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The Effect of SCH 23390 on Extinction of Conditioned Hyperactivity in Swiss Webster Mice

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Submitted in partial fulfillment of Honors Requirements for the Department of Neuroscience

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May 14, 2012
The Department of Neuroscience at Dickinson College hereby accepts this senior honors thesis by Kristen Ratner, and awards departmental honors in Neuroscience.

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May 2012
Abstract

Previous research has suggested the dopamine subtype-1 (D$_1$) receptor system has a role in extinction of a conditioned response. However, the precise role of the D$_1$ receptor system in different memory processes (i.e., retrieval, reconsolidation) involved with extinction of conditioned responding is unknown. Thus, the present experiment determined the effect of a selective D$_1$ receptor antagonist, SCH 23390, on extinction of methamphetamine-conditioned hyperactivity when SCH 23390 was administered immediately after the extinction sessions (i.e., targeting memory reconsolidation). Male Swiss-Webster mice received an injection (s.c.) of methamphetamine (1.0 mg/kg) associated with a locomotor activity chamber (paired) or in their home cages (unpaired) during the training phase (conditioning). Following conditioning, mice received an injection of saline prior to exposure to the locomotor activity chamber (extinction). Paired and unpaired mice received an injection (i.p.) of either SCH 23390 (0.0125, 0.025, 0.05 mg/kg) or saline immediately after the extinction sessions. Methamphetamine produced robust conditioned hyperactivity followed by extinction of conditioned hyperactivity in all paired mice. However, no dose of SCH 23390 significantly altered the rate of extinction. These results suggest that the D$_1$ receptor may not be involved in reconsolidation of a drug-environment memory.

*Keywords:* methamphetamine, conditioning, D$_1$ receptor, SCH 23390, extinction
The Effect of SCH 23390 on Extinction of Conditioned Hyperactivity in Swiss Webster Mice

One of the greatest challenges in treating addiction is the tendency of the addict to relapse in an environment previously associated with the drug of abuse, a result attributed to maladaptive classically conditioned responses (Milton & Everitt, 2010). It has been previously shown that behavioral sensitization (i.e., heightened response to a drug) as a result of repeated administration of psychostimulant drugs of abuse (e.g., methamphetamine) is partly due to the drug’s pharmacological action and partly due to drug-environment pairings (Mazurski & Beninger, 1987; Rauhut & Bialecki, 2011). Rauhut and Bialecki (2011) demonstrated that in keeping with the principles of classical conditioning, Swiss-Webster mice learn to associate the locomotor-activating effects of methamphetamine (unconditioned stimulus - US) with the environment in which the drug is received (e.g., locomotor activity chamber; conditioned stimulus - CS). After several US-CS pairing sessions, the animal shows conditioned hyperactivity (conditioned response - CR) in response to the CS by itself. If the experimenter continues to present the CS in the absence of the US, the animal undergoes extinction, or the weakening and eventual expiration of the conditioned response over time (Pezze & Feldon, 2004). Extinction is a type of new learning and cognitive flexibility in which the animal adapts to a sudden change in its environment (i.e., the removal of the US). Indeed, extinction shares some, but not all, of the molecular mechanisms underlying long-term memory formation (El-Ghundi, O’Dowd, & George, 2007; Berman & Dudai, 2001). Thus, extinction does not erase the memory of a conditioned pairing – such as conditioned fear produced by delivering a shock in a certain context – but rather blocks the expression of the association, which remains intact. This is evident in the ability of renewal
and reinstatement tests (in which animals are reintroduced to the original context and the US, respectively) to restore the CR even after the CR has been completely extinguished (Bouton & King, 1983).

The dopaminergic system has been implicated as a mediator of the new learning processes, with particular emphasis on two of its receptor sub-types. According to El-Ghundi, O'Dowd, and George (2007), D1 and D2 receptors modulate different types of learning and memory, particularly those types involved in spatial memory. There is strong evidence in animal models such as rodents and monkeys to suggest that D1 receptor activation, in particular, is critical for acquisition and early consolidation in spatial learning (El-Ghundi, O'Dowd, & George, 2007). Examples of spatial learning in animal models include navigation of the Morris water-maze task and association of a particular place with a reward. It appears that D1 receptors’ contribution to spatial learning, particularly long-term spatial memory formation, may be localized in the hippocampus. A recent study reports that bilateral infusions of a D1 antagonist, SCH 23390, into the CA1 of the hippocampus impair retention of the water-maze task in rats when given immediately post-training but not after a 3-hour delay (i.e., during a critical window for the progression from short- to long-term memory) (da Silva, Köhler, Radiske, & Cammarota, 2012).

