Title

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Journal

Behavioural Brain Research, 239(1)

ISSN

0166-4328

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Publication Date

2013-02-01

DOI

10.1016/j.bbr.2012.10.042

Peer reviewed

Behavioural Brain Research 239 (2013) 155–163

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The competitive NMDA receptor antagonist CPP disrupts cocaine-induced conditioned place preference, but spares behavioral sensitization

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HIGHLIGHTS

- CPP co-administration with cocaine altered the acute response to cocaine.
- NMDAR antagonism with CPP had no effect on cocaine-induced behavioral sensitization.
- NMDAR antagonism with CPP abolished cocaine-induced conditioned place preference.

ARTICLE INFO

Article history:
Received 24 October 2012
Accepted 29 October 2012
Available online xxx

Keywords:
Addiction
Memory
Plasticity
N-methyl-D-aspartate
Stimulant
Mice

ABSTRACT

Recently, the notion that memory and addiction share similar neural substrates has become widely accepted. N-methyl-D-aspartate receptors (NMDAR) are the cornerstones of synaptic models of memory. The present study examined the effect of the competitive NMDAR antagonist CPP on the induction of behavioral sensitization and conditioned place preference to cocaine. Conditioned place preference is an associative memory model of drug seeking, while sensitization is a non-associative model of the transition from casual to compulsive use. There were three principal findings: (1) co-administration of CPP and cocaine altered the acute response to cocaine, suggesting a direct interaction between the two drugs; (2) NMDAR antagonism had no effect on behavioral sensitization; and (3) NMDAR antagonism abolished conditioned place preference. A review of prior evidence supporting a role for NMDARs in sensitization suggests that NMDAR antagonists directly interfere with cocaine's psychostimulant effects, and this interaction could be misinterpreted as a disruption of sensitization. Finally, we suggest that addiction recruits multiple kinds of plasticity, with sensitization recruiting NMDAR-independent mechanisms.

1. Introduction

Addiction is a chronic disease characterized by pathological drug use, an overwhelming involvement with the taking of a drug, the securing of its supply, and a chronic tendency to relapse, even after withdrawal and detoxification [1,2]. Indeed, although rehabilitation clinics have become quite successful in detoxing patients and sustaining abstinence for a few weeks or months, relapse rates remain very high [3]. Therefore, in recent years, research has increasingly focused on long-term changes that contribute to relapse. Several long-lasting neurobehavioral adaptations to repeated drug exposure have been found, including memories of the circumstances surrounding drug use, as well as neural and behavioral sensitization.

In one conception, addiction-related neuroadaptation in the brain, such as sensitization, is thought to reflect the same neurobiological processes as memory, especially at the molecular level, because the brain may have limited plasticity mechanisms to remodel synapses [4]. In a similar conception, addiction may represent aberrant learning, whereby addictive drugs hijack the natural reinforcement (incentive) pathway and recruit maladaptive habit learning directed at drug seeking and taking [5,6]. By these views, addiction persists as a memory or memory-like process long after drug exposure, which is consistent with high rates of relapse in addicts even after prolonged abstinence. Thus, behavior is driven by the approach and pursuit of incentive stimuli: contexts, people, and emotions previously associated with drug use [5,7].

Complementary to this view, the *incentive sensitization theory* of addiction suggests that in certain predisposed individuals, addictive drugs sensitize neural circuits, assigning abnormally high

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http://dx.doi.org/10.1016/j.bbr.2012.10.042

incentive salience to psychostimulant drugs and associated cues that elicit pathological drug craving, seeking, and use [8–10]. This process is manifest as an increase in drug response after repeated administration and can be measured as increases in locomotor activity or reward [11,12]. It is relevant to study the mechanisms underlying these synaptic changes because extinction or induced amnesia of addiction-related memories could become a useful treatment for relapse. From this perspective, it is logical to explore the role of N-methyl-D-aspartate receptors (NMDARs) in sensitization and other addiction-related memories, as NMDARs are the cornerstone of synaptic models of associative learning [13]. Through pairing of extensive depolarization and NMDAR activation by glutamate, the influx of calcium through NMDARs triggers a signaling cascade that causes long-term synaptic remodeling underlying long-term potentiation (LTP) and memory. It follows, then, that blocking NMDARs during initial drug exposure should inhibit the development of addiction-related memory.

