSF or B/M was administered 5 min prior to placing the animal in the operant chamber. For methamphetamine-induced reinstatement, animals were microinjected with either aCSF or B/M, injected with a single, non-contingent dose of methamphetamine (1 mg/kg, i.p.) and were returned to home cages for 20 min before being placed in the operant chamber for a 60 min reinstatement test. For methamphetamine-induced reinstatement the conditioned cues were not presented in response to a lever press.

A portion of animals implanted with IL microinjection cannula received a final injection of B/M alone on the first microinjection trial in order to compare to our previous study with rats trained to self-administer cocaine showing that B/M alone elicited a significant increase in active lever pressing (Peters et al., 2008). Otherwise, no injections of aCSF or B/M were made in animals separate from a cue- or methamphetamine-primed reinstatement trial.

Histology and Statistics

After reinstatement testing, subjects were overdosed with pentobarbital anesthesia (100 mg/kg, i.p.) and then perfused transcardially with 0.9% physiological saline, followed by 10% Formalin. Brains were stored in Formalin for at least 24 hr before being sectioned. Brains were blocked and sliced in coronal sections (50µm thick) through the prefrontal cortex and nucleus accumbens to examine placement of microinfusion cannulas. Sections were mounted on gel-coated slides, stained with cresyl violet, and examined for cannula placement by an individual unaware of each subject's behavior or group.

Data were statistically evaluated using a two-way, repeated measures analysis of variance (ANOVA) comparing extinction levels of active lever pressing with levels following microinjection of B/M or aCSF within each reinstatement modality (i.e. separate ANOVA for cue- and methamphetamine-induced reinstatement within each microinjection site). The

extinction comparison value used for comparison with each trial was the average of the 2 extinction days before that trial. Post hoc analysis was a Bonferonni test.

RESULTS

Self-administration, extinction and escalation of methamphetamine intake

Figures 1 and 2 show the active lever responding for the short access (days 1-10), the long access (days 11-22) phases of self-administration training, as well as the extinction training period for each microinjection group. Table 1 demonstrates that animals in the IL, PL and NAcCore groups showed escalated methamphetamine intake over the long access self-administration period. In contrast, the NAcShell group showed increased methamphetamine intake, but the escalation was not statistically significant. Interestingly, no microinjection group showed a significant change in extinction lever pressing over the 10 days of initial training. This was due largely to the fact that most rats achieved the extinction criterion (<25 active lever presses) on the first day of extinction training (day 23).

Inactivation of the prefrontal cortex

Figure 1A shows that B/M administration into the PL reduced both methamphetamine- and cue-induced reinstatement of lever pressing. In contrast, B/M into the IL inhibited only cue-induced reinstatement, with no effect on Meth reinstatement (figure 1B). Because previous studies revealed that B/M alone into the IL reinstated lever pressing in rats extinguished from cocaine self-administration (Peters et al., 2008), five animals were also tested for reinstatement of lever pressing by B/M alone. While a modest increase in active lever pressing was associated with B/M microinjection into the IL, the effect was not statistically different from extinction baseline.

Inactivation of the nucleus accumbens

Figure 2A shows that B/M into the NAcCore inhibited both reinstatement modalities. While methamphetamine reinstatement after B/M was significantly reduced compared to aCSF, cue-induced reinstatement was reduced to a level that was not statistically different from extinction level pressing, but also not different from aCSF pretreatment. In contrast, B/M into the NAcShell did not alter either cue- or methamphetamine-induced reinstatement of active lever pressing. However, there was a tendency for NAcShell B/M microinjection to inhibit cue-induced reinstatement ($p = 0.078$ comparing cued reinstatement after B/M versus aCSF; Wilcoxon paired sign test).

Histology

Figure 3 shows the location of microinjection cannula tips for animals that were used in data analysis. Of the 44 animals that completed the behavioral training and testing, 36 were determined to have correct cannula placement and used for data analysis.

DISCUSSION

These data reveal that akin to reinstated cocaine seeking in animals extinguished from cocaine self-administration (Kalivas & Volkow, 2005), cue- or methamphetamine-induced

reinstatement in methamphetamine extinguished animals requires neuronal activity in the PL and NAc core. Also similar to cocaine-induced reinstatement in cocaine-trained animals, inhibition of the IL and NAc shell with microinjected B/M was without effect of reinstated methamphetamine-induced drug seeking. However, in contrast with cocaine training, the IL, and to a lesser extent the NAc shell, appeared important for cue-induced reinstatement in methamphetamine-trained animals. Finally, although inhibition of the IL alone produced a near significant trend to reinstate lever pressing in methamphetamine trained animals, the previously reported robust reinstatement by intra-IL B/M in cocaine trained animals was not observed (Peters *et al.*, 2008).

