THE ROLE OF NMDA RECEPTORS IN EXTINCTION OF COCAINE SELF-ADMINISTRATION

by

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ABSTRACT

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Relapse is highly prevalent among recovering addicts, and can be triggered by associations made between the rewarding effects of the drug and cues, such as drug paraphernalia or contexts. Inhibiting these associations, through new extinction learning, could help reduce relapse rates. Extinction is formed in phases, like other types of memory. The memory first is acquired in short-term memory, then is consolidated into long-term storage from which it can be retrieved at a later time (Quirk & Mueller, 2008). NMDA receptors are necessary for extinction in other paradigms (Santini, Muller, & Quirk, 2001), and we previously found that blocking NMDA receptors before four 45-minute extinction sessions disrupts retention when tested during a subsequent full extinction session. However, it is unclear which learning phase was disrupted. To determine how NMDA receptors affect extinction of cocaine self-administration, rats were trained to lever press for i.v. infusions of cocaine. Following training, rats underwent four 45-minute extinction sessions and received post-extinction session systemic injections of either the NMDA receptor antagonist CPP or the NMDA receptor coagonist D-serine. I hypothesized that CPP would disrupt extinction learning, and D-serine would facilitate extinction learning. Post-extinction session injections of CPP did not disrupt the consolidation of extinction, but instead appears to facilitate extinction.

Alternative explanations such as pharmacological side effects or disrupted reconsolidation could explain these results. Post-session injections of D-serine facilitated consolidation of extinction compared to controls. These studies indicate that NMDA receptors are necessary for acquisition and maybe consolidation of extinction, and potentiating NMDA receptors will facilitate extinction learning. Additionally, western blotting was conducted on ventral medial prefrontal cortex (vmPFC) and nucleus accumbens (NAc) tissue to determine glutamate receptor expression following extinction of cocaine self-administration, withdrawal from cocaine, or extinction of sucrose reinforcement. Results indicated a trend for increased NR2B-containing NMDA receptor expression in the vmPFC after extinction of sucrose reinforcement and increased expression of NR2A- and NR2B-containing NMDA receptors in the NAc following cocaine withdrawal compared to expression following extinction of sucrose reinforcement. Thus, these studies indicate that cocaine use and extinction learning can induce changes with NMDA receptors in the NAc and possibly vmPFC.

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Introduction

Drug addiction and extinction learning

Humans have been abusing psychoactive substances for thousands of years, presumably because the psychoactive substances are able to induce positive or reduce negative affective states (Nesse & Berridge, 1997). However, prolonged use can lead to tolerance, dependence, or addiction that is characterized by compulsive drug seeking and taking despite negative consequences (Koob et al., 2004). Drug addiction directly impacts roughly 23.5 million people ages 12 and over in the United States, and indirectly impacts more individuals through family and friends (National Survey on Drug Use and Health, NIDA, 2009). Addiction is considered to be a chronic disorder, because most addicts relapse even after long periods of abstinence or after undergoing treatment (McLellan, Lewis, O'Brien, & Kleber, 2000).

Addiction can also be viewed as a disorder of learning and memory (Kelley, 2004). Individuals learn associations between the rewarding effects of a drug and cues, such as drug paraphernalia (e.g., pipe or syringe) or drug-associated contexts (e.g., place where drugs were purchased or used) (Hyman & Malenka, 2001). When drug addicts are exposed to these drug cues, they experience increased craving and withdrawal-like symptoms, which could trigger relapse (Childress, McLellan, & O'Brien, 1986). By viewing addiction as a disorder of learning and memory, new learning-based therapies can be developed that focus on inhibiting drug-associated memories, such as extinction-based exposure therapy (Kantak & Nic Dhonnchadha, 2011). Despite the progress in understanding drug addiction, much is unknown about the neural mechanisms underlying drug-associated learning and extinction.

When an addict is exposed to drug-associated cues repeatedly in the absence of the drug, the association between the cues and the rewarding effects of the drug will be inhibited; in other words, the addict will undergo extinction. Extinction learning results in the formation of a new memory that inhibits the original associations made between cues and the rewarding properties of a drug. This new inhibitory memory is formed in stages, like other forms of learning. The memory is first acquired in short-term memory and is then consolidated into long-term storage. The memory can then be retrieved and subsequently reconsolidated back into long-term storage (Quirk & Mueller, 2008). Extinction learning can also be manipulated at each of these stages. The initial acquisition of extinction can be manipulation prior to the first extinction session to either strengthen or weaken the memory, and consolidation of extinction can be manipulated following the initial extinction session. Similarly, retrieval of the extinction memory can be manipulated before subsequent extinction trials, and reconsolidation can be manipulated following subsequent extinction trials (Millan, Marchant, & McNally, 2011). In a clinical context, extinction is utilized in extinction-based exposure therapy. Thus, by understanding the underlying mechanism of extinction learning, pharmaceutical adjuncts can be developed to strengthen extinction therapy and reduce relapse rates. To study the underlying mechanisms of extinction learning, the self-administration paradigm can be used to model drug addiction in animals.

The self-administration paradigm

Drug addiction can be modeled with animals using the self-administration paradigm. This model allows researchers to study the acquisition of drug taking, model a

form of exposure therapy with extinction learning, and study the mechanisms underlying withdrawal and reinstatement of drug seeking. Animals are trained to lever press for intravenous infusions of a drug, and these lever presses are often paired with salient cues such as tones and lights. Throughout training sessions, animals learn to associate the tone and light cues with the administration of the drug (Thomsen & Caine, 2005). Rats, mice, and monkeys will consistently and reliably learn to administer a number of drugs that are commonly abused in humans and will not reliably administer drugs not abused by humans (Koob, 2012; Thomsen & Caine, 2005).

Following stable drug taking, animals can then be tested under extinction conditions. During extinction learning, animals are presented with the salient cues without administration of drug until lever pressing is substantially reduced (Myers, Carlezon, & Davis, 2011; Thomsen & Caine, 2005). With repeated non-reinforced pairings, the cue becomes associated with the omission of the reinforcer, and a new memory is formed. This new memory inhibits the original memory that associates the cues with drug intake. Lever pressing can be reinstated, once extinction criterion is met, by exposing the animals to a priming injection of the drug, a stressor, or a non-extinguished drug-associated cue (Epstein, Preston, Stewart, & Shaham, 2006; Lynch, Nicholson, Dance, Morgan, & Foley, 2010).

The neural mechanisms of extinction learning and the resulting effects this learning has on relapse can be studied in the self-administration paradigm. As previously mentioned, extinction memory is formed in phases and each phase can be manipulated (Millan et al., 2011; Quirk & Mueller, 2008). Prior to the first extinction session, acquisition of the new extinction memory can be manipulated; likewise, immediately

after the first extinction session, consolidation of the new extinction memory can be manipulated. In addition, by shortening this initial extinction session to half the amount of time spent during acquisition of cocaine self-administration (e.g., 45 minutes), the time between the learning event (e.g., consolidation) and the pharmacological manipulation can be reduced (LaLumiere, Niehoff, & Kalivas, 2010). Retention of learning that occurs during the initial shortened extinction session can be tested during a subsequent full drug-free (e.g., 90 minutes) extinction session. Therefore, the self-administration paradigm can be used to investigate the learning that occurs during extinction training. A number of neural mechanisms have been determined necessary for both learning and addiction. For example, glutamate transmission has been implicated in the plasticity underlying learning and memory, addiction (Kalivas, 2004), and extinction learning in other paradigms (Quirk & Mueller, 2008).

Learning, memory, and glutamatergic signaling

Glutamate is the primary excitatory neurotransmitter in the central nervous system, and glutamatergic transmission has been implicated in the plasticity underlying learning and memory. Glutamate binds to three groups of ligand-gated ionotropic receptors: kainate (2-carboxy-3-carboxy-methyl-4-isopropenylpyrrolidine), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), and NMDA (N-methyl-D-aspartate). NMDA receptors are considered coincidence detectors, as they require not only binding of presynaptically released glutamate, but also require additional postsynaptic depolarization (e.g., through AMPA receptor activation) to remove the magnesium blocking the channel pore. The NMDA receptor is a heteromeric complex

composed of a combination of three gene families: NR1, NR2, and NR3 (Low & Wee, 2010). The NR1 subunit is contained in all NMDA receptors and is required for the receptor to be active (Tsien, Huerta, & Tonegawa, 1996). All NMDA receptors also contain at least one NR2 subunit, which can be further divided into four subtypes: NR2A, NR2B, NR2C, and NR2D. In an adult rat brain, the NR2A and NR2B subtypes are largely expressed in the forebrain, and the NR2C and NR2D subtypes are expressed in the cerebellum and brain stem regions (Wenzel et al., 1995).

The NMDA receptor is permeable to calcium (Ca^{2+}), which triggers a number of downstream events important for regulating synaptic strength and neuronal excitability (Kawamoto, Vivar, & Camandola, 2012). Thus, due to this influx of Ca^{2+}, NMDA receptors have been heavily studied for their role in synaptic plasticity and learning and memory. For example, mice that over-express NR2B-containing NMDA receptors in the forebrain had improved performance relative to wild-type mice on a number of learning and memory tasks such as novel-object-recognition, context and cued fear conditioning and extinction, and Morris water maze (Tang et al., 1999).

On the other hand, drugs that act as NMDA receptor antagonists such as phencyclidine (PCP) and its analog ketamine impair learning and memory tasks in humans (Newcomer & Krystal, 2001) and monkeys (Thompson, Winsauer, & Mastropaolo, 1987). Administration of MK-801, another NMDA receptor antagonist, disrupts learning specific operant behaviors in Rhesus monkeys (Buffalo, Gillam, Allen, & Paule, 1993) and acquisition of water maze learning in rats (McLamb, Williams, Nanry, Wilson, & Tilson, 1990) and gerbils (Mondadori, Weiskrantz, Buerki, Petschke, & Fagg, 1989). Moreover, in a fear conditioning paradigm, systemic injections of MK-

801 disrupt acquisition and consolidation of extinction learning when given before or four, but not 12, hours after the extinction session (Liu et al., 2009). Similarly, rats given a systemic injection of the NMDA receptor antagonist CPP (3-(2-carboxypiperazin4-yl)propyl-l-phosphonic acid) prior to an extinction of conditioned fear session demonstrated normal extinction learning during the session, but were unable to express this learning 24 hours later, suggesting the consolidation of the new extinction memory was disrupted (Santini et al., 2001; Suzuki et al., 2004). In summary, over-expression of NMDA receptors enhances learning, and NMDA receptor antagonists impair learning, including the consolidation of extinction of conditioned fear. However, the role of NMDA receptors in extinction of cocaine self-administration is poorly understood.