Several behavioral studies have examined the role of NMDARs in the development of addiction-related memory, primarily by co-administering dizocilpine (MK801), a noncompetitive NMDAR antagonist, with psychostimulants (for a review see [14]). Co-administration of MK801 prevents the development of sensitization to cocaine [15–23], amphetamine [24–28] and methamphetamine [29,30]. Moreover, MK801 similarly prevents the development of cocaine, amphetamine, and methamphetamine-induced conditioned place preference, a prominent associative memory model of drug seeking behavior [31–33].

Although these studies suggest a role for NMDARs in behavioral sensitization and conditioned place preference, such findings are difficult to interpret because of several serious side effects associated with MK801 administration [34–36]. MK801 acts within the NMDA calcium channel in a manner similar to phencyclidine (PCP) and ketamine, and can produce psychosis [37], catalepsy, analgesia, and locomotor hyperactivity. Indeed, most noncompetitive NMDAR antagonists, because of their similarity to PCP, have been proposed as animal models of schizophrenia [38]. As with PCP, MK801 induces large neuronal vacuoles (Olney's lesions) within 30 min of administration [39]. Furthermore, MK801 paradoxically induces behavioral sensitization and place preference to itself, while blocking the development of these behaviors to other drugs [28,34,40–44]. Thus, it is difficult to interpret the chronic behavioral effects of co-administering MK801 with psychostimulants as anything other than ambiguous.

A few studies have used cleaner, competitive NMDAR antagonists in examining psychostimulant-induced sensitization [45,46,17,20,27,29,41,47–54]. For example, Wolf et al. [27] used CGS19755 and found a "clean block" of amphetamine sensitization – i.e., stereotyped and locomotor behavior did not increase with repeated administration in rats co-administered CGS19755 with amphetamine. However, CGS19755 dramatically altered the acute locomotor response to amphetamine, in particular the time course of the drug response (see for e.g., Wolf et al., 1995, their Fig. 3). This difference in time course makes a later sensitization challenge test difficult to interpret. It seems that most competitive NMDAR antagonists interfere with the acute behavioral response to psychostimulants; CPP, CGS19755, and AP5 have all been shown to alter the acute responses to cocaine and amphetamine [45,46,27,47,48,51–55]. These findings highlight the need for more studies using selective NMDAR antagonists and more attention to the effect of NMDAR antagonism on acute psychostimulant-induced behaviors. In the present report, we emphasize the difference in acute versus sensitized response as a measure of sensitization, which we believe is more transparently interpreted.

We examined the effects of a well-tolerated competitive NMDAR antagonist, ±CPP [D-3-(2-Carboxypiperazin-4-yl) propyl-1-phosphate] on the induction of cocaine-induced locomotor sensitization and conditioned place preference. CPP is a lipid soluble AP7 analog, and a highly potent antagonist that binds to the glutamate site on NR2A/2B subunits, in a manner similar to AP5 [56,57]. CPP produces only mild unconditional changes in behavior (e.g., muscle relaxation), unlike the profound ataxia seen even at low doses of MK801 [58]. We found that co-administration of CPP with cocaine abolished conditioned place preference and dramatically altered the acute behavioral response to cocaine, but did not affect the development of cocaine-induced sensitization of horizontal or vertical activity. To confirm that severe anterograde amnesia is present at the dose of CPP used in this study, we also report a dose-response curve for CPP using Pavlovian fear conditioning. The results are discussed in the context of theories that describe parallels between addiction and memory. Rather than arguing that addiction is a form of memory, we argue that addiction involves multiple associative memories (e.g., place preference), along with non-associative neuroadaptationist changes (e.g., sensitization).