There is evidence from fear conditioning paradigms to support dopamine’s modulatory role in extinction learning. For example, Ponnusamy, Nissim, and Barad (2005) found that a dopamine D2 receptor antagonist, sulpiride, facilitated extinction of fear-conditioning involving the pairing of a white noise (CS) with a footshock (US) in mice. The same study provided somewhat weaker evidence that that the D2 agonist quinpirole helps block extinction. Pezze and Feldon (2004) have reviewed previous studies of dopaminergic
agents used in fear-conditioning paradigms. These authors suggest that increasing synaptic levels of dopamine by systemic administration of indirect dopamine agonists (e.g., amphetamine and cocaine) slows down extinction of the conditioned fear response.

More recently, research has focused on the role of D₁ and D₂ receptors in reward-related paradigms of classical conditioning involving food or drugs of abuse in addition to conditioned fear paradigms, although more attention has been focused on renewal of reward-seeking behavior (*after* extinction) rather than the process of extinction itself. For example, El-Ghundi et al. (2007) proposed that D₁ receptor activation facilitates extinction learning in both reward-related and conditioned fear paradigms, as indicated by the delay or impairment of extinction in mice with knocked-down expression of the D₁ receptor, relative to wild-type controls, in operant responding for sucrose and in a one-trial step-through passive avoidance task involving a footshock. Mice with half the normal expression of D₁ receptors (heterozygotes) also took longer than wild-types to undergo extinction in the conditioned fear experiments. Specifically, these studies showed that D₁ knockout mice are slower to learn that pressing a particular lever is no longer paired with delivery of sucrose, or that stepping into a compartment will no longer deliver a footshock (El-Ghundi, O'Dowd, & George, 2001; El-Ghundi, O'Dowd, Erclik, & George, 2003).

Several studies have investigated the role of dopamine receptors in renewal or reinstatement tests after extinction. Rauhut, Fenton, and Bardo (2010) found that administering the D₂ receptor antagonist eticlopride to rats inhibited the renewal of reward-seeking behavior following extinction, in which the animals had been reintroduced to the context in which they had originally responded for a sucrose reward. Similarly, Liu et al. (2010) observed that both the D₁ antagonist, SCH 23390, and the D₂ antagonist, eticlopride,
decrease lever-press responding in rats during a reinstatement test (reintroduction to environmental cues, such as a brief tone, that were originally paired with the drug) following extinction of lever responding for nicotine. An analogous study of cocaine self-administration in rats also demonstrated the ability of both SCH 23390 and the D₂ antagonist, raclopride, to inhibit drug-seeking behavior after extinction (Crombag, Grimm, & Shaham, 2002).

Focusing on the process of extinction as well as reinstatement after extinction, Brenhouse, Dumais, and Andersen (2010) found that microinjections of a D₁ agonist, SKF 38393, in the prelimbic prefrontal cortex (a brain region implicated in the formation of drug-environment pairings) of adolescent rats accelerated extinction of cocaine conditioned-place preference relative to vehicle controls, in addition to lowering preference scores in a reinstatement test following extinction. Another study also focusing on D₁ manipulation in rats during extinction observed that pretreatment with the D₁ antagonist, SCH 23390, significantly lowered conditioned responding for sucrose on the first day of withholding the sucrose reward (i.e., accelerated extinction on the first day of extinction), relative to saline controls (Grimm et al., 2011). Collectively, these studies suggest a critical role for these dopamine receptor sub-types in mediating extinction learning, as well as the potential to use dopaminergic agents to enhance or delay extinction.

It is very important to note, however, that much of the extinction literature focusing on the dopaminergic system has produced contradictory results. Regarding the studies discussed thus far, it is presently unclear why some have demonstrated an ability of D₁ or D₂ agonists to block extinction, while others suggest these agents facilitate extinction. Findings
are also inconsistent across studies assessing the ability of D$_1$ or D$_2$ antagonists to block or facilitate extinction.