NMDA receptors and extinction of drug seeking

Few studies have been conducted to determine the role of NMDA receptors in extinction of drug self-administration despite the role that NMDA receptors have in learning and memory, particularly in extinction of conditioned fear. Contrary to reports on fear extinction, one study found that systemic injections of a low dose (5 mg/kg, i.p.) of the NMDA receptor antagonist CPP did not disrupt extinction learning in a self-administration paradigm (Kelamangalath, Swant, Stramiello, & Wagner, 2007). However, we recently found that a higher dose (10 mg/kg, i.p.) of CPP was effective at disrupting extinction of cocaine self-administration. After rats had acquired cocaine self-administration, they received systemic injections of either saline or CPP before four 45-minute extinction sessions. The shortened 45-minute extinction sessions were selected to reduce the amount of learning occurring during a session, thus reducing the time between

the learning event and the pharmacological manipulation (LaLumiere et al., 2010). During the subsequent drug-free 90 minute extinction session (day five), rats previously treated with CPP had disrupted extinction retention compared to controls (see figure 1; Hafenbreidel, Twining, & Mueller, unpublished data). Interestingly, Burgos-Robles et al., (2007) demonstrated that a 5mg/kg dose did not disrupt recall of fear extinction when tested 24 hours later, but the 10mg/kg dose did disrupt extinction when tested 24 hours later. Taken together, these data suggest that CPP is only effective at disrupting extinction learning when a sufficient dose is used.

Figure 1

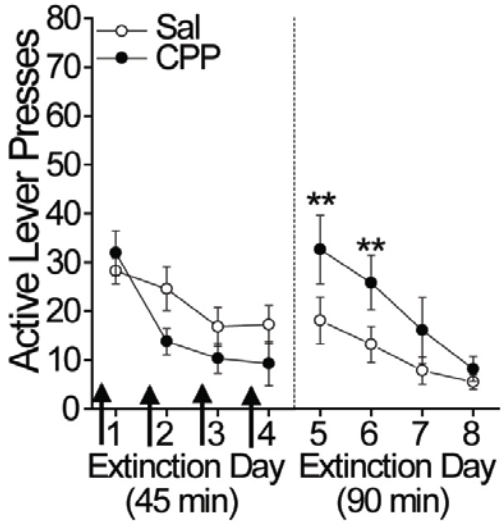

Figure 1. NMDA receptors are necessary for extinction.
Rats treated with either CPP (black) or saline (open) during the 45-minute extinction session reduced lever presses across trials (left). However, rats treated with CPP did not retain extinction learning when given a full drug-free extinction session compared to rats treated with saline (right). **P<0.05 compared to saline, and compared to CPP extinction day four.

As mentioned previously, an extinction memory is formed in stages like other types of memory (Quirk & Mueller, 2008). The original drug-memory is retrieved during extinction day one, however, as the session progresses the animal will acquire a new

memory that inhibits the original associations between the cues and the rewarding effects of the drug. Following this first extinction session, the new inhibitory memory is consolidated into long-term storage. In the self-administration paradigm, extinction occurs across multiple trials, during which the inhibitory extinction memory is strengthened. Acquisition of the new extinction memory can be pharmacologically manipulated before the first extinction session, and consolidation can be manipulated immediately after the first extinction session (Millan et al., 2011). CPP remains active for over 18 hours (Abraham & Mason, 1988) and thus pre-extinction injections are likely to affect both acquisition and consolidation. Thus, it is not possible to dissociate whether NMDA receptors are necessary for the acquisition or consolidation of the new inhibitory extinction memory from this experiment. These discrepancies warrant further investigation to determine the specific role of NMDA receptors in extinction of drug seeking.

Clinical implications: Enhancing NMDA receptor function

Cues associated with the rewarding effects of a drug can trigger drug craving and increase the risk of relapse (Hyman & Malenka, 2001). Thus, inhibiting cues from eliciting craving through extinction training could help reduce relapse rates. With other cue association type disorders, such as phobias, extinction-based exposure therapy is a very effective treatment (Hofmann & Smits, 2008). However, without a pharmacological adjunct, extinction-based exposure therapy has had limited success in the treatment of drug addiction (Conklin & Tiffany, 2002) and currently there is no FDA-approved medication for the treatment of cocaine addiction. A pharmacological manipulation of

NMDA receptors may prove to be a possible method for treatment. Inhibiting NMDA receptors disrupts learning and memory in humans (Newcomer & Krystal, 2001), monkeys (Buffalo et al., 1993; Thompson et al., 1987), and rats (Burgos-Robles et al., 2007; Santini et al., 2001). Conversely, enhancing NMDA receptor function may improve extinction learning and enhance extinction-based exposure therapy.

NMDA receptors bind glutamate, but also can bind a coagonist, such as glycine or D-serine. D-serine is an endogenous ligand that binds to the NMDA receptor and acts as a coagonist to potentiate the function of the NMDA receptor by increasing the frequency of the receptor opening (Johnson & Ascher, 1987; Mothet et al., 2000; Schell, Molliver, & Snyder, 1995). A coagonist differs from a partial agonist in that it binds in addition to the full agonist (e.g., glutamate) to enhance receptor function and is not sufficient to open the receptor alone (Johnson & Ascher, 1987). A partial agonist, on the other hand, can bind and activate the receptor on its own, but has a reduced biological effect or lower efficacy compared to a full agonist (Mayer, 2006; Nestler, Hyman, & Malenka, 2009). In summary, the NMDA receptor coagonist D-serine is a naturally occurring ligand in the brain and has its biological effect as an additive to the normal function at the synapse. The affect of D-serine on NMDA receptors suggests a more natural way to enhance synaptic transmission, and perhaps facilitate new extinction learning.

D-serine can facilitate learning in other learning and memory paradigms, such as extinction of fear conditioning (Fiorenza, Rosa, Izquierdo, & Myskiw, 2012; Matsude et al., 2010). D-serine can also facilitate extinction in another paradigm that is used to study addiction: the conditioned place preference paradigm (Hammond, Seymour, Burger, & Wagner, 2012). Furthermore, D-serine administered before or after one or five extinction

trials following cocaine self-administration will reduce low dose cocaine-induced reinstatement (Kelamangalath, Seymour, & Wagner, 2009; Kelamangalath & Wagner, 2010). In conclusion, D-serine can facilitate learning, and may act as protection against reinstatement. Therefore, because of D-serine's affect on learning, it is likely that D-serine will enhance extinction learning and thus could be used to improve therapy success by reducing relapse rates.

Aims

In the present study, I aimed to dissociate the role of NMDA receptors in the acquisition and consolidation of extinction of cocaine self-administration. In addition, I aimed to determine the effect of NMDA receptor potentiation on extinction by administrating the coagonist D-serine. To dissociate acquisition from consolidation, rats received systemic injections of either saline or the NMDA receptor antagonist CPP immediately after each of four 45-minute extinction sessions. Retention of extinction learning was then determined during a subsequent drug-free 90-minute extinction session. Post-extinction session injections were selected to target consolidation and eliminated possible drug-state effects, as the rat was drug free during the extinction session. Additionally, to determine the effects of NMDA receptor potentiation on extinction learning, another set of rats received systemic injections of either saline or the NMDA receptor coagonist D-serine immediately after each of four 45-minute extinction sessions. Retention of extinction learning was then determined during a subsequent drug-free full-length extinction session. I hypothesized that post-extinction session injections of CPP would disrupt consolidation of extinction learning, and post-injections of D-serine would facilitate extinction learning.

Methods

Subjects

A total of 57 male Long-Evans rats (Harlan Laboratories, Madison, WI) weighing 250-300 g at the start of the experiment were housed individually in clear plastic cages in a temperature and humidity controlled room with a 14 hour light/ 10 hour dark cycle (lights on at 7am). Rats had unlimited access to water, but were food restricted (13-28 g standard rat chow daily ration) throughout the experiment except during surgery and recovery from surgery. All experimental protocols have been approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee in accordance with National Institutes of Health guidelines.

Sucrose and self-administration apparatus

Sucrose and cocaine self-administration procedures were conducted using twelve standard operant chambers (MED Associates, St. Albans, VT) that measure 30.5 cm long x 24.1 cm wide x 21.0 cm high. Each chamber has two retractable levers with a light placed above them, a food hopper placed between the two levers, a house light (25 W), a rod grid floor (23 4.8-mm stainless steel bars spaced 1.3 cm apart), and a speaker/tone generator (65 Hz, 65 dB). The operant chamber is located within a sound- and light-attenuating enclosure with a ventilation fan. Each chamber has a clear Plexiglas top, front, and back wall. The sidewalls are made of aluminum and Plexiglas. The syringe pump is located outside of the enclosure and is connected to a single channel swivel (Instech Solomon, Plymouth Meeting, PA) by tygon tubing. Connected to the swivel is a

connector cannula (Plastics One, Roanoke, VA; a metal spring attached to a metal spacer with PE50 tubing inserted down the center) that is connected to the catheter anchored in the back of the rat (see Surgery).

Sucrose lever press training

After a one week adjustment period, rats were placed in the operant chamber daily for ten-hour sessions on a fixed ratio (FR1) for sucrose reinforcement until minimum criteria of 100 lever presses was achieved. At the beginning of a session, the house light turned on, the right lever extended, and the right stimulus light turned on. When a rat pressed the lever, a single 20mg sucrose pellet was dispensed into the food hopper. When criteria were met, sessions were shortened to 90 minutes daily on a FR1 fixed schedule with a minimum criterion of 50 rewards per session. After rats met these criteria, they underwent catheter surgery (see figure 2 for methods summary).

Figure 2

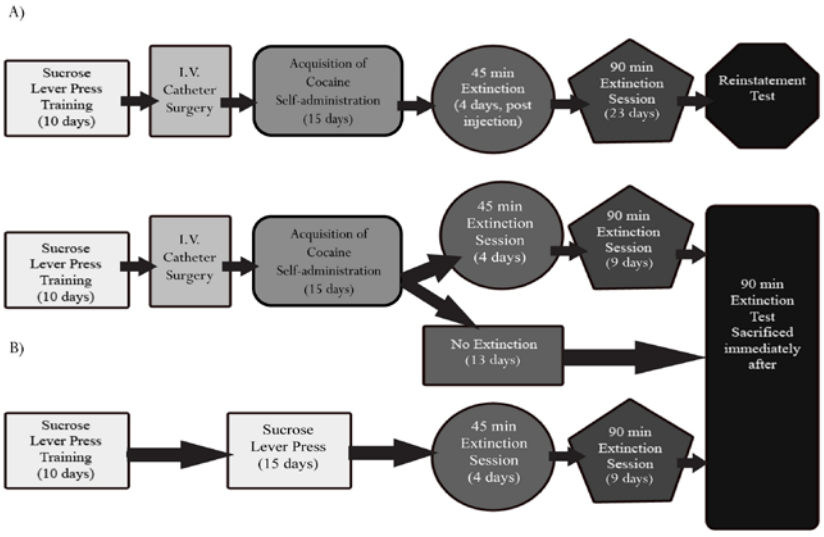

Figure 2. Summary and timeline of methods.
A) Rats were first trained to lever press for sucrose for 10 days, before being implanted with an intravenous (i.v.) catheter. Following recovery, rats acquired cocaine self-administration for 15 days. Next, rats underwent four shortened extinction sessions of 45 minutes and were injected immediately after each session with either saline, CPP, or D-serine. Extinction retention was tested drug-free in a subsequent longer 90-minute extinction session. When rats met extinction criterion, they then underwent reinstatement tests. B) Two groups of rats were trained to lever press with sucrose for 10 days. The first group was then implanted with an i.v. catheter, before undergoing 15 days of cocaine self-administration. The other group lever pressed for sucrose for the same 15 days. The first group was then divided into two groups: one group underwent extinction similar to A) and the other did not undergo extinction. The group that was lever pressing for sucrose underwent the same extinction protocol as the group that received cocaine. When both groups met extinction criterion, all rats underwent an extinction test and were sacrificed immediately after the session.