2. Materials and methods

2.1. Subjects

Experiments were conducted using C57BL6/J inbred mice from the Jackson Laboratory (West Sacramento, CA) in approximately equal numbers of males and females balanced across groups. Mice were weaned at 3 weeks of age and group housed (4–5 mice per cage) with continuous access to food and water. Mice were at least 10 weeks old before testing and were handled for 5 days prior to experimental procedures. The vivarium was maintained on a 14:10 h light:dark schedule and all testing was performed during the light phase of the cycle. All animal care and testing procedures were approved by the UCSD IACUC and were compliant with the National Research Council Guide for the Care and Use of Laboratory Animals, 7th/8th editions.

2.2. Drugs

Cocaine HCl and ±CPP [±3-(2-carboxy-piperazine-4-yl)-propyl-1-phoshonic acid] (Sigma–Aldrich Co., St. Louis, MO) were dissolved in physiological (0.9%) saline. All saline and drug injections were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. In conditioned place preference and sensitization experiments, cocaine HCl was given 15 mg/kg (salt weight), a moderate dose previously found to induce substantial sensitization and place preference [59]. CPP was given 20 mg/kg (salt weight), a dose that produces severe amnesia in standard learning and memory tasks [60]. In fear conditioning experiments, CPP injections (salt weight: 5, 10, or 20 mg/kg) were given 20 min before introduction to the testing equipment.

2.3. Apparatus

2.3.1. Conditioned place preference and sensitization

Eight mice were tested concurrently in individual place preference chambers (43.2 cm × 43.2 cm × 30.5 cm; Med-Associates Inc., St. Albans, VT) housed in a windowless room. Each chamber consisted of two compartments (21.6 cm × 43.2 cm × 30.5 cm; Med-Associates Inc., St. Albans, VT) bisected by an opaque wall with a removable insert. The compartments each had distinct visual, tactile, and odor cues, designed to maximize their contextual differences. One compartment had a wire-mesh floor and walls decorated with colorful stickers, while the other compartment had a metal rod floor and standard clear polycarbonate walls. Half of the compartments were cleaned and scented with 7% isopropanol and half with plain water. The compartments were counterbalanced across Paired and Unpaired side designations. Each of the chambers was equipped with two rows of 16 × 16 infrared beam arrays and sensors that were evenly spaced and juxtaposed around the four peripheral sides of the chamber. The data acquisition and analysis software (Activity Monitor v 5.5; Med-Associates Inc., St. Albans, VT) used the interruption of infrared beams in *x*, *y*, and *z* space to detect mouse position and to derive distance traveled (horizontal activity), and rearing (vertical activity).

2.3.2. Fear conditioning

2.3.2.1. Conditioning context. Three to four mice were tested concurrently in individual conditioning chambers housed in a windowless room. Conditioning chambers were set up as described previously ([61,62]; [91]). Each conditioning chamber (32 cm × 25 cm × 25 cm; Med-Associates Inc., St. Albans, VT) was located within a sound-attenuating chamber (63.5 cm × 35.5 cm × 76 cm) and equipped with a speaker in the sidewall. The context consisted of a stainless steel grid floor (36 rods, each rod 2 mm in diameter, 8 mm center to center) and a stainless steel drop pan. The sidewalls were white acrylic and the front wall was clear polycarbonate to allow for viewing. Between each trial, the chambers were cleaned and scented with 7% isopropanol to provide a background odor. Ventilation fans provided

Table 1

Group	Injection		
	1: Unpaired	2: Home	3: Paired
Saline	Saline	Saline	Saline
Cocaine	Saline	Saline	Cocaine
CPP	Saline	CPP	Saline
CPP + Coc	Saline	CPP	Cocaine

background noise (65 dBA). Each sound-attenuating chamber was equipped with an overhead LED light source, providing white and near-infrared light. The mice were continuously monitored by a front-mounted IEEE 1394 progressive scan video camera with a visible light filter connected to a computer in an adjacent room. Each chamber was connected to a solid-state scrambler, providing AC constant current shock, an audio stimulus-generator, controlled via an interface connected to a Windows computer running Video Freeze (Med-Associates, Inc., St. Albans, VT), a program designed for the automated assessment of freezing and locomotor activity. Computer and human scored freezing had a correlation of 0.971 and a fit of computer = −.007 + 971 × human (for more detail see [61,63]).