Milton and Everitt (2010) suggest that memories produced by classical conditioning are subject to reconsolidation, which is the restabilization of the original memory trace after it has been retrieved and returned to a labile state. Sarantis, Antoniou, Matsokis, and Angelatou (2012) provided evidence of a specific pathway involving the D$_1$ receptor for consolidation of a novel environmental memory that may also apply to reconsolidation, since both memory processes seem to share the MAPK signaling cascades (Kelly, Laroche, & Davis, 2003). Sarantis et al. (2012) observed that simultaneous activation of dopamine D$_1$ receptors and glutamate NMDA receptors results in phosphorylation of NMDA and AMPA receptor subunits, which then activates (by phosphorylation) ERK1/2 kinases (MAPK signaling cascade) in the hippocampus and pre-frontal cortex of rats. Activation of this ERK1/2 signaling pathway was critical for expression of the immediate early-genes c-Fos and zif-268 in the CA1 region of the hippocampus (which appears to also depend on activation of muscarinic acetylcholine receptors, in addition to D$_1$ and NMDA receptors, for full expression of these proteins). Chromatin remodeling (specifically, the phosphorylation and acetylation of histone H3) in the CA1 of the hippocampus was also dependent on the simultaneous activity of D$_1$, NMDA, and muscarinic receptors. These genetic changes in hippocampal neurons that occur downstream from the ERK signaling cascade are associated with long-term synaptic plasticity and memory consolidation processes (Sarantis et al., 2012; Kelly et al., 2003). Therefore, this may be a potential pathway to explain a link between D$_1$ receptor activation and memory reconsolidation at the molecular level.
Reconsolidation is thought to be an active memory process during extinction learning that may be independent and in direct opposition to the formation of extinction memories (i.e., reconsolidation may strengthen contextual memories) (Myers & Davis, 2002; Taylor, Olausson, Quinn, & Torregrossa, 2009). By extension to drug-environment conditioning, therapeutics that disrupt the reconsolidation process following retrieval of a drug-environment memory might facilitate extinction learning and lower the probability of relapse in addicts (Milton & Everitt, 2010). Therapeutics targeting the D₁ dopamine receptors are of particular interest due to previously mentioned evidence of their prominent role in memory processes and extinction.

A preliminary study in the Rauhut laboratory provided evidence for the role of D₁ receptors in retrieval of drug-environment memories. It was observed that i.p. administration of the D₁ receptor antagonist, SCH 23390 (0.05 mg/kg), 30 minutes prior to test sessions (thereby targeting retrieval) facilitated extinction of methamphetamine-conditioned hyperactivity relative to saline controls (unpublished manuscript). The present study sought to build on this previous study by examining the role of the dopamine D₁ receptor in reconsolidation of drug-environment memories. Specifically, this study assessed the effect of the dopamine D₁ receptor antagonist, SCH 23390, when administered immediately after extinction sessions, on the rate of extinction of methamphetamine-conditioned hyperactivity in Swiss Webster mice. SCH 23390 is an ideal antagonist for this behavioral assay because it is highly selective for the D₁ receptor (Hyttel, 1983; Andersen & Gronvald, 1986). All mice were initially divided into two groups, paired and unpaired, for the conditioning phase; paired animals were injected with methamphetamine immediately prior to sessions in a locomotor-activity recording chamber, whereas unpaired animals received methamphetamine
in their home cages. Paired animals were expected to show a sensitized response in the form of increased locomotor activity (hyperactivity), as indicated by a greater distance travelled in the recording chamber. Following conditioning, mice were subjected to an extinction phase in which the paired and unpaired groups were split further into groups receiving vehicle or a low (0.0125 mg/kg), moderate (0.025 mg/kg), or high (0.05 mg/kg) dose of SCH 23390 immediately after each extinction test session. If the D₁ receptor does in fact play a critical role in the reconsolidation of a drug-environment paired memory during extinction learning, then mice treated with SCH 23390 immediately following expression (retrieval) of the meth-chamber memory are expected to undergo extinction at a faster rate than paired control mice. This should be apparent in a steeper decline in the locomotor response (i.e., distance travelled in the recording chamber). Additionally, the ability of SCH 23390 to disrupt reconsolidation of memory and thereby facilitate extinction should be dose-dependent.

**Method**

**Experiment 1**

**Subjects**

Subjects were naïve, male Swiss Webster mice (N = 30) purchased from Charles River Co., NJ. Mice were group housed (3 – 4 mice/tub) in ventilated plastic tubs with wire tops measuring 9 3/8 x 5 7/16 x 5 1/8 inches (L x W x H). Mice were provided with *ad libitum* food (Fortified Purina Rodent Chow) and water and kept in a room at a temperature of ~72°F with a 12 hr light/dark cycle. At the start of the experiment, the animals' body weights were between 23 - 36 g. Mice were handled for 1 minute/day by the experimenters for 2 weeks prior to the start of the experiment. All procedures followed regulations set forth
by the *APA Ethical Standards of Care and Treatment of Animals* and approved by the Dickinson College Animal Care and Use Committee.

**Apparatus**

Fifteen open-field locomotor activity chambers (MED-OFA-510; Med-associated, Vt., USA) were used for conditioning and extinction sessions. Chamber compartments consisted of Plexiglas walls and measured 27.9 x 27.9 cm (L x W). Breakage of 16 infrared photobeams was used to track the animals' locomotor activity and compute the distance travelled (cm), and these data were recorded by an IBM personal computer (MED-PC Activity Software) connected to the chambers. A bottle cap containing a few drops of Pure Anise Extract (McCormick) was placed adjacent to each chamber before every session to serve as an environmental cue. After every session, all chambers were cleaned with a disinfectant solution following removal of the mice.