Surgery

Self-administration catheter

The catheters were designed by R. Twining (UW, Milwaukee, WI) and Access Technology (Skokie, IL). The catheter consists of a smaller polyurethane tube (3.5 french gauge by 8.1cm), with a fixed bead placed 3.5cm from the end of the small tubing, inserted and glued into a larger polyurethane tube (2 french gauge by 6cm), which is

attached to a backmount pedestal (313-001BM-10-5up; Plastics One, Roanoke, VA) with polyester mesh above the side tubing to facilitate tissue regrowth. The 3.5cm length of tubing past the fixed bead is placed in the jugular vein and anchored to the vein with silk sutures. The catheter is assembled and sent from Access Technologies (Skokie, IL) in a sterile pouch. The sterile pouch is opened and a 2.5 by 2.5 cm mesh screen (Small Parts, Miramar, FL) is permanently fixed to the base via cyanoacrylic gel and functions to stabilize the base subcutaneously. The entire catheter is soaked in betadine for a minimum of 1 hour, rinsed, and flushed with reagent ethanol and sterile saline before implantation.

Catheter implantation and maintenance

Surgery was conducted by using a modified procedure described by Grigson and Twining (2002). Rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine/xylazine (87 mg/kg/13 mg/kg). The hair was shaved on the back of the rat between the shoulder blades and over the right jugular vein. The first incision was made diagonally, approximately 25 mm in length, across the mid-back of the rat. The second incision was made horizontally between the shoulder blades, approximately 5 mm in length. The skin was separated from the muscle by hemostats. The final incision was made over the right jugular, approximately 10 mm in length, at a 30° angle away from the midline. The tissue was gently cleared from the muscle by blunt microforceps. A stainless steel rod (0.095 mm in diameter) was moistened with saline and gently pushed subcutaneously from the incision over the right jugular, over the right foreleg, and through the larger incision on the back. The polyurethane tubing was then inserted into the rod and rod was removed, bringing the tubing to the incision above the jugular. Next,

the jugular vein was isolated and the surrounding tissue was gently removed with blunt microforceps. Another stainless steel rod (0.058 mm in diameter) was moistened with saline and gently placed under the jugular vein to enable the experimenter to make a small incision (approximately 0.5 mm) in the vein. The polyurethane tubing up to the fixed bead of the catheter was then inserted through the incision into the vein. To verify that the catheter was in the jugular vein, a syringe filled with saline was attached to the other end of the catheter (coming out of the back of the rat) and blood was drawn back into the syringe. After verification of proper placement, the catheter was secured by tying silk sutures gently around both the tubing and the jugular vein immediately anterior and posterior to the fixed bead. Next, the superficial and exterior skin layers were sutured closed and antibiotic ointment was applied over the sutures. The rat was then laid on its ventral surface, and the top of the catheter was inserted into the smaller incision between the shoulder blades. A stylet was placed in the exposed catheter opening to ensure debris did not enter the catheter. The larger incision was closed with wound clips (Fisher Scientific, Pittsburgh, PA) and antibiotic ointment was applied over the wound clips. Rats were treated prophylactically with a subcutaneous (s.c.) injection of the antibiotic penicillin g procaine (75,000 units in a 0.35 mL) to control infection and s.c. injection of the nonsteroidal anti-inflammatory carprofen (5.0 mg in 0.1 mL) to relieve pain following surgery. Each rat was given a minimum of 5 days to recover before patency was first verified with 0.3 ml of 1% Propofol (i.v.), a short acting general anesthetic that causes an immediate loss of muscle tone. Rats were given a minimum of 7 days to recover prior to behavioral testing and wound clip removal. Catheter patency is

maintained with heparinized saline (~0.2 cc of 50 international units / ml) daily during the experiment.

Drugs

Cocaine HCl (National Institute of Drug Abuse) was dissolved in sterile 0.9% saline and administered intravenously (i.v.) at a concentration of 0.25 mg per infusion for three seconds or at 10 mg/kg, i.p. for cocaine-induced reinstatement. (±)3-(2-carboxypiperazin-4yl)propyl-1-phosphonic acid (CPP) was dissolved in sterile 0.9% saline and administered at a dose of 10 mg/kg, i.p. D-Serine was dissolved in sterile 0.9% saline and administered at a dose of 100 mg/kg, i.p. These doses were determined based on previous research using CPP (Burgos-Robles et al., 2007; Santini et al., 2001) or D-Serine (Kelamangalath et al., 2009).

Cocaine self-administration

Rats were placed in the self-administration chamber daily for 90 minute sessions on a fixed ratio (FR1) or until the daily cap was achieved. Rats were first capped at 20 infusions a session but were progressively moved up to a cap of 35 infusions per session, in order to reduce the risk of overdosing. At the beginning of a session, the house light turned on, the right and left lever extended, and the right stimulus light turned on. When a rat pressed the right lever, the stimulus light turned off for 20 seconds, a tone (65 dB) sounded for five seconds, and a cocaine infusion was initiated for three seconds. To further reduce the risk of overdosing, another cocaine infusion was not available until the stimulus light turned back on. Responding to the left lever had no programmed

consequences, but was recorded. Rats underwent 15 days of cocaine self-administration, at which a minimum criterion of 25 infusions across the last three days was obtained. Rats that did not consistently meet the minimum daily criterion received priming infusions, a second 90-minute session to reach the cap, or long access sessions (10 hours, with a cap of 35 infusions). Out of the total number of rats used, two rats failed to meet the criterion. They were removed from the experiment and their data discarded.

Experimental manipulation

Experiment one.

Following cocaine self-administration, rats were pseudo-randomized into three groups: saline control, CPP post-extinction session injection, and D-serine post-extinction session injection. Rats were matched into the three groups based on their overall average number of infusions, the average of their last three days, and if they received (and how many) priming infusions, second sessions, or long-access sessions. This type of pseudo-randomized matching was necessary due to the high level of individual differences found in self-administering rats.

Extinction training began three days after the last day of cocaine self-administration in order to control for any withdrawal symptoms that might occur. During an extinction session, a right lever press had the same-programmed consequences as during self-administration, but no cocaine was administered. For the first four days of extinction training (extinction days 1-4), rats were placed into the operant chamber for only 45 minutes. The shortened 45-minute extinction session was selected in order to reduce the time between the learning event and the pharmacological manipulation

(LaLumiere et al., 2010). We showed previously that when rats undergo 45-minute extinction sessions, or half the time they spent self-administering, they do retain some extinction learning as evident by their slightly reduced lever pressing during subsequent extinction sessions (See figure 1; Hafenbreidel et al., unpublished data). Allowing for some learning to occur, but not as much as would occur during a long session, permits for a more timely manipulation of the consolidation of the extinction memory. At the end of the session, rats received either an injection of saline (1 ml/kg; i.p.), the NMDA receptor antagonist CPP (10 mg/kg; i.p.), or the NMDA receptor coagonist D-serine (100 mg/kg; i.p.) before being placed in their home cage. Post injections were selected in order to determine the role of NMDA receptors in the consolidation of extinction learning without the possibility of a state effect. Following the last day of the 45-minute sessions, rats were not tested for two days to ensure no residual drug was left before extinction retention was tested in the full 90-minute extinction session. The full 90-minute extinction sessions were conducted with the same extinction procedures as during the 45-minute sessions.

Reinstatement and locomotion procedures

After reaching the minimum requirement for extinction, rats were given either a priming injection of cocaine (10 mg/kg; i.p.) or a stressor consisting of 10-minute unsignaled variable interval (v.i.) footshock (1 mA) before undergoing normal extinction procedures to test for reinstatement. Rats underwent reinstatement procedures to determine if drug treatment during extinction would protect against reinstatement. To test for possible drug effects, rats were injected with either saline, CPP (10 mg/kg, i.p.),

or D-serine (100 mg/kg, i.p.) one hour before being placed in an open access conditioned place preference apparatus and photobeam breaks were recorded for 20 minutes.

Experiment two

At the end of cocaine self-administration, rats were pseudo-randomized as described in experiment one into two groups (extinction or no extinction). An additional control group was included, in which rats were reinforced with sucrose, rather than cocaine, to control for drug use. Half the rats from the group that self-administered cocaine (extinction group) and the rats that lever pressed for sucrose underwent the same extinction procedures as in experiment one. The other half of rats from the group that self-administered cocaine (no extinction) did not undergo extinction, and instead underwent forced withdrawal and remained in their home cages.

After the rats undergoing extinction had met extinction requirements (day 13), all rats, including the no extinction group, were tested for extinction retention on the following day with the same extinction procedure used throughout the experiment. Immediately following the extinction test session, rats were anesthetized with isoflurane gas, sacrificed, and their brains were extracted and frozen in -80˚C for western blot analysis.

Western blots

The ventral-medial prefrontal cortex (vmPFC) and the nucleus accumbens (NAc) were dissected out rapidly on dry ice. The vmPFC was dissected out from blocked tissue by making a vertical cut along the medial forceps minor corpus callosum and a horizontal cut midway through and at the ventral edge of the forceps minor corpus callosum (see

figure 8A for a representative image). The NAc was dissected out from blocked tissue by making a horizontal cut along the ventral edge of the forceps minor corpus callosum and lateral vertical cuts along the edge of the anterior commissure (see figure 9A for a representative image). Tissue samples were stored at -80˚C until needed. All tissue samples were resuspended in a 1:50 weight/volume dilution in lysis buffer containing phosphate and protease inhibitors, and were homogenized by sonication. Protein concentration of the homogenates was determined using a Bio-Rad protein assay (Bio-Rad, Hercules, CA). All samples were then normalized to 2 μg/μl using 5X SDS/PAGE loading buffer and 1X lysis buffer, and were boiled for 5 minutes to denature proteins. Next, 10 μl aliquots of sample was loaded onto 4-15% Tris-HCl polyacrylamide gels (Bio-Rad, Richmond, CA) and underwent electrophoresis for protein separation. Following separation, samples were transferred from the gel to a membrane using a Trans Blot Midi transfer pack and Turbo transfer system (Bio-Rad, Hercules, CA). Membranes were next incubated in blocking solution of 5% milk for one hour and then incubated overnight at 4˚C in primary antibodies for NR2B, NR2A, GluR1, (1:1000 for NR2B and NR2A; 1:800 for GluR1; Cell Signaling, Danvers, MA) and β-Actin (1:5000; Cell Signaling, Danvers, MA) for normalization. Following primary incubation, membranes were incubated in a secondary anti-rabbit IgG (1:5000 for GluR1 and NR2A; 1:20000 for NR2B and β-Actin; Cell Signaling, Danvers, MA) for one hour, and developed using a chemiluminescence (SuperSignal West Dura, Fisher Scientific, Pittsburgh, PA). Images of protein expression were taken using a Gel Logic Pro Molecular Imager (Carestream Woodbridge, CT), and were quantified by densitometry through accompanying

Carestream analysis/quantification software. To determine changes in protein expression; GluR1, NR2B, NR2A were normalized to β-Actin levels.