2.3.2.2. Alternate context. The conditioning context was altered along several dimensions for tone testing trials. White acrylic sheets were placed over the grid floors and a black plastic, triangular teepee (23 cm, each side), translucent to near-infrared light, was placed inside each box. Only near-infrared light was used, creating a dark environment visible only to the video camera. Between trials, the chambers were cleaned and scented with a 5% vinegar solution.

2.4. Experimental procedures

2.4.1. Conditioned place preference and sensitization

2.4.1.1. Training. On each of 7 training days, mice were placed into both compartments of a conditioned place preference chamber with the insert in place for 15 min per compartment per day. Mice were randomly assigned to one of four groups: Saline, Cocaine, CPP + Coc, and CPP. All mice received 3 injections per day (Table 1). All mice were first given an injection of saline immediately prior to placement into the first compartment (Unpaired side) for 15 min. Following this, Saline control mice ($n = 18$) were given saline and placed in their home cages. Fifteen minutes later, they were given a third saline injection and placed into the second compartment (Paired side). The Cocaine group ($n = 25$) was given saline for 15 min in their home cages and then given cocaine prior to placement in the second compartment (Paired side). The CPP + Coc group ($n = 25$) was given CPP and placed in their home cages for 15 min. They were then injected with cocaine and placed into the second compartment (Paired side) for 15 min. To control for unconditional effects of CPP, the CPP group ($n = 18$) was treated with CPP for 15 min in their home cages and then injected with saline prior to placement into the second compartment (Paired side) for 15 min.

2.4.1.2. Place preference testing. Seven days after the final training session mice were tested for place preference. The insert between the two compartments was removed and the subjects were allowed free access to both compartments for 15 min following a saline injection. The amount of time spent in each compartment was recorded by Activity Monitor software (Med-Associates Inc., St. Albans, VT). A higher percentage of time spent on the Paired side was taken as a measure of place preference.

2.4.1.3. Behavioral sensitization. Locomotor (horizontal distance traveled) and vertical activity were recorded during training on the Paired side for each of the 7 training days. Behavioral sensitization was measured as the difference between the response on Day 7 (sensitized response) and Day 1 (acute response) of training.

2.4.2. Fear conditioning

2.4.2.1. Training. Mice were injected with saline or CPP 20 min prior to training. Mice were randomly assigned to groups labeled by dose of CPP administered: 0 (saline control, $n = 19$), 5 ($n = 14$), 10 ($n = 13$), or 20 mg/kg ($n = 20$). Training consisted of a 2 min baseline period, followed by three tone-shock pairings, each separated by 30 s. A tone-shock pairing consisted of a 30 s tone (2.8 kHz, 85 dB, A Scale) that co-terminated with a scrambled, constant current AC 2 s footshock (0.75 mA, RMS). Mice were inside the fear conditioning chambers for a total of 10 min before being returned to their home cages [64]. Freezing behavior, defined as the absence of all movement with the exception of respiration [65], was scored automatically using Video Freeze software (Med-Associates, Inc., St. Albans, VT) [61,63].

2.4.2.2. Testing. Mice were returned to the conditioning context, without drug, 7 days after training. Freezing was scored for 5 min. Mice were placed in the alternate context 24 h later, also off drug. Tone testing consisted of a 2 min baseline, followed by 3, 30 s tones separated by 30 s, identical to the training tones. Freezing was scored for the entire 5 min period.