**Drugs**

All drugs were purchased from Sigma Chemical Company (St. Louis, MO). Methamphetamine HCl (1.0 mg/kg) was dissolved in a physiological saline (0.9%) solution and injected subcutaneously (SC) in a volume of 10 ml/kg body weight. SCH 23390 (0.0125, 0.025 and 0.05 mg/kg) was also prepared in physiological saline and injected intraperitoneally (IP) in a volume of 10 ml/kg. Drug doses are expressed as the salt weights.

**Procedure**

*Conditioning Phase*

Mice were subjected to 4 conditioning sessions (Chamber Days) that alternated with injections in their home cages (Home Cage Days), for a total of 8 days. On Chamber Days (Days 1, 3, 5 and 7), paired mice \( n = 16 \) received methamphetamine (1.0 mg/kg)
immediately before a 30 min recording session in the locomotor activity chamber, while unpaired mice \((n = 14)\) received saline immediately prior to the recording session.

Locomotor activity was quantified as distance travelled in cm. After every recording session in the chambers, mice were returned to their home cages. On Home Cage Days (Days 2, 4, 6 and 8), paired mice received saline and unpaired mice received methamphetamine in the animal colony. There was a 2-day lapse in between Days 4 and 5.

**Extinction Phase**

Extinction began two days after the conditioning phase. Both paired and unpaired groups received injections of saline immediately prior to a 30 min recording session in the locomotor activity chamber. Immediately after the session ended, mice \((n = 4\) per group, except for the Unpaired-Vehicle and Unpaired-0.0125 mg/kg SCH 23390 groups, for which \(n = 3\)) received either vehicle or SCH 23390 at doses of 0.0125 mg/kg (low), 0.025 mg/kg (moderate), or 0.05 mg/kg (high) and were returned to their home cages. This procedure was repeated for a total of 4 consecutive days. On the fifth day, all mice received injections of saline before a final 30 min recording session in the locomotor activity chamber and subsequent return to the colony. Similar to the conditioning phase, all locomotor activity counts were recorded as distance travelled in cm.

**Data Analysis**

A three-way repeated measures analysis of variance (ANOVA) was performed on the between-subjects factors of Conditioning (Paired vs. Unpaired) and Dose (vehicle, 0.0125, 0.025, and 0.05 mg/kg) and the within-subjects factor of Chamber Day (Day 1 vs. Day 4) on the distance travelled in the locomotor activity chambers. Conditioning was evaluated at two levels, paired and unpaired. Similarly, a three-way ANOVA was performed on the factors of
Conditioning, Dose, and Extinction Day (Extinction Days 1 – 5). Post-hoc contrasts were made with independent-samples $t$ tests. The $\alpha$ level of significance was set at 0.05, two-tailed.

**Experiment 2 (Replication)**

A replication study was performed approximately 4 months later in February 2012 with a new set of naive, male Swiss Webster mice ($N = 30$). All methods were identical to those of Experiment 1 with the following exceptions: the acclimation/handling period was 5 days prior to the start of the experiment, and a 4-day break separated Days 1 – 4 and Days 5 – 8 of the Conditioning Phase. The data of Experiments 1 and 2 were analyzed separately and then pooled to achieve a final $N = 60$, with 6 – 8 mice per group.

**Results**

**Experiment 1**

Paired mice demonstrated sensitization to methamphetamine as well as the drug’s stimulant property by itself, as indicated in the paired animals’ greater average locomotor response relative to unpaired animals on Chamber Day 1 and increase in activity by Day 4, while unpaired mice remained at baseline (Figure 1A). For the extinction phase, all paired groups showed hyperactivity on the first day but then became less active with each session, thus demonstrating extinction of the conditioned hyperactive response; unpaired animals did not show any obvious changes in locomotor activity and remained around baseline (Figures 1B and 1C). Regarding the effects of SCH 23390, the moderate dose (0.025 mg/kg) decreased locomotor activity in paired animals at a greater rate overall compared to the Paired-Vehicle group, suggesting the moderate dose of SCH 23390 (0.025 mg/kg) facilitated extinction. No dose of SCH 23390 significantly decreased locomotor activity in the unpaired
mice during the extinction phase, suggesting that doses of SCH 23390 in this range do not produce motor problems (Figure 1C). These conclusions were supported by the following statistical results.