Data analysis

Drug-seeking behavior between groups was determined by averaging active lever presses during the last three days of cocaine self-administration and was analyzed using an independent sample *t*-test. Drug-seeking behavior during extinction conditions was analyzed by comparing lever presses between saline controls and drug groups (CPP or D-serine) using a repeated measures analysis of variance (ANOVA). The extinction data for the 45-minute extinction sessions (extinction days 1-4), the full 90-minute extinction sessions (extinction days 5-13 and 23; day 23 is the average of each rats last two extinction days to account for individual differences), and between day four and five were analyzed separately. Tukey's Honestly Significant Difference (HSD) *post hoc* tests were used, when appropriate, to determine the difference between lever-presses between and within each drug group per day or across multiple days. Reinstatement was measured by the average number of lever presses made during the last two extinction trials compared to the number of lever presses made after a priming-injection of cocaine or stressful event (10 minute v.i. footshock), and was analyzed with ANOVA. Tukey's HSD *post hoc* tests were used, when appropriate, to determine differences in lever pressing between days or within previous treatment groups. Locomotor activity was measured as the number of total photobeam breaks made during the 20-minute trial, and was analyzed using an independent sample *t*-test. Of the total number of rats used, 13 rats were removed from the analysis due to a blocked or non-patent catheter, sickness, or

being two or more standard deviations above the group mean throughout extinction training. For western blot analysis, mean optical densities were determined for each group, and data was analyzed using a one-way ANOVA. Outliers greater than two standard deviations were removed from the analysis. Tukey's HSD *post hoc* tests were used, when appropriate, to determine expression differences between groups.

Results

Consolidation of extinction of cocaine self-administration was not disrupted by post-injections of the NMDA receptor antagonist CPP

To determine the necessity of NMDA receptors in the consolidation of extinction of cocaine self-administration, rats were injected with the NMDA receptor antagonist CPP immediately following four shortened 45-minute extinction sessions. The shortened 45-minute extinction sessions were selected to ensure consolidation was manipulated by reducing the amount of time between the pharmacological manipulation and the learning event (LaLumiere et al., 2010). Extinction memory retention was tested on day five with a full 90-minute extinction session. Rats were then given 20-30 more daily 90-minute extinction sessions until extinction criterion was met. There were no significant differences between active lever presses between groups using an independent *t*-test (*t(14)=0.941, P=0.362)* across the average of the last three days of cocaine self-administration (see figure 3A). The left side of figure 3B shows the average number of active lever presses during the 45-minute extinction days 1-4, and on the right side are the average number of active lever presses during the 90-minute extinction day 5-13 and 23. Day "23" is the average number of lever presses during the last two days of extinction for

each rat. Averaging the last two extinction days of each individual rat was necessary due to individual differences between rats and reaching extinction criteria. For the 45-minute extinction days 1-4, a repeated measures ANOVA revealed no significant differences across extinction days ($F_{3,51}$= 1.924, P=0.140) or between treatment groups ($F_{1,52}$=2.279, P=0.138). For the 90-minute extinction days 5-23, a repeated measures ANOVA found no significant differences across days ($F_{18,246}$=0.424, P=0.982), but there was a significant difference between treatment groups ($F_{1,246}$=49.368, P<0.0001). Thus, these data suggest that rats treated with CPP during the 45-minute extinction sessions extinguished faster than saline controls during extinction days 5-23. Lastly, ANOVA revealed no significant differences between the 45-minute extinction day four to the 90-minute extinction day five by day ($F_{1,24}$=2.880, P=0.104) or by treatment group ($F_{1,24}$=0.821, P=0.375), indicating both groups had retained learning during the 45-minute extinction sessions when tested during the longer extinction sessions. Thus, blocking NMDA receptors with CPP following four 45-minute extinction sessions did not disrupt consolidation of extinction learning. Rather, post-extinction CPP injections appeared to unexpectedly facilitate extinction, had pharmacological side effects, or impair reconsolidation.

Figure 3

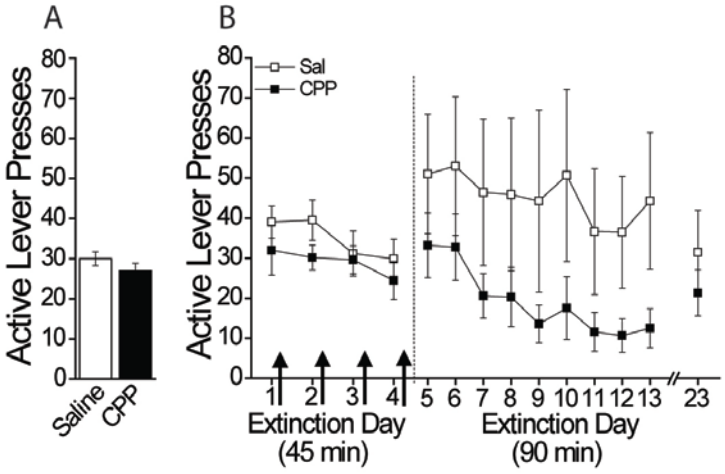

Figure 3. Blocking NMDA receptors, post-extinction session does not disrupt consolidation of extinction.
A) There was no significant difference between rats treated during extinction with either saline (open) or CPP (black) during the average of the last three days of cocaine self-administration. B) (Left) Rats injected with CPP (black) immediately following four 45-minute extinction sessions were not significantly different that rats injected with saline (open). (Right) When tested during a full 90-minute extinction session on day five, neither group significantly increased drug seeking. However, rats treated with CPP appear to extinguish faster across extinction days 5-23 compared to rats treated with saline.

To test for previous drug treatment affects on reinstatement, reinstatement of drug seeking was tested once both groups were at extinction criteria. Rats were given either a non-contingent priming injection of cocaine (i.p.) or a stressor, consisting of 10-minute variable interval (v.i.) footshocks, before a regular extinction session (see Figure 4A for cocaine-induced reinstatement and 4B for stress-induced reinstatement). ANOVA revealed significant differences between the average of the last two extinction days and cocaine-induced reinstatement test day ($F_{1,25}$=21.763, P<0.0001), but not by treatment group ($F_{1,25}$=0.424, P=0.522). After cocaine-induced reinstatement, rats underwent extinction training again before receiving stress-induced reinstatement. An ANOVA

found no significant differences between the average of the last two extinction days and stress-induced reinstatement test day ($F_{1,25}$=2.230, *P*=0.150) or by group ($F_{1,25}$=2.620, *P*=0.120). Therefore, both groups previously treated with either saline or CPP significantly increased lever pressing when given a priming injection of cocaine but not when given a stressor. Lastly, to determine if systemic injections of CPP had any effect on locomotor activity, saline or CPP was injected one hour before being placed in an open access conditioned place preference apparatus and photobeam breaks were recorded for 20 minutes. There were no significant differences in photobeam breaks between rats treated with saline or CPP using an independent *t*-test (*t*(10)=0.211, *P*=0.874) (see Figure 4C). Thus, indicating systemic injections of CPP did not affect locomotor activity.

Figure 4

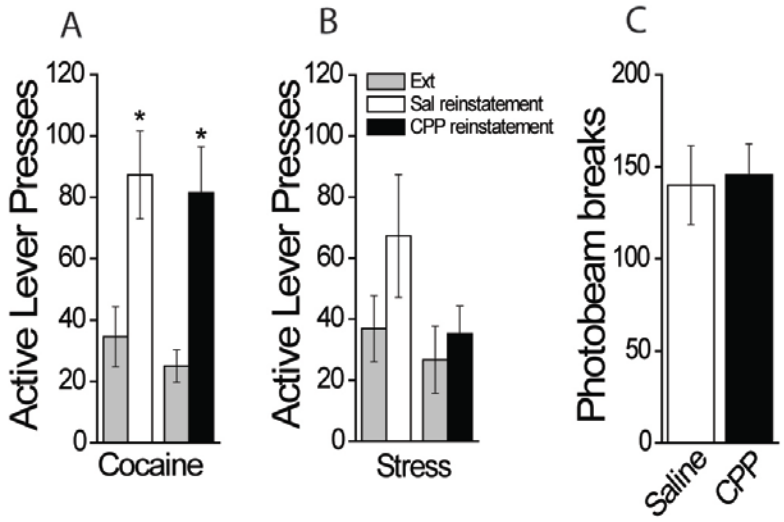

Figure 4. Previous treatment with the NMDA receptor antagonist CPP had no effect on reinstatement

A) Rats previously treated with either saline (right) or CPP (left) significantly increased drug seeking when given a priming injection of cocaine (*P<0.05, compared to average of the last two extinction days), but did not significantly increase lever presses when given a stressful event (B). C) CPP (black) had no effect on locomotor activity, as measured by photobeam breaks, compared to saline when administered before being placed in an open access conditioned place preference chamber.

Potentiating the NMDA receptor with D-Serine facilitated extinction learning and reduced stress-induced reinstatement

To determine if NMDA receptor potentiation would facilitate extinction of cocaine self-administration, rats were injected with the NMDA receptor coagonist, D-Serine, immediately following four 45-minute extinction sessions. Retention of extinction learning was tested on a subsequent 90-minute extinction session (day 5), and rats were given 20-30 additional 90-minute extinction sessions until extinction criteria were met. There were no significant differences between active lever presses between treatment groups across the average of the last three days of cocaine self-administration using an independent *t*-test ($t(14)=0.820$, $P=0.425$) (see Figure 5A). The left side of figure 5B shows the average number of active lever presses during the 45-minute extinction days 1-4, and the right side shows the average number of active lever presses during the 90-minute extinction days 5-13 and 23. Day "23" is the average number of lever presses during the last two days of extinction for each rat. Averaging the last two extinction days of each individual rat was necessary due to individual differences between rats and reaching extinction criteria. A repeated measures ANOVA revealed no significant differences by day during the 45-minute extinction days 1-4 ($F_{3,51}=1.769$, $P=0.167$) or by treatment group ($F_{1,51}=1.803$, $P=0.186$). For the 90-minute extinction days 5-23, a repeated measures ANOVA revealed no significant differences by day ($F_{18,235}=0.562$, $P=0.923$), but did indicate significant differences by treatment group ($F_{1,235}=31.310$, $P<0.0001$). These data indicate that rats treated with D-Serine during the 45-minute extinction sessions have facilitated extinction compared to saline controls

during the 90-minute extinction days 5-23. Finally, ANOVA revealed significant differences between extinction day four to extinction day five ($F_{1,25}$=4.694, P<0.05) but not between treatment groups ($F_{1,25}$=0.225, P=0.618), indicating both groups retained little from the four 45-minute extinctions sessions when tested on the full 90-minute session. However, this difference was not due to previous drug-treatment as there were no differences between groups. In summary, potentiating NMDA receptors with the coagonist D-Serine facilitated extinction of cocaine self-administration.