The capacity of the IL to regulate cue-induced reinstatement of methamphetamine seeking is the primary distinction observed in this study relative to the cocaine literature. The IL has robust projections to the NAc shell and the intercalated cell masses in the amygdala (McDonald, 1996), and in turn receives reciprocal innervation from the basolateral amygdala (McDonald *et al.*, 1996). This circuit has been characterized in both the fear conditioning and the cocaine self-administration literature as important in extinction training (Quirk & Mueller, 2008; Peters *et al.*, 2009). Thus, it is proposed that the IL is activated during extinction training and its activity is required for extinction-mediated inhibition of behavior. In this regard, it is interesting that in all groups of animals there was not a significant learning curve during extinction training from methamphetamine self-administration. This resulted from the fact that nearly full extinction of behavior was achieved on the first day of extinction training. This rapid extinction learning in methamphetamine trained animals has been observed before [(Shepard *et al.*, 2004; Schwendt *et al.*, 2009); but see (Harrod *et al.*, 2003; Anggadiredja *et al.*, 2004; Ping & Kruzich, 2008; Rogers *et al.*, 2008a; Shelton & Beardsley, 2008)]. In examining this literature, two procedural aspects of the experiments may contribute to the differences. First, in the Schwendt *et al.* (2009) where both short (1 hr) and long-access (6 hr) to methamphetamine were examined, the short-access group showed first day extinction pressing akin to the active lever pressing during self-administration training and demonstrated significant reductions in pressing over extinction training. In contrast, akin to the present study, the long-access subjects showed no change in active lever pressing during extinction training. Along these lines, the Shepard *et al.* (2004) study that also employed a long-access (6 hr) methamphetamine self-administration protocol, and failed to show significant changes over extinction training due largely to a lack of pressing on the first day. In contrast, with the exception of the Rogers *et al.* (2009a) study, the studies showing significant extinction learning used a relatively short access methamphetamine self-administration protocol [e.g. 1-2 hrs daily exposure; (Harrod *et al.*, 2003; Anggadiredja *et al.*, 2004; Ping & Kruzich, 2008; Shelton & Beardsley, 2008)]. The second experimental parameter to consider is strain differences. While this does not explain the differences between the present study and others using Long Evans rats, Kruzich and coworkers have noted marked strain differences in active lever responding on the first day of extinction training after short-access methamphetamine self-administration (Kruzich & Xi, 2006; Xi & Kruzich, 2007).

It is interesting to speculate that the apparent rapid extinction training and role of the IL in cue-induced methamphetamine seeking may be mechanistically related. In this regard, rapid extinction may occur from either more rapid learning or the fact that the drug-paired context is not motivating methamphetamine seeking to the extent it does cocaine seeking. Context-induced drug seeking in cocaine self-administration trained animals undergoing forced abstinence, or extinction in an environment not paired with drug, show an important involvement of the basolateral amygdala projection to the dorsal hippocampus (Fuchs *et al.*, 2007). Importantly, long access contingent or high dose noncontingent methamphetamine results in impaired spatial memory in a novelty recognition task that has been linked in part to dorsal hippocampus function (Bisagno *et al.*, 2002; Schroder *et al.*, 2003; Kesner & Rogers, 2004; Belcher *et al.*, 2005; Rogers *et al.*, 2008b). Given the well-established role for the basolateral amygdala in cue-induced reinstatement, perhaps a methamphetamine-induced dysfunction in the BLA projections to the dorsal hippocampus and/or the IL could result in a simultaneous impairment in context-induced and increased involvement of the IL in cue-induced methamphetamine seeking. However, this possibility requires direct experimental examination of the role played by the circuit containing the BLA, IL and hippocampus in reinstated methamphetamine seeking, as well as more detailed examination of possible changes in cue salience produced by long access to self-administered methamphetamine.

Other classes of drugs of abuse have been shown to involve a more elaborated drug seeking circuitry than cocaine, notably involvement of the IL in heroin- and cue-induced drug seeking (Rogers *et al.*, 2008b). As well, amphetamine and cocaine may induce somewhat different effects on glutamate transmission in the nucleus accumbens. For example, although both amphetamine and cocaine administration are reported to elicit increased glutamate overflow in the accumbens in animals showing behavioral sensitization after repeated drug administration (Vanderschuren & Kalivas, 2000; Kim *et al.*, 2005), the alterations in glutamate receptor subunit expression on the neuron surface produced after chronic cocaine are not present after chronic amphetamine administration (Nelson *et al.*, 2009). Thus, even within the family of addictive drugs that bind to dopamine transporters the circuitry recruited to mediate reinstated drug seeking may be partly distinct. Accordingly, while neuronal activity in the PL to NAc core projection appears to be obligatory for both methamphetamine and cocaine, only in the methamphetamine-trained animals did conditioned cue-induced reinstatement require neuronal activity in the IL. Finally, it is worth noting that in some studies using cocaine-trained rats this dose of B/M alone into the NAc core suppresses extinguished lever pressing (Peters *et al.*, 2008). While a motor suppressant effect is not apparent following B/M microinjection into the NAc core, nor does B/M in the NAc core suppress food-reinstated food-seeking (McFarland & Kalivas, 2001), it is possible that the effect on extinguished pressing may have contributed to the reduction in methamphetamine- and cue-induced active lever pressing.