**Conditioning Phase**

Three-way repeated measures ANOVA for the conditioning phase revealed a significant main effect of Chamber Day, \( F(1, 14) = 18.3, p < .05 \). The main effect of Conditioning on distance travelled was likewise significant, \( F(1, 14) = 29.0, p < .05 \). Dose did not show a significant main effect, nor were there any significant interactions involving Dose. There was a significant interaction between Chamber Day x Conditioning such that paired mice were more active than unpaired mice and even more so by Day 4, \( F(1, 14) = 20.9, p = .000 \). The power was interrupted on Chamber Day 4 and resulted in early termination of the recording session program for 8 mice. Therefore, the activity counts for these subjects (paired and unpaired, \( n = 4 \) per group) had to be excluded from the analyses for this day. All mice were still allowed to run in the chamber for the entire 30 min session.

**Extinction Phase**

For the extinction phase, three-way ANOVA showed a significant main effect of Conditioning, \( F(1, 22) = 7.50, p < .05 \), and of Extinction Day, \( F(4, 88) = 27.3, p < .05 \), but not of Dose. The interactions between Extinction Day x Dose, Conditioning x Dose, and Conditioning x Extinction Day x Dose were not significant. However, there was a significant interaction between Conditioning and Extinction Day such that paired animals' activity differed from that of unpaired animals depending on the day, \( F(4, 22) = 7.19, p < .05 \). Despite the lack of a significant Conditioning x Dose x Extinction Day interaction, an exploratory two-way (Group x Extinction Day) ANOVA, isolating only the Paired-Vehicle
and the Paired-0.025 SCH 23390 groups, revealed a significant main effect of Group, $F(1, 6) = 11.5, p = 0.015$. The significant main effect of Group motivated further exploratory contrasts involving independent-samples $t$ tests comparing each of these groups on each day of extinction. These contrasts revealed that Paired-0.025 SCH 23390 was less active on Extinction Day 2, $t(6) = 2.9, p = 0.028$, and tended to be less active on Extinction Day 3, $t(6) = 2.1, p = 0.077$) than Paired-Vehicle. These latter results suggest that the moderate SCH 23390 dose (0.025 mg/kg) facilitated extinction, as predicted.

**Experiment 2 (Replication)**

Similar to Experiment 1, the stimulant effect of methamphetamine and its ability to produce sensitization was evident in that paired mice were more active than unpaired mice on Chamber Day 1 and demonstrated greater hyperactivity by Day 4, while unpaired mice remained at baseline (Figure 2A). Paired animals also displayed a strong conditioned response and underwent extinction (Figure 2B). However, none of the paired groups that had received a low, moderate or high dose of SCH 23390 were significantly more or less active than the Paired-Vehicle group on any Extinction Day (Figure 2B). Thus, no dose of SCH 23390 had a significant effect on the rate of extinction in this replication study. Differences between Experiments 1 and 2 are also observed in the unpaired groups, as seen in greater activity in the Unpaired-0.0125 mg/kg SCH 23390 group relative to the other unpaired groups, which remained around baseline, from Extinction Days 1 – 5 (Figure 2C). These conclusions were supported by the following statistical results.

**Conditioning Phase**

Three-way repeated measures ANOVA for the conditioning phase of the replication study revealed a significant main effect of Chamber Day, $F(1, 22) = 60, p = .000$, and of
Conditioning, $F(1, 22) = 122.93, p = .000$, on distance travelled in the chamber. There was a significant Chamber Day $\times$ Conditioning interaction such that paired animals were more active than unpaired animals on Conditioning Day 1, and this difference was more pronounced by Day 4, $F(1, 22) = 48.27, p = .000$. Neither the main effect of Dose nor any interactions involving Dose were significant.

**Extinction Phase**

Three-way ANOVA for the extinction phase of the replication study showed a significant main effect of Extinction Day, $F(1, 22) = 47.48, p = .000$, and of Conditioning, $F(1, 22) = 10.34, p = .004$, but not of Dose. There was a significant Extinction Day $\times$ Conditioning interaction such that paired animals’ activity differed from that of unpaired animals depending on the day of extinction, $F(1, 22) = 48.93, p = .000$. The interactions of Extinction Day $\times$ Dose, Conditioning $\times$ Dose, and Extinction Day $\times$ Dose $\times$ Conditioning were not significant. *Post-hoc* analysis revealed that Unpaired-0.0125 SCH 23390 was significantly more active than Unpaired-Vehicle on Extinction Day 1, $t(4) = 4.35, p = .012$; Extinction Day 2, $t(4) = 2.82, p = .048$; Extinction Day 4, $t(4) = 4.40, p = .012$; and Extinction Day 5, $t(4) = 3.60, p = .023$.