Figure 5

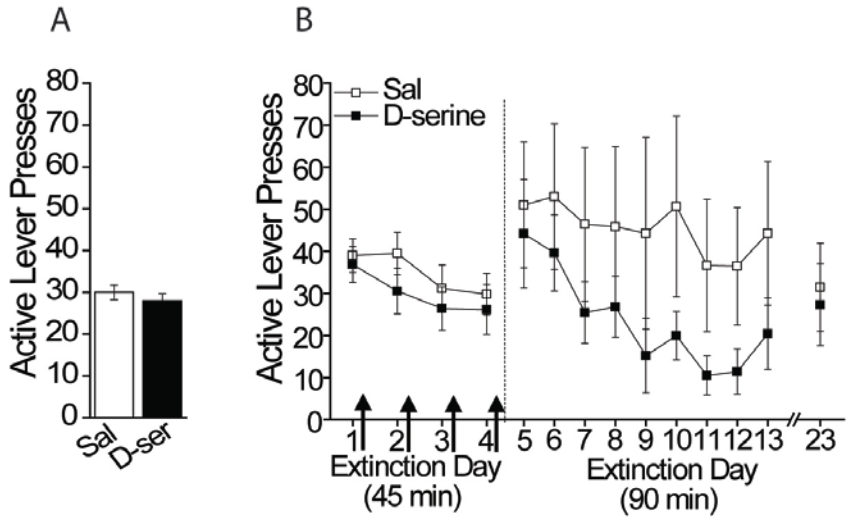

Figure 5. Potentiating NMDA receptors with D-serine facilitates extinction learning.
A) There was no significant difference between rats treated during either extinction with saline (open) or D-serine (gray) during the average of the last three days of cocaine self-administration. B) (Left) Rats injected with D-serine (gray) immediately following four 45-minute extinction sessions were not significantly different that rats injected with saline (open). (Right) When tested during a 90-minute extinction session on day 5, both groups significantly increased drug seeking. However, rats treated with D-serine had facilitated extinction across extinction days 5-23 compared to rats treated with saline.

To test for previous drug treatment affects on reinstatement, reinstatement of drug seeking was tested once both groups were at extinction criteria. Rats were either given a

non-contingent priming injection of cocaine or underwent a stressful event of 10-minute v.i. footshock to induce reinstatement, before undergoing a normal extinction session (see Figure 6A for cocaine-induced reinstatement and 6B for stress-induced reinstatement). ANOVA revealed significant differences between the average of the last two extinction days and the cocaine-induced reinstatement day ($F_{1,21}$=16.691, P<0.001), and no effect of treatment ($F_{1,21}$=0.076, P=0.786). Rats underwent two more extinction sessions before undergoing stress-induced reinstatement. ANOVA did not reveal a significant difference between the average of the last two extinction days and the stress-induced reinstatement day ($F_{1,21}$=1.289, P=0.271), but did reveal a non-significant but strong trend between groups ($F_{1,21}$=3.383, P=0.082). In summary, both groups previously treated with either saline or D-Serine significantly increased lever pressing when given a priming injection of cocaine, but not a stressor. However, there was a trend for D-serine to protect against stress-induced reinstatement. Lastly, to determine if D-serine had any effect on locomotor activity, saline or D-serine was injected one hour before being placed in an open access conditioned place preference chamber and photobeam breaks were recorded for 20 minutes. There were no significant differences in photobeam breaks between rats treated with saline or D-serine using an independent *t*-test (t(10)=0.039, P=0.97) (see Figure 6C). Thus, indicating systemic injections of D-serine did not affect locomotor activity.

Figure 6

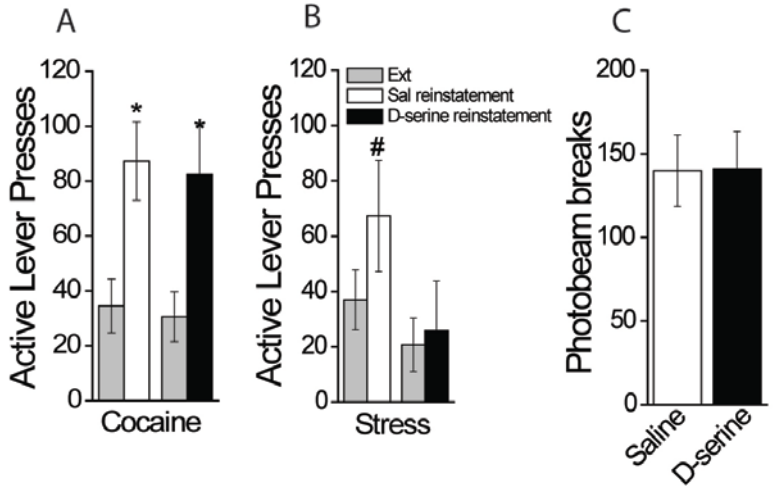

Figure 6. Previous treatment with D-serine reduces stress-induced, but not cocaine-induced, reinstatement.
A) Rats previously treated with either saline (right) or D-serine (left) significantly increased drug seeking when given a priming injection of cocaine (*P<0.05, compared to average of the last two extinction days). B) Rats previously treated with saline (right) or D-serine did not significantly increase drug seeking when given a stressful event. However, rats previously treated with D-serine had a strong trend to lever press less than rats previously treated with saline following the stress-prime (#P=0.082, compared to saline). C) D-serine had no effect on locomotor activity compared to saline, as measured by photobeam breaks, when administered before being placed in an open access conditioned place preference chamber.

NMDA receptor, but not AMPA receptor, expression is altered in the vmPFC and NAc following extinction

To determine if specific glutmatergic receptor expression was altered in the ventral medial prefrontal cortex (vmPFC) and the nucleus accubens (NAc) following extinction of cocaine self-administration, rats were trained to lever press for either cocaine or sucrose. Following training, half the rats that lever pressed for cocaine and all the rats that lever pressed for sucrose underwent extinction training identical to experiment one. The other half of rats that lever pressed for cocaine received no

extinction training and remained in their home cages. There were no significant differences between active lever presses between groups that either underwent extinction or no extinction across the average of the last three days of cocaine self-administration using an independent *t*-test ($t(13)=0.413$, $P=0.686$) (see Figure 7A). After rats undergoing extinction had reached criteria (see Figure 7B for extinction of cocaine self-administration, and 7C for extinction of sucrose reinforcement), all groups were tested for extinction retention (see Figure 7D). An ANOVA revealed a significant difference between treatment groups ($F_{2,23}=19.806$, $P<0.0001$). *Post hoc* analysis confirmed that rats that received extinction of cocaine self-administration lever pressed significantly less than those who did not received extinction ($P<0.005$), rats that received sucrose extinction lever pressed significantly less than rats that did not receive extinction of cocaine self-administration ($P<.0001$), and both groups that underwent extinction training were not significantly different. Following the extinction retention test, rats were sacrificed and their brains were extracted. The vmPFC and NAC were later collected for analysis (see figure 8A and 9A for representative collection areas).

Figure 7

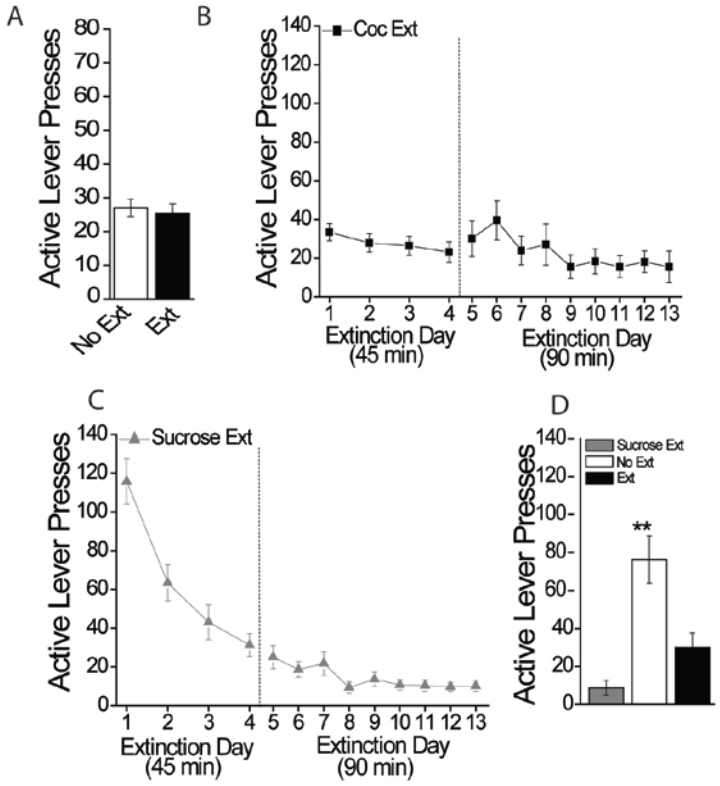

Figure 7. Extinction of either cocaine self-administration or sucrose reinforcement A) There was no significant difference between rats that either underwent extinction (black) or no extinction (open) during the average of the last three days of cocaine self-administration. Rats underwent extinction of cocaine self-administration (B) or extinction of sucrose reinforcement (C). D) On test day, rats that underwent extinction (black, cocaine extinction; gray, sucrose extinction) lever pressed significantly less than rats that did not undergo extinction (open) (*P<0.05, no extinction compared to extinction or sucrose).

Glutamate receptor expression in the vmPFC

To determine glutamate receptor expression following extinction of cocaine self-administration in the vmPFC, tissue was collected following an extinction retention test from rats that underwent cocaine or sucrose reinforcement extinction, or did not undergo cocaine extinction training. There were no significant differences using an ANOVA between groups of GluR1-containing AMPA receptor expression ($F_{2,23}$=0.758, *P*=0.481)

(see figure 8A) or of NR2A-containing NMDA receptor expression ($F_{2,23}$=0.245, *P*=0.785) (see figure 8B). Finally, an ANOVA revealed a strong trend for reduced expression of NR2B-containing NMDA receptors in rats that received cocaine compared to rats that received sucrose reinforcement ($F_{2,21}$=3.299, *P*=0.059; see figure 8D). These results indicate a trend for cocaine use to reduce NR2B-containing NMDA receptors that may not be affected by extinction training.

Figure 8

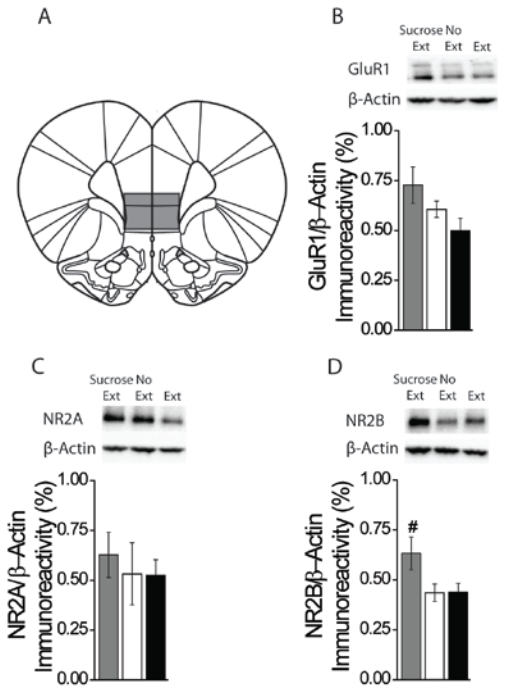

Figure 8: Glutamate receptor expression following extinction in the vmPFC
A) Coronal drawings (bregma, 3.24 mm; Paxinos and Watson, 2007; Copyright 2007) showing representive tissue collected from the vmPFC. There were no significant differences of either GluR1-containing AMPA receptor expression (B) or NR2A-containing NMDA receptor expression (C) between extinction of sucrose reinforcement (gray), no extinction of cocaine self-administration (open), or extinction of cocaine self-administration (black). D) However, there was a trend for decreased expression in rats that did not undergo extinction of cocaine self-administration (open) and rats that underwent extinction of cocaine self-administration (black) compared to rats that underwent extinction of sucrose reinforcement (gray) (#P=0.059).