Acknowledgements

This research was supported in part by USPHS grant #DA022658, DA015369, DA003906, DA012513.

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Figure 1.

Animals pretreated into the PFC with B/M show reduced reinstatement responding relative to aCSF injections. A) Rats were pretreated into the PL and both cue- and methamphetamine (Meth)-induced reinstatement of active lever pressing were reduced (methamphetamine: interaction $F_{[1,14]} = 19.89$, $p < 0.001$, extinction vs reinstatement $F_{[1,1]} = 14.86$, $p = 0.002$, aCSF vs B/M $F_{[1,1]} = 35.40$, $p < 0.001$; Cue: interaction $F_{[1,12]} = 8.84$, $p = 0.012$, aCSF vs B/M $F_{[1,1]} = 10.92$, $p = 0.045$). B) Microinjection of B/M into the IL antagonized cue-induced (interaction $F_{[1,10]} = 5.87$, $p = 0.051$, aCSF vs B/M $F_{[1,1]} = 15.90$, $p = 0.003$), but was without effect on methamphetamine-induced reinstatement (extinction vs reinstatement $F_{[1,1]} = 25.36$, $p < 0.001$). Animals were also microinjected with B/M alone into the IL that induced a trend towards an increase in active lever pressing ($p = 0.063$, Wilcoxon signed rank; $p = 0.093$, paired Student's t-test). Closed arrow- switch from 1 hr short access to 6 hr long access protocol. Open arrow- switch from long access self-administration to extinction training. Data were statistically analyzed using a one-way ANOVA with repeated measures over day followed by a Bonferroni post hoc analysis.

+ $p < 0.05$, compared to extinction (Ext)

* $p < 0.05$, comparing aCSF to Bac/Mus within each reinstating modality

Figure 2.

Animals pretreated into the NAc_{core}, but not the NAc_{shell}, with B/M show reduced reinstatement responding relative to aCSF injections. A) Microinjection of B/M into the NAc_{core} antagonized methamphetamine- (interaction $F_{[1,14]} = 9.77$, $p = 0.008$, extinction vs reinstatement $F_{[1,1]} = 7.68$, $p = 0.015$, aCSF vs B/M $F_{[1,1]} = 29.34$, $p < 0.001$;) and cue-induced reinstatement (interaction $F_{[1,8]} = 6.61$, $p < 0.001$, aCSF vs B/M $F_{[1,1]} = 41.53$, $p < 0.001$). B) Rats were pretreated into the NAc_{shell} and neither cue- nor methamphetamine-induced reinstatement of active lever pressing was reduced by B/M (Meth: extinction vs reinstatement $F_{[1,1]} = 52.73$, $p < 0.001$; Cue: extinction vs reinstatement $F_{[1,1]} = 43.76$, $p < 0.001$). Closed arrow- switch from 1 hr short access to 6 hr long access protocol. Open arrow- switch from long access self-administration to extinction training. Data were statistically analyzed using a one-way ANOVA with repeated measures over day followed by a Bonferroni post hoc analysis.

+ $p < 0.05$, compared to extinction (Ext)

* $p < 0.05$, comparing aCSF to Bac/Mus within each reinstating modality

Prefrontal Cortex

Nucleus Accumbens

Figure 3.

Location of microinjection cannula tips in the brain for all animals used for data analysis in this study. In the prefrontal cortex, the IL (open circles) and PL (filled circles) are separated by the bold line. In the nucleus accumbens, the NAshell is indicated by the open, and the NAcore by the filled circles.

Table 1

Methamphetamine infusions and active lever presses during long access and the escalation of intake.

Injection Site	Presses Ave days 11-13 [*]	Infusions Ave days 11-13	Presses Ave days 20-22 [*]	Infusions Ave days 20-22	Statistics comparing infusions
Prelimbic cortex (PL)	161 ± 12	83 ± 6	193 ± 19	111 ± 14	t= 2.92[7] p= 0.023
Infralimbic cortex (IL)	180 ± 25	90 ± 12	261 ± 60	118 ± 11	t= 2.41[10] p= 0.039
NAcore	133 ± 18	81 ± 12	168 ± 14	105 ± 7	t= 2.40[7] p= 0.048
NAshell	143 ± 25	67 ± 11	193 ± 46	91 ± 16	t= 1.76[8] p= 0.116

** Paired Student's t-test (t value[degrees of freedom], probability value)

* Data for individual days in each group shown in figures 1 and 2.