**Pooled Analysis of Experiments 1 and 2**

The combined data from Experiment 1 and its replication, Experiment 2 (for a total $N = 60$), revealed robust sensitization to methamphetamine as well as the drug’s initial stimulant effect, as indicated by greater activity in paired mice, relative to unpaired mice, on Chamber Day 1 and a more pronounced hyperactive response in paired animals by Chamber Day 4 (Figure 3A). All paired mice demonstrated a strong conditioned hyperactive response on Extinction Day 1 and significantly less activity by Day 5 (Figure 3B). Furthermore, all
paired groups underwent extinction at about the same rate, suggesting that SCH 23390 had no reliable effect on the rate of extinction over this dose range (Figure 3B). The locomotor activity of unpaired groups remained around baseline from Extinction Days 1 – 5 (Figure 3C). These conclusions were supported by the following statistical results.

**Conditioning Phase**

Analysis of the pooled data by three-way ANOVA revealed a significant main effect of Conditioning, $F(1, 44) = 108.25, p = .000$, and of Chamber Day, $F(1, 44) = 74.67, p = .000$. There was no significant main effect of Dose, nor were Conditioning x Dose, Chamber Day x Dose, and the three-way interaction significant. The interaction between Chamber Day and Conditioning was significant such that paired animals were more active than unpaired animals on Conditioning Day 1 and even more so by Day 4, $F(1, 44) = 67.69, p = .000$.

**Extinction Phase**

Three-way ANOVA for the pooled data showed a significant main effect of Extinction Day, $F(1, 52) = 82.94, p = .000$, and of Conditioning, $F(1, 52) = 16.56, p = .000$, but not of Dose. The Extinction Day x Conditioning interaction was significant such that the activity between paired and unpaired groups was different depending on the day of extinction, $F(1, 52) = 51.17, p = .000$. None of the interactions involving Dose were significant. Independent-samples $t$ tests did not show any significant differences between paired groups on any day of extinction.

**Discussion**

Taken together, the results of Experiments 1 and 2 reveal three important findings. First, methamphetamine produced behavioral sensitization followed by conditioned
hyperactivity in mice. These results are consistent with a previous study in the laboratory suggesting that methamphetamine (1.0 mg/kg) produces robust sensitization and conditioned hyperactivity in mice (Rauhut & Bialecki, 2011). Second, presentation of the locomotor activity chamber in the absence of methamphetamine pre-treatment, following the conditioning phase, resulted in a weakening of the CR over time (i.e., extinction). This result too is consistent with a previous study showing that contextual cues undergo extinction, in which rats had been similarly trained to pair the stimulant effect of amphetamine with a locomotor activity chamber (Stewart & Vezina, 1991). To our knowledge, this is the first report to demonstrate extinction of conditioned hyperactivity as produced by methamphetamine. Third, and most importantly, the administration of SCH 23390 post-retrieval of the meth-chamber memory did not significantly alter the rate of extinction, relative to the paired control group, over this particular dose range. Therefore, these findings do not support a role for the D₁ receptor in the reconsolidation of a drug-environment paired memory produced by classical conditioning. It should be noted that this study, as well as similar behavioral studies, is limited in its ability to firmly link molecular events at the synapse to whole-animal behavior, especially since administration of the drug was systemic (i.e., not targeting receptors in any specific region of the brain). Nonetheless, this result contradicts some of the previous literature supporting a role for the D₁ receptor in memory reconsolidation. For example, previous research has shown that 0.5 mg/kg SCH 23390 disrupts reconsolidation of a contextual memory (the association of a red bead with the aversive taste of methyl anthranilate) in the day-old chick (Sherry, Hale, & Crowe, 2005). Furthermore, it was recently shown that SCH 23390 (0.1 – 1 mg/kg) dose-dependently impaired the extinction of cocaine-conditioned place preference in rats when administered
immediately after retrieval (i.e., targeting reconsolidation), which also implies a role for the D1 receptor in reconsolidation of contextual drug memories and contradicts the findings of the present study (as well as the prediction that SCH 23390 should facilitate extinction if interfering with reconsolidation) (Fricks-Gleason, Khalaj, & Marshall, 2012). It is possible, however, that these inconsistencies in findings and other discrepancies in the extinction literature, as mentioned in the introduction, exist because of the unique conditioning paradigms (e.g., fear conditioning vs. appetitive conditioning) and behavioral assays (e.g., locomotor activity recording vs. conditioned place preference) used in each study (Myers & Davis, 2002).