Glutamate receptor expression in the NAc

To determine glutamate receptor expression following extinction of cocaine self-administration in the NAc, tissue was collected following cocaine or sucrose extinction, or following no cocaine extinction training. ANOVA revealed no significant difference between groups in expression of GluR1-containing AMPA receptors ($F_{2,23}$=1.324, P=0.288) (see figure 9B). On the other hand, ANOVA did reveal a significant difference between groups in expression of NR2A-containing NMDA receptors ($F_{2,22}$=7.534, P<0.005). *Post hoc* analysis confirmed that rats that underwent sucrose extinction had reduced expression compared to rats that did not undergo extinction of cocaine self-administration (P<0.005; see figure 9C). Furthermore, ANOVA also revealed a significant difference between groups in expression of NR2B-containing NMDA receptors ($F_{2,22}$=5.097, P<0.05). *Post hoc* analysis confirmed that rats that underwent extinction of sucrose reinforcement had reduced expression compared to rats that did not undergo extinction of cocaine self-administration (P<0.05; see figure 9D). In summary, these results suggest that expression of NMDA receptors containing the NR2A or NR2B subunits is increased following withdrawal of cocaine self-administration, and is reduced following extinction of sucrose reinforcement.

Figure 9

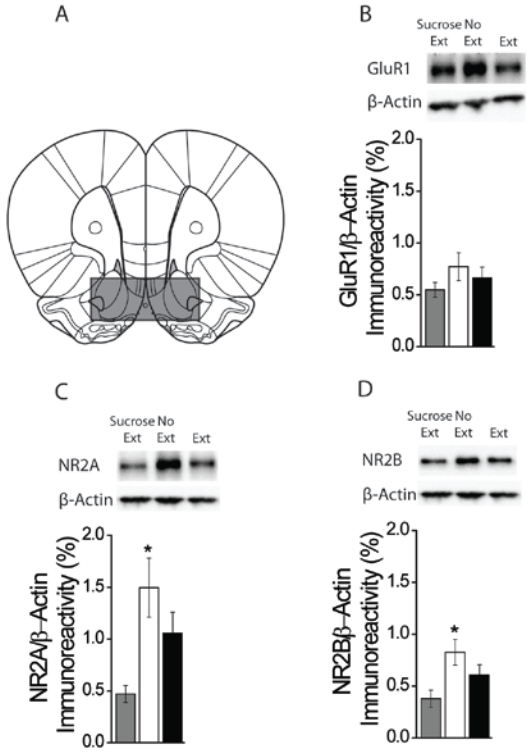

Figure 9: Glutamate receptor expression following extinction in the NAc
A) Coronal drawings (bregma, 2.76 mm; Paxinos and Watson, 2007; Copyright 2007) showing representive tissue collected from the NAc. B) There was no significant difference of GluR1-containing AMPA receptor expression between rats that underwent extinction of sucrose reinforcement (gray), rats that did not undergo extinction of cocaine self-administration (open), or rats that underwent extinction of cocaine self-administration (black). There was a significant decrease in rats that underwent sucrose reinforcement (gray) compared to rats that did not undergo extinction (open) in expression of NR2A-containing NMDA receptors (C) and NR2B-containing NMDA receptors (D) (**P<0.005, compared to sucrose extinction; *P<0.05, compared to sucrose extinction).

Discussion

The aim of these experiments was to dissociate the role of NMDA receptors in the acquisition and consolidation of extinction of cocaine self-administration, and to determine the effect of NMDA receptor potentiation on extinction learning. The original hypothesis postulated that post-extinction injections of the NMDA receptor antagonist CPP would disrupt consolidation of extinction learning and post-extinction injections of

the NMDA receptor coagonist D-serine would facilitate extinction learning. This hypothesis was only partially supported. Injections of CPP following four 45-minute extinction sessions did not disrupt consolidation when tested on the subsequent 90-minute extinction session. Rather, CPP administration appeared to facilitate extinction learning, have pharmacological side effects, or disrupt reconsolidation of the original drug-memory. On the other hand, post-extinction injections of D-serine following four 45-minute extinction sessions facilitated extinction learning as hypothesized. We previously demonstrated that pre-extinction session injections of CPP disrupted acquisition and possibly consolidation of extinction. These previous findings alongside our results with post-extinction injections of D-serine, suggest that NMDA receptors are necessary for extinction learning, and implicate a role for NMDA receptors in consolidation of extinction of cocaine self-administration. However, our results with post-extinction injections of CPP suggest either a pharmacological issue or disruption of memory reconsolidation. Thus, the overall evidence supports that NMDA receptors contribute to consolidation of extinction, but systemic administration of CPP may also affect other processes.

To further examine the role of NMDA and AMPA receptors in extinction, we examined glutamate receptor expression following extinction of cocaine self-administration. Tissue collected from the vmPFC and NAc was used to analyze expression of GluR1-containing AMPA receptors, and expression of NR2A- and NR2B-containing NMDA receptors following extinction or no extinction (withdrawal) from cocaine self-administration, or extinction of sucrose reinforcement to control for lever pressing. In the vmPFC, none of the targeted receptors types were significantly altered in

any of the groups. However, expression of the NR2B subunit had a strong trend toward being significantly reduced in rats that lever pressed for cocaine compared to rats that lever pressed for sucrose. In the NAc, NR2A- and NR2B-containing NMDA receptor expression was significantly increased in rats that did not undergo cocaine extinction compared to rats that did undergo sucrose reinforcement extinction. There was no change in expression of GluR1 subunit in any group in the NAc. Overall, these results indicate a trend for cocaine use to reduce NR2B-continaing NMDA receptor expression in the vmPFC, and increase NR2A- and NR2B-containing NMDA receptor expression in the NAc in rats that did not undergo extinction.

NMDA receptors are necessary for extinction of cocaine self-administration

In a previous experiment, we found that pre-extinction injections of CPP before four 45-minute extinction sessions disrupted extinction retention when tested during a subsequent drug-free full extinction session. These data demonstrated that NMDA receptors mediate extinction of cocaine self-administration. Our results were consistent with previous research concerning NMDA receptors and their role in extinction in other paradigms. For example, the NMDA receptor antagonist MK-801 disrupted acquisition and consolidation of fear extinction (Liu et al., 2009) and CPP disrupted consolidation of fear extinction when retention was tested 24 hours later (Burgos-Robles et al., 2007; Santini et al., 2001). Furthermore, the NMDA receptor antagonist APV also disrupted extinction learning in an amphetamine conditioned place preference paradigm (Hsu & Packard, 2007). In order to dissociate acquisition of extinction learning from consolidation, we replicated our first study, but instead administered post-extinction

injections to disrupt consolidation alone. The finding that post-extinction injections of an NMDA receptor antagonist did not disrupt consolidation of extinction of cocaine self-administration is surprising. More surprising is that it appears that post-extinction injections facilitated extinction. However, due to our previous experiment and other studies conducted with fear conditioning and the conditioned place preference paradigm, it seems unlikely that NMDA receptor blockade would enhance extinction learning.

A possible explanation for our current results is that a few limitations in the experiment might have influenced the interpretation of the results. The first limitation is the small sample size due to unforeseen complications with the animals' health (saline, n=6; CPP, n=7). A larger group size would help to reduce variability and provide a more accurate representation of the data. The second limitation is the use of CPP. CPP is a potent and competitive NMDA receptor antagonist, but 10mg/kg systemic injections can reduce spontaneous activity in rats (Sotres-Bayon, Bush, & LeDouz, 2007) and increase ataxia/muscle relaxation compared to controls (Jerram, Smith, & Darlington, 1996; Lehmann et al., 1986). However, we found that systemic injections of CPP did not affect locomotion compared to saline controls when measured by photobeam breaks. Furthermore, the behavioral effects of CPP were controlled for with post-extinction injections, which would not have affected the preceding behavior. Thus, post-extinction CPP injections should not have affected the rats' behavior during the extinction session.

There is evidence that CPP alters extracellular levels of neurotransmitters, which could explain the unexpected findings with post-extinction injections. For instance, microinfusions of CPP into the mPFC increase extracellular levels of dopamine (DA) in the NAc (Del Arco, Segovia, & Mora, 2008) and amygdala (Del Arco, Ronzoni, & Mora,

2011). DA is critical for the rewarding and reinforcing aspects of cocaine (Wise, 2004), and injections of a DA receptor antagonist following each extinction sessions in a cocaine conditioned place preference paradigm disrupts extinction learning compared to saline controls (Fricks-Gleason, Khalaj, & Marshall, 2012). Thus, increased DA levels following CPP administration might have lead to an enhancement of extinction learning not related to the effects of NMDA receptor blockade. Furthermore, CPP infusions into the mPFC increases acetylcholine (ACh) in the NAc (Del Arco et al., 2008), the hippocampus, and the amygdala (Del Arco et al., 2011). ACh has also been implicated in learning and memory, and injections of an antagonist specific to the muscarinic ACh receptor before or after fear extinction disrupts extinction recall 24 hours later (Santini, Sepulveda-Orengo, & Porter, 2012). Therefore, increased ACh levels after CPP administration might have also lead to an enhancement of extinction learning that is unrelated to NMDA receptor blockade. Additionally, CPP infusions into the mPFC also increase corticosterone (Cort) levels in the hippocampus (Del Arco et al., 2011). Stress and the release of Cort are well known to have an important role in drug addiction and relapse (Sinha, 2008), as well as having a complicated role in learning and memory. Stress can either enhance or impair cognitive function depending on a number of factors including gender, type of stress, and duration of stress (Campeau, Liberzon, Morilak, & Ressler, 2011). Consequently, increased Cort levels following CPP administration also could have affected extinction learning in an unrelated way to NMDA receptor blockade. Taken together, the side effects from infused CPP (increased dopamine, acetylcholine, and corticosterone), could also occur following systemic injections, and could have influenced consolidation of the extinction memory. If so, then it is unclear if these side

effects, NMDA receptor blockade, or a combination of effects is responsible for our results. In order to address these possible confounds, future studies should use a more specific NMDA receptor antagonist.

Another possible explanation for our results is that CPP did not affect the new extinction memory, but instead affected the original drug-memory by disrupting reconsolidation. Reconsolidation is the process of re-stabilizing a memory after the memory has been retrieved and made labile again, and is classically defined as a process that requires protein synthesis (Misanin, Miller, & Lewis, 1968; Nader, 2003; Nader, Schafe, & LeDoux, 2000). Protein synthesis inhibitors administered after reactivation of drug-associated memories can impair subsequent recall of those memories (von der Goltz et al., 2009). Depending on the experimental parameters, NMDA receptors have been implicated in both reconsolidation and extinction learning. Following reactivation of an alcohol self-administration memory, injections of the NMDA receptor antagonist MK-801 result in a strong trend for reconsolidation disruption when tested the following day (Wouda et al., 2010). In a conditioned place preference paradigm, repeated injections of an NMDA receptor antagonist following reactivation tests disrupt reconsolidation of the drug-associated memory (Alaghband & Marshall, 2012; Sadler, Herzig, & Schmidt, 2007). Taken together, these studies indicate that drug-associated memories can be disrupted during reconsolidation and that NMDA receptors are necessary for this process. Thus, it is possible that our results are due to disrupted reconsolidation instead of facilitated extinction.