3. Results

3.1. Conditioned place preference and behavioral sensitization

3.1.1. Locomotor activity

Fig. 1 depicts locomotor (horizontal) activity across the 7 days of training. Sensitization of locomotor activity for the average activity of each day is shown in Fig. 1A. These data were fed into a multivariate analysis of variance (MANOVA), which revealed a significant group × day interaction [$F(3,82) = 6.58$, $p < 0.0001$]. Visual inspection of Fig. 1A suggests that this reflects the fact that groups receiving cocaine sensitized, while the others did not. Fig. 1B shows the time course of the locomotor response for day 1. There were significant group differences [$F(3,82) = 20.4$, $p < 0.0001$]. The unusual response to the combination of CPP and cocaine suggests a direct drug interaction. Although there was an obvious group × time interaction [$F(3,82) = 20.6, p < 0.0001$] due to the abnormal response in CPP + Coc mice, for the sake of simplicity, only the average response is discussed thoroughly. Similar conclusions are reached if the min-by-min response is used. In terms of average response, mice in the Cocaine group showed greater locomotor activity than all other groups (p values <0.03, pairwise Wald tests). CPP + Coc mice were reduced from Cocaine mice, but elevated compared to

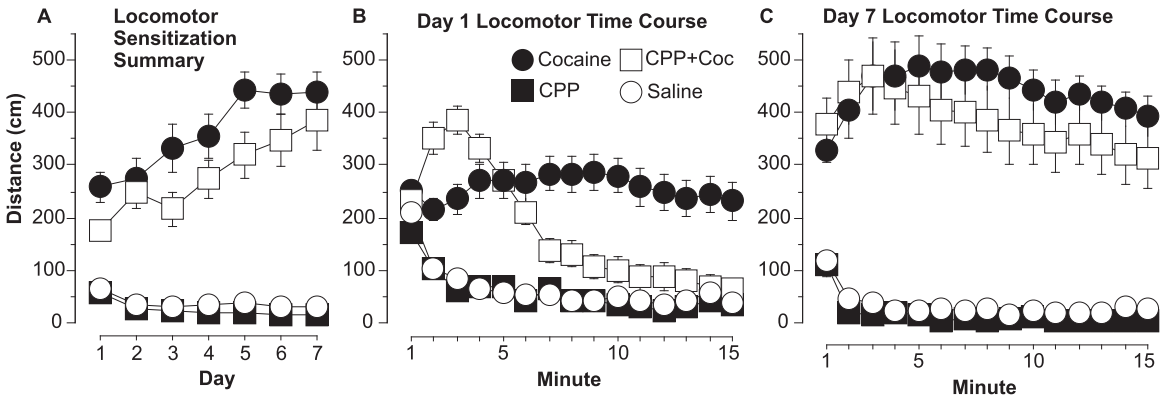

Fig. 1. Sensitization of Locomotor Activity. (A) Average locomotor activity after drug treatment on the Paired side by day. Cocaine and CPP + Coc mice show sensitization, an increase in locomotor activity from Day 1 to 7, while CPP and Saline control mice do not. (B) Time course of drug action on Day 1 (acute response). CPP + Coc initially enhances and then reduces locomotor activity as compared to mice given Cocaine only. Neither Saline nor CPP alone stimulate locomotor activity. (C) Time course of drug action on Day 7 of training. Cocaine and CPP + Coc groups are elevated with respect to Saline and CPP control mice, but do not differ from one another. Each point represents the mean ± 1 standard error.