There are clear differences in the results between Experiments 1 and 2. Although Experiment 1 demonstrated some ability of the moderate dose of SCH 23390 (0.025 mg/kg) to accelerate the decline in locomotor activity over the extinction phase (i.e., facilitate extinction), these findings were not replicated in Experiment 2. It is possible that the differences between the Paired-0.025 mg/kg SCH 23390 and Paired-Vehicle groups in Experiment 1 were not due to any specific effect of the drug but rather can be explained by a weaker CR developed by the mice in this group that is evident at the beginning of the extinction phase (See Figure 1B). Paired-0.025 mice were less active than the other groups on Extinction Day 1, which was before treatment with SCH 23390 had begun (i.e., injections occurred after the 30 minute extinction sessions in the locomotor activity chambers).

SCH 23390 begins to have slight cataleptic effects at 0.05 mg/kg and induces highly significant catalepsy at 0.1 mg/kg in mice (Zarrindast & Habibi-Moini, 1991). Collectively, the results of Experiments 1 and 2 indicate that SCH 23390 does not produce motor side effects at the doses used. Unpaired animals receiving SCH 23390 were not significantly
more or less active than the unpaired control group over the 5 days of extinction testing. However, Experiment 2 showed higher activity in the Unpaired-0.0125 mg/kg SCH 23390 group relative to the Unpaired-Vehicle group, suggesting that the low dose may have had a slight locomotor-activating effect (although this is opposite to the literature that D₁ antagonists suppress motor activity at sufficiently high doses). Since the drug was administered after each extinction session, and the half-life of SCH 23390 binding to D₁ receptors in the mouse brain is only about 33 minutes, it can be assumed that any motor side effects in the short-term and effects on extinction sessions the following day were avoided (Andersen & Gronvald, 1986). Additionally, the highest dose of SCH 23390 (0.05 mg/kg) did not significantly lower activity in unpaired mice relative to saline controls in both replications. In conclusion, it appears to be highly unlikely that this phenomenon can be explained by motor problems produced by SCH 23390.

A more probable explanation to account for differences between Experiments 1 and 2 in general is that there was greater individual variability in Experiment 2, which greatly impacts a study of relatively small sample size (n = 3 – 4/group for Experiments 1 and 2, final n = 6 – 8/group). Additionally, procedural differences between the replications, such as the 2-day lapse between Conditioning Days 4 and 5 in Experiment 1 versus the 4-day gap in Experiment 2, may or may not have impacted the results. Effects of season and/or the time of year on neurotransmitter systems and pharmacological manipulations cannot be ruled out, as Experiment 1 took place in October and Experiment 2 was about 4 months later in February. One study has suggested that behavior of male Swiss Webster mice in morphine withdrawal varies according to season (Kemmling, Rubio, & Baliero, 2002).
It is possible that the D$_1$ receptor plays a role in the retrieval of contextual drug memories rather than memory reconsolidation. A previous experiment in the Rauhut laboratory found that 0.05 mg/kg SCH 23390 facilitates extinction of methamphetamine-conditioned hyperactivity in Swiss Webster mice when injected i.p. 30 minutes prior to extinction testing in a locomotor activity chamber (i.e., targeting retrieval of the meth-chamber memory) (unpublished manuscript). Furthermore, studies that have demonstrated a significant effect of SCH 23390 to block reinstatement of food- or sucrose-seeking behavior in rats, both on the first day of extinction testing and after the CR has extinguished, may be targeting retrieval of the contextual memory because SCH 23390 is administered prior to presentation of the CS in these studies (Ball, Combs, & Beyer, 2011; Grimm et al., 2011).

Perhaps D$_2$-like receptors (D$_2$, D$_3$, and D$_4$), rather than D$_1$-like receptors, are involved in reconsolidation of contextual memories. Ponnusamy et al. (2005) speculates that D$_2$ receptors are modulatory and not necessarily critical for extinction, but still may be involved in memory consolidation processes active during extinction. These authors observed that conditioned fear as measured by level of freezing behavior, invoked by presentation of the CS (white noise) in the absence of the US (footshock) and without any drug pre-treatment, was significantly lower 24 hours after mice had received an i.p. injection of the D$_2$ antagonist sulpiride. The drug had no significant effect on freezing behavior on the same day of treatment, when sulpiride was administered 45 minutes prior to extinction sessions. This suggested that antagonism of D$_2$ receptors facilitates extinction and does so by affecting reconsolidation, but not retrieval, of a contextual memory. Another report on conditioned fear in rats found that the specific D$_4$ antagonist, L-741,741, also had an effect on extinction testing on the following day but not the same day as drug treatment, suggesting a role for D$_4$
receptors in consolidation processes active during extinction learning (Pfeiffer & Fendt, 2006). However, this study demonstrated that antagonism of D₄ receptors blocks extinction of conditioned fear, rather than facilitates it (i.e., the conditioned freezing response was stronger). In addition, these experiments were specific to D₄ receptors in the medial prefrontal cortex and did not examine systemic administration of L-741,741. Regardless, it would still be worthwhile to examine the effect of a D₂ or D₄ antagonist on the extinction of conditioned hyperactivity in future studies. Interpretation of drug-memory extinction studies by Taylor et al. (2009) brings forth an important question that pertains to most studies discussed in this paper, as well as the present study – is dopamine receptor blockade affecting reconsolidation of the original association, or instead targeting the distinct, competing process of consolidation of the new extinction memory? The distinction between the processes of reconsolidation and extinction (if one truly exists), and the means by which one can reliably differentiate their respective influences in behavioral studies, remain unclear (Taylor et al., 2009). This may also account for the inconsistencies and contradictory interpretations of results in the extinction literature discussed previously.