Reconsolidation cannot be definitively measured in the present study. Although, it is possible that the original memory was weakened by a partial reconsolidation

disruption and therefore appears as if extinction was facilitated across days compared to saline controls. There are a few problems with this explanation, however. First, when given a priming injection of cocaine, rats previously treated with CPP reinstate drug seeking similarly to those previously treated with saline. This implies that the rats were still able to retrieve the original drug-memory. However, there is some evidence from the conditioned place preference paradigm that suggests that it is possible for animals to reinstate drug seeking after a priming injection of cocaine despite previous disruption of reconsolidation (Kelley, Anderson, & Itzhak, 2007). Moreover, new research is beginning to suggest that reconsolidation disruption is not as persistent as previously believed (Amaral, Osan, Roesler, & Tort, 2008; Stafford & Lattal, 2009). Perhaps a reconsolidation disruption is not completely blocking the memory, and thus the memory can still be retrieved if the cues are adequate. Therefore, it is possible that reconsolidation was partially impaired with post-extinction injections of CPP.

Second, impaired reconsolidation may not fit our results, because rats previously treated with CPP did not drug seek differently than those treated with saline when tested during the full 90-minute session on day five. If reconsolidation of the original drug-memory was disrupted following the four 45-minute extinction sessions, then it would have been expected that rats previously treated with CPP would have a reduction in lever presses compared to controls on day five. Conversely, if extinction was disrupted, then lever pressing should have been significantly higher on day five in rats treated with CPP compared to controls, as demonstrated previously (LaLumiere et al., 2010). However, neither group significantly increased nor decreased lever pressing from day four to day five, despite doubling the session length. This indicates that both groups underwent

extinction learning. However, extinction learning does not occur in isolation. Extinction learning can take place at the same time that the original memory is being retrieved or reconsolidated. Under our current methods, it is not possible to dissociate extinction learning from the original drug-memory. Therefore, future experiments should aim to determine the role of NMDA receptors in extinction and reconsolidation of the original memory separately.

A possible future experiment to test the role of NMDA receptors in reconsolidation of the original drug-memory could be conducted similar to our current methods. Instead of using 45-minute sessions, a single or multiple shortened sessions of 5 or 15 minutes could be used. The 5- or 15-minute session would be short enough to reactivate the original memory, but not long enough for extinction learning to occur as demonstrated previously (LaLumiere et al., 2010). After each reactivation session, rats would receive an injection of an NMDA receptor antagonist. Retention of the original drug-memory would be tested on a subsequent longer session. Experiments such as these should be conducted to determine if NMDA receptors are necessary for reconsolidation of extinction of cocaine self-administration, as well as to dissociate extinction from reconsolidation.

In summary, we previously found that injections of CPP before four 45-minute extinction sessions disrupted extinction learning when retention was tested during a full extinction session on day five. Here, we found that injections of CPP immediately after four 45-minute extinction sessions did not disrupt consolidation of extinction learning when tested on day five. On the other hand, CPP post-extinction injections appeared to result in facilitated extinction learning compared to saline. Neither previous research nor

our own data support the view that NMDA receptor blockade enhanced learning. Thus, more likely alternative explanations include side effects induced by CPP on extinction learning or disrupted reconsolidation.

Potentiating NMDA receptors can facilitate extinction of cocaine self-administration

The finding that potentiation of NMDA receptor function with the NMDA receptor coagonist D-serine facilitated extinction learning is consistent with previous research. Post-session injections of D-serine facilitated extinction learning when tested 24 hours later in an inhibitory avoidance procedure (Fiorenza et al., 2012), and pre-extinction injections of D-serine facilitated extinction of conditioned fear (Matsuda et al., 2010). Furthermore, injections of D-serine given before each extinction session for five days resulted in reduced lever pressing on extinction day one and day three compared to saline controls (Kelamangalath et al., 2007) and facilitated extinction of a conditioned place preference when injected before each extinction session (Hammond et al., 2012). Thus, administration of D-serine can facilitate extinction learning in a number of paradigms.

Additionally, the NMDA receptor partial agonist d-cycloserine (DCS) can also facilitate different types of learning. For example, DCS facilitates extinction of fear-potentiated startle when injected before an extinction session (Walker, Ressler, Lu, & Davis, 2002), facilitates extinction of fear conditioning when injected before (Langton & Richardson, 2008) or after (Ledgerwood, Richardson, & Cranney, 2003) the extinction session, and facilitates extinction in a conditioned place preference paradigm when injected immediately after, but not four hours after, the extinction session (Botreau,

Paolone, & Stewart, 2006). Taking these studies together, NMDA receptor agonists will facilitate learning when given before or after an extinction session. This indicates that NMDA receptor agonists could facilitate extinction during either the acquisition or consolidation phase of learning. However, the effect that extinction can be facilitated with post-extinction session injections indicates that NMDA receptor agonists are more likely enhancing the consolidation of extinction, rather than acquisition.

The effect of administering D-serine immediately after four 45-minute extinction sessions appears similar to the effect of administering CPP immediately after four 45-minute extinction sessions. However, unlike the results with CPP injections, it is unlikely that reconsolidation was disrupted by D-serine. First, this interpretation seems unlikely because previous research demonstrates that administration of an NMDA receptor agonist either before or after a learning event can facilitate learning in a number of paradigms. Second, it seems unlikely that an antagonist and coagonist could have opposite biological functions and yet yield the same result. It is more likely that NMDA receptor agonists would facilitate reconsolidation, which has been demonstrated with DCS in fear conditioning (Lee, Milton, & Everitt, 2006). Thus, it seems more likely that injections of D-serine are affecting extinction learning instead of the original drug-memory.

In addition, treatment with D-serine during extinction resulted in a strong trend for reduced stress-induced reinstatement, but had no effect on cocaine-induced reinstatement. Although the effect of D-serine on stress-induced reinstatement has not yet been tested, the trend for reduced stress-induced reinstatement compared to controls agrees with other research. D-serine reduces low-dose (0.5 or 1 mg/kg, i.v.) cocaine-induced, but not cue- or context-induced, reinstatement when D-serine is administered

during one or five days of extinction training following both a short- and long-access (6+ hour daily sessions) cocaine self-administration procedure (Kelamangalath et al., 2009; Kelamangalath & Wagner, 2010). Interestingly, we used a higher dose of cocaine (10 mg/kg, i.p.) to induce reinstatement and saw no effect of previous D-serine treatment. Perhaps D-serine during extinction is only effective at reducing cocaine-induced reinstatement when the cocaine dose is low, or when the route of administration matches the original self-administration parameters. As mentioned above, one issue in the present study is that there was a small sample size for the treatment groups (saline, n=6; D-serine, n=7) due to unforeseen complications with the animals' health. A larger group size would reduce overall variability, and would likely result in a significant effect of D-serine treatment on subsequent stress-induced reinstatement. Thus, indicating that D-serine might be able to protect against some types of reinstatement.

In summary, NMDA receptor agonists appear to facilitate extinction learning by enhancing consolidation of the new extinction memory. This is likely because pharmacological manipulation following the learning event rules out effects on acquisition (Millan et al., 2011). Thus, in combination with our previous results with pre-extinction session injections of CPP, NMDA receptors appear to be necessary for the consolidation of extinction of cocaine self-administration. Additionally, administering D-serine during extinction might protect against stress-induced reinstatement. These results begin to explain the role that NMDA receptors have in extinction of cocaine self-administration and also demonstrate a possible use for NMDA receptor potentiation as a pharmacological adjunct to extinction-based exposure therapy that could help reduce relapse rates.

NMDA receptors are altered following cocaine exposure or extinction learning in the vmPFC and NAc

Glutamate binds to both AMPA and NMDA receptors, and each receptor type has been implicated in learning and memory, plasticity, and drug-use (Nakanishi, 1992; Pickens et al., 2011). AMPA receptors are composed of GluR1-4 subunits, (Keinanen et al., 1990; Nakanishi, 1992) and the GluR1 subunit is upregulated following the induction of long-term potentiation (LTP), and is downregulated after induction of long-term depression (LTD) (Carroll, Lissin, von Zastrow, Nicoll, & Malenka, 1999; Malinow & Malenka, 2002; Turrigiano & Nelson, 2000). LTP and LTD are considered good models of experience-dependent plasticity and are believed to be important in learning and memory (Hyman, Malenka, & Nestler, 2006). Thus, alterations in expression of AMPA and NMDA receptors would be expected following a learning event, such as extinction.

Overall, in the mPFC, there were no significant changes in any of the targeted proteins between any of the groups. Our results indicate a strong trend for a reduction in NR2B-containing NMDA receptors in the groups that had prior exposure to cocaine compared to the group that had prior exposure to sucrose reinforcement. In the NAc, GluR1 subunit expression did not change in any of the groups. However, NR2A and NR2B subunit expression was increased in rats that did not undergo extinction compared to rats that underwent extinction of sucrose reinforcement. Taking these results together, there is a trend for NMDA receptor expression to be reduced following cocaine use in the vmPFC and for extinction to reduce NMDA receptor expression in the NAc.

Depending on if short- or long-access cocaine self-administration is used, glutamatergic signaling in the mPFC can vary substantially. For example, following 17

days of short-access cocaine self-administration, glutamate in the mPFC is reduced; but this reduction is even more pronounced following long-access cocaine self-administration (Ben-Shahar et al., 2012). These results indicate that cocaine use can induce changes glutamatergic signaling in the vmPFC, and these changes are related to the amount of drug exposure. These results also fit with our findings, which indicated a trend for reduced NR2B subunit expression in rats that received cocaine compared to rats received sucrose.

Even though we did not find significant difference between rats that underwent extinction compared to rats that did not undergo extinction, previous research suggests that withdrawal from cocaine can alter glutamate receptor expression in the mPFC. For instance, after 20 minutes of withdrawal from short-access cocaine self-administration, total NMDA receptor densities in the mPFC were reduced (Ben-Shahar et al., 2007), and following one day of withdrawal from short-access cocaine self-administration, GluR1 subunit expression in the mPFC is decreased (Ben-Shahar et al., 2009). Additionally, following long-access cocaine self-administration, NR2A subunit expression is increased after 60 days of withdrawal, and NR2B subunit expression is increased after 14 days of withdrawal in the mPFC (Ben-Shahar et al., 2009). Taken together, these studies indicate initial reduction in AMPA and NMDA receptor expression in the mPFC, but after prolonged withdrawal NMDA receptor subunit expression in differentially increased. Including our findings, it does not appear that GluR1 subunit expression increases after prolonged withdrawal from cocaine in the mPFC.