Regarding previously mentioned signaling cascades that link co-activation of D₁ receptors and NMDA receptors to synaptic plasticity and memory consolidation processes, it would be interesting to replicate the present study but with administration of the selective NMDA receptor antagonist, MK-801, as well as co-administration of SCH 23390 and MK-801 (Sarantis et al., 2012). If reconsolidation processes in extinction learning share similar signaling pathways to those of consolidation of novel environmental information proposed by Sarantis et al. (2012), then perhaps it can be demonstrated that activation of NMDA receptors, and not D₁ receptors, is necessary for reconsolidation processes active during
extinction of conditioned drug memories. Indeed, Brown, Lee, and Sorg (2008) provided evidence that i.p. administration of MK-801 in rats impairs reconsolidation of a cocaine-chamber association in a conditioned place preference task, when the drug is given immediately before a reactivation session (i.e., "reminder") in the chamber (after extinction has occurred). Rats receiving MK-801 demonstrated significantly lower preference scores in a cocaine-induced reinstatement test that followed reactivation sessions, relative to rats that had not undergone reactivation. Additionally, Myers and Davis (2002) suggest that memory consolidation processes in fear extinction may be dependent on NMDA receptor activation.

It remains unclear whether the D₁ receptor subtype, other dopamine receptor subtypes (e.g., D₂-like), or perhaps even glutamate NMDA receptors play a critical role in the reconsolidation of drug-environment memories produced by classical conditioning, and whether antagonism of these receptors may facilitate or block extinction learning. Greater understanding of the role of dopamine receptor sub-types in extinction learning may permit the development of specific pharmacological approaches to combating maladaptive associations and preventing renewal of drug-seeking in addicts.
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Figure 1. Experiment 1: Conditioned Hyperactive Response and Effect of SCH 23390 on Extinction. The level of locomotor activity was measured by the distance travelled (in cm) in the open-field activity chamber over a 30 min session, and data points are the mean values for each group on a given day. Extinction of paired and unpaired groups was plotted on separate graphs for clarity. Error bars represent the ± 1 standard error of the mean. (A) Conditioning Phase. (*) indicates a significant difference between Paired and Unpaired groups and (#) indicates a significant difference between Chamber Days 1 and 4. (B) Extinction Phase: Paired Groups. (*) indicates a significant difference between Paired-Vehicle and Paired-0.025 mg/kg SCH 23390. (C) Extinction Phase: Unpaired Groups. All ps < .05.
Figure 2. Experiment 2: Conditioned Hyperactive Response and Effect of SCH 23390 on Extinction. The level of locomotor activity was measured by the distance travelled (in cm) in the open-field activity chamber over a 30 min session, and data points are the mean values for each group on a given day. Extinction of paired and unpaired groups was plotted on separate graphs for clarity. Error bars represent the ± 1 standard error of the mean. (A) Conditioning Phase. (*) indicates a significant difference between Paired and Unpaired groups and (#) indicates a significant difference between Chamber Days 1 and 4. (B) Extinction Phase: Paired Groups. (C) Extinction Phase: Unpaired Groups. (*) indicates a significant difference between Unpaired-Vehicle and Unpaired-0.0125 mg/kg SCH 23390. All ps < .05.
Figure 3. Pooled Analysis: Conditioned Hyperactive Response and Effect of SCH 23390 on Extinction. The level of locomotor activity was measured by the distance travelled (in cm) in the open-field activity chamber over a 30 min session, and data points are the mean values for each group on a given day. Extinction of paired and unpaired groups was plotted on separate graphs for clarity. Error bars represent the ±1 standard error of the mean. (A) Conditioning Phase. (*) indicates a significant difference between Paired and Unpaired groups and (#) indicates a significant difference between Chamber Days 1 and 4. (B) Extinction Phase: Paired Groups. (C) Extinction Phase: Unpaired Groups. All $p < .05$. 