Few studies have determined glutamate receptor expression following extinction of cocaine self-administration in the vmPFC, despite the role of glutamate and the mPFC

in learning (LaLumiere et al., 2010; Nakanishi, 1992; Peters, Lalumiere, & Kalivas, 2008; Pickens et al., 2011). After one day of extinction from short-access cocaine self-administration, GluR1 is increased (Nic Dhonnchadha et al., 2013). However, following 12-14 days of either extinction or withdrawal from long-access cocaine self-administration in the vmPFC neither the GluR1 subunit nor total NMDA receptor expression was not changed compared to saline controls (Ghasemzadeh et al., 2011). We also found no significant difference in glutamate receptor expression following either withdrawal or extinction of cocaine self-administration. However, we did find a trend for reduced expression of the NR2B subunit in rats that received cocaine compared to rats that received sucrose. This discrepancy is likely due to distinguishing between NMDA receptor subunits. In summary, cocaine use can reduce glutamate in the mPFC and possibly reduce NR2B subunit expression. Withdrawal from cocaine use can initially reduce total NMDA receptor and GluR1 subunit expression in the mPFC, but after prolonged withdrawal from cocaine, expression of NR2A and NR2B subunits is increased. Finally, extinction learning initially increases GluR1 subunit expression, but after multiple extinction days, GluR1 and NMDA receptor expression is not different than controls.

Similarly to the mPFC, glutamatergic receptor expression in the NAc depends on drug consumption and the time frame used. The interesting difference with the NAc is the occurrence of incubation. Following long-access cocaine self-administration, AMPA receptor expression is increased in the NAc following a minimum of 30 days withdrawal, which is often referred to as incubation (Lu, Grimm, Hope, & Shaham, 2004; Wolf & Ferrario, 2010). Our results did not indicate any changes between groups in GluR1

expression in the NAc. These results are likely due to not differentiating the core and shell subregions. For example, GluR1 subunit expression was increased in the NAc shell subdivision following one week of extinction training, compared to one week of withdrawal or sucrose extinction (Sutton et al., 2003), but this effect was not significant in the NAc core (Self, Choi, Simmons, Walker, & Smagula, 2004). An increase in GluR1 subunit expression in the NAc shell, but not core, has also been observed following 14 days withdrawal in either long- or short-access self-administration (Ben-Shahar et al., 2009). In summary, following withdrawal or one week of extinction, GluR1 expression is increased in the NAc shell, but not the core.

Even though we did not separate the core and shell, our results still indicated an increase in NR2A and NR2B subunit expression in rats that did not undergo extinction. In agreement with our results, NR2B subunit expression was increased in the NAc shell, but not core, after 14 days of withdrawal from short-access, but not long-access, cocaine self-administration. However, in disagreement with our results, NR2A subunit expression in the NAc core or shell was not altered after 1, 14, or 60 days withdrawal in either short- or long-access self-administration (Ben-Shahar et al., 2009). Again, it is possible that pooling the NAc core and shell together inflated NR2A subunit expression, which lead to our significant result that was not observed in the core or shell alone. In summary, cocaine use and time spent in withdrawal appears to increase NMDA receptor expression, in the NAc shell particularly. However, extinction learning might reduce the increased expression following cocaine use.

The specific role of NMDA receptor subunits has not yet been directly tested with extinction of cocaine self-administration, but there is some evidence of the role of

NMDA receptors in extinction from other paradigms. In a fear conditioning paradigm, NR2A-containing NMDA receptors are necessary for acquisition of conditioned fear, but had no effect on fear extinction (Dalton, Wu, Wang, Floresco, & Phillips, 2012). In addition, mice lacking the NR2A subunit of the NMDA receptor also have disrupted spatial working memory measured with an elevated T maze (Bannerman et al., 2008) and impaired discrimination operant learning (Brigman et al., 2008). On the other hand, NR2B-containing NMDA receptors are necessary for fear extinction (Dalton et al., 2012; Sotres-Bayon et al., 2007; Sotres-Bayon, Diaz-Mataiz, Bush, & LeDouz, 2009). Furthermore, over-expression of NR2B-containing NMDA receptors in the forebrain of mice improves performance on certain learning and memory tasks, such as extinction of fear conditioning and Morris water maze, compared to controls (Tang et al., 1999). In summary, both subunits are necessary in learning and memory. The NR2A subunit is particularly important in fear acquisition and discrimination learning, whereas the NR2B subunit is particularly important in fear extinction and spatial learning.

Our results indicate expression of NR2A- and NR2B-containing NMDA receptors changes following cocaine experience or extinction learning. Furthermore, NMDA receptor subunits have different roles in specific learning and memory tasks, such as fear conditioning and extinction. NMDA receptor subunits may also have specific roles in acquisition or consolidation of extinction of cocaine self-administration, and perhaps this could explain the conflicting results with pre- verses post-extinction session injections data. CPP has a higher affinity for NR2A-containing NMDA receptors than the other subunits (Lehmann et al., 1986; Lozovaya et al., 2004), and perhaps NR2A-containing NMDA receptors are more important in the acquisition, and not consolidation,

of extinction. Thus, the next step is to determine what the potentially different roles the NR2A and NR2B subunit have in acquiring and consolidating extinction of cocaine self-administration.

Possible brain loci in which NMDA receptors mediate extinction: Future directions

Despite our currently conflicting effect with CPP post-extinction injections, previous research and our other findings suggest NMDA receptors are necessary for extinction of cocaine self-administration. The next step is to determine the brain locus in which NMDA receptors mediate extinction. Our western blot results suggest the vmPFC and NAc are prime candidates. A few other brain structures that are potential candidates, because of their role in learning and extinction in other paradigms, include the amygdala and the hippocampus. These brain structures will be discussed next as potential targets of NMDA receptor function in mediating extinction of cocaine self-administration.

The medial prefrontal cortex (mPFC) is strongly implicated in executive function, planning, and emotional regulation, and in neuroimaging studies were demonstrated to be important in drug addiction (Goldstein & Volkow, 2002). The mPFC is also necessary for learning and memory. For example, blocking NMDA receptors into the mPFC disrupts consolidation of fear extinction (Burgos-Robles et al., 2007), and disrupts extinction learning in an amphetamine conditioned place preference paradigm (Hsu & Packard, 2007). The mPFC is comprised of two subregions: the ventral infralimbic (IL) and the dorsal prelimbic (PL). Inactivation or lesions of the IL-mPFC disrupts extinction of cocaine self-administration (LaLumiere et al., 2010), and inactivation after extinction results in reinstatement of drug seeking (Peters et al., 2008). On the other hand, the PL-

mPFC is necessary for the initiation of drug seeking (McFarland & Kalivas, 2001). Taking these studies together, the PL-mPFC is necessary for drug seeking, and the IL-mPFC is necessary for inhibiting drug seeking after extinction training. However, the role of NMDA receptors in these brain regions during extinction of drug seeking is unclear.

The nucleus accumbens (NAc) is important in reward, reinforcement, and gating behaviors, such as drug seeking (Koob & Volkow, 2010). The NAc integrates glutamate inputs, modulated by dopamine projections from the ventral tegmental area (VTA), from cortical and limbic structures and sends projections onward to motor structures. Thus, the NAc is believed to be important in translating motivation to movement or action (Mangiavacchi & Wolf, 2004). In addition, the PL-mPFC has projections to the core subregion of the NAc and is believed to drive the expression of drug seeking behavior, whereas the IL-mPFC has projections to the shell subregion and is believed to drive the expression of extinction of drug seeking (McFarland & Kalivas, 2001; Peters et al., 2008). Furthermore, microinfusions of the NMDA receptor antagonist APV into either the NAc core or shell reinstates drug seeking after extinction of cocaine self-administration is learned (Famous, Schmidt, & Pierce, 2007). However, it is unclear whether the NAc core or shell is necessary for extinction learning or if the NAc is solely important for the expression of extinction after it has been learned. The NAc core and shell might be differentially important for extinction learning and extinction expression, as one study found that inactivating the NAc core, but not shell, before the first extinction session of cocaine self-administration resulted in increased lever pressing on extinction day two (Peters et al., 2008). Conversely, another study found that AMPA receptors are

necessary in the NAc shell for extinction of alcohol self-administration (Millan & McNally, 2011). These studies indicate a role for AMPA receptors in the NAc for extinction of drug seeking, however, the role of NMDA receptors in the NAc core or shell for extinction of drug seeking is unclear.

The amygdala is critical in emotional learning and fear conditioning (Milad & Quirk, 2012; Myers et al., 2011), as well as extinction of fear conditioning (Myers & Davis, 2007; Phillips & LeDoux, 1992; Quirk & Mueller, 2008; Sotres-Bayon et al., 2007) and extinction of cocaine self-administration (Szalay, Morin, & Kantak, 2011). However, if NMDA receptors are necessary in the amygdala for extinction of cocaine self-administration has not been directly tested. In one study, blocking NMDA receptors with APV in the basolateral nucleus of the amygdala (BLA) immediately following two cue-induced reinstatement tests, did not reduce lever pressing across reinstatement tests as seen with controls (Feltenstein & See, 2007). Future studies should directly test the role of NMDA receptors in the amygdala in extinction of cocaine self-administration.

Finally, the hippocampus has a major role in contextual-based learning and memory. For example, bi-lateral lesions of the dorsal hippocampus (dHipp) disrupted acquisition of contextual-cued fear conditioning (Phillips & LeDoux, 1992). Furthermore, the dHipp is also necessary for fear extinction (Myers & Davis, 2007), and NMDA receptors within this structure are also necessary for fear extinction retention when tested 24 hours later (along with the BLA and mPFC) (Fiorenza et al., 2012). The dHipp has also been implicated to be important for context-induced reinstatement after rats have undergone extinction of drug self-administration (Crombag, Bossert, Koya, &

Shaham, 2008; Myers et al., 2011). However, the role of NMDA receptors in the hippocampus during extinction of self-administration has yet to be determined.

In summary, previous evidence and our results from the western blots suggest NMDA receptors mediate extinction in the mPFC, NAc, amygdala, and hippocampus. Future studies should aim to determine each structures role in the different phases of extinction learning in a cocaine self-administration model.

Conclusions

We previously demonstrated that pre-extinction injections of the NMDA receptor antagonist CPP disrupted extinction learning when tested during a subsequent extinction retention test. In the current study, we found that post-session injections of the NMDA receptor coagonist D-serine facilitated extinction learning when tested during a subsequent full extinction session. However, we also found that post-session injections of CPP did not disrupt consolidation of extinction, but instead appeared to facilitate extinction learning. These results conflict with our previous findings, as well as previous research conducted in other paradigms. Thus, it seems likely that CPP was either having an unexpected pharmacological effect or reconsolidation was being disrupted. Thus, future studies should be conducted to better determine the role of NMDA receptors in consolidation of extinction of cocaine self-administration and reconsolidation of the original drug-memory.

Our findings that pre-extinction injections of CPP disrupted extinction learning, and post-extinction injections of D-serine facilitated extinction learning suggest that extinction of cocaine self-administration is mediated by NMDA receptors. Additionally,

post-extinction session injections target consolidation alone, thus, it is likely that NMDA receptors are required for consolidation of extinction of cocaine self-administration. Furthermore, facilitating extinction with D-serine later reduced stress-induced reinstatement and may thus have therapeutic benefit by helping reduce relapse rates.

Surprisingly few studies have aimed to determine the role of NMDA receptors in extinction of cocaine self-administration, specifically their role in consolidation of extinction learning. Despite the progress these studies have made, future directions include determining the brain loci of NMDA receptors involved in consolidation of extinction, and differentiating the role of NR2A- and NR2B-containing NMDA receptors in extinction of cocaine self-administration. These studies will further the understanding of how extinction learning occurs at a neurological level and thus could help develop pharmacological adjuncts to extinction-based exposure therapy and help reduce relapse rates.

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