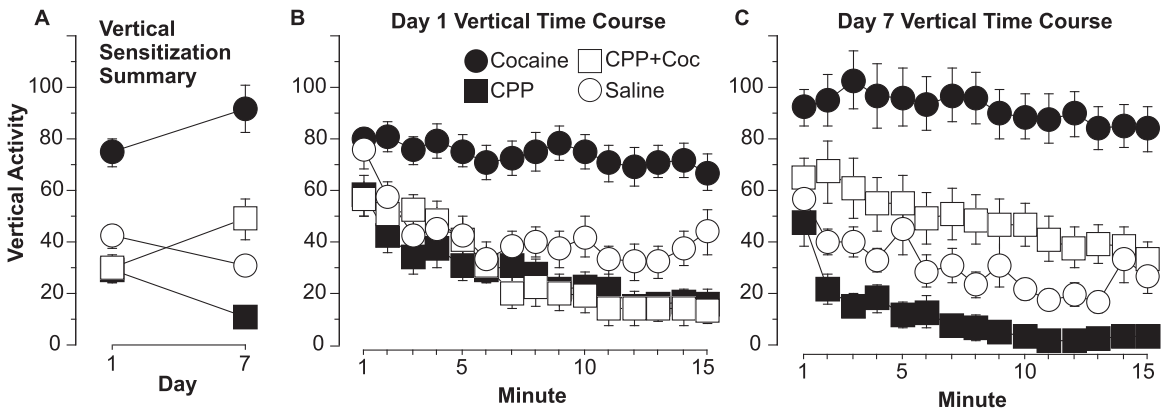

Fig. 2. Sensitization of Vertical Activity. (A) Average vertical activity after drug treatment on the Paired side by day. Both Cocaine and CPP + Coc mice show sensitization, an increase in vertical activity from Day 1 to 7, but CPP co-administration reduces the overall amount of vertical activity. CPP alone reduced activity from Day 1 to 7. (B) Time course of drug action on Day 1 (acute response). Cocaine mice show higher vertical activity than all other groups. CPP + Coc have a dramatically reduced response and do not differ from mice given CPP or Saline alone. (C) Time course of drug action on Day 7. Cocaine mice again show higher vertical activity than all other mice. CPP + Coc mice have reduced activity compared to Cocaine mice, but are elevated as compared mice given CPP or Saline alone. CPP alone dramatically depresses vertical activity, even as compared to Saline controls. Each point represents the mean ± 1 standard error.

CPP and Saline controls (p values <0.0001), which did not differ from one another ($p > 0.3$). Thus, CPP alone did not alter locomotor activity, but it dramatically altered the acute response to cocaine, without totally abolishing it.

Fig. 1C shows the locomotor response for day 7. There were significant group differences [$F(3,82) = 30.0$, $p < 0.0001$]. Mice in the Cocaine and CPP + Coc group did not differ ($p > 0.4$) and were elevated with respect to the CPP alone and Saline control groups (p values <0.01). The CPP alone group exhibited a slight depression of activity relative to Saline controls ($p < 0.0001$), but numerically the difference was quite small. Overall, by day 7, cocaine produced a robust locomotor response, which was reduced when CPP was co-administered. CPP had little effect when given alone, producing a small, but significant, reduction in distance traveled.

3.1.2. Vertical activity

Fig. 2A depicts average vertical activity across the 7 days of training. Each day was considered separately as there was a significant group × day interaction [$F(3,82) = 6.58$, $p < 0.0001$]. The time course of vertical activity for day 1 is shown in Fig. 2B. There were significant group differences [$F(3,82) = 18.2$, $p < 0.0001$]. Cocaine mice exhibited higher vertical activity than all other groups (p values ≤ 0.0001). CPP + Coc mice had a dramatically reduced response compared to Cocaine mice ($p < 0.0001$) and did not differ from mice given CPP alone or Saline (p values >0.1). CPP alone also produced a substantial reduction in vertical activity compared to Saline controls ($p < 0.05$).

The time course for day 7 is shown in Fig. 2C. There were significant group differences [$F(3,82) = 22.5$, $p < 0.0001$]. Again, Cocaine mice exhibited more vertical activity than all other groups (p values ≤ 0.001). CPP + Coc mice were depressed compared to Cocaine mice, but exhibited higher activity than mice given CPP alone ($p < 0.0001$) and were nearly different than mice given Saline alone ($p = 0.077$). Finally, CPP alone depressed vertical activity relative to Saline controls ($p < 0.0001$). Overall, CPP not only dramatically disrupted the vertical response to cocaine, it also reduced vertical activity in general.

3.1.3. Sensitization (activity difference) scores

Despite evidence that CPP reduced the acute response to cocaine, it seems that mice given CPP + Coc exhibited sensitization of both locomotor and vertical activity (see Figs. 1A and 2A). We quantified sensitization as the difference in response from day 7 to day 1, both on an average and min-by-min basis. The difference in locomotor activity (day 7–day 1) for each group on a min-by-min basis is shown in Fig. 3A. It is apparent that substantial and similar sensitization exists in the Cocaine and CPP + Coc groups, while none is present in the CPP and Saline groups. In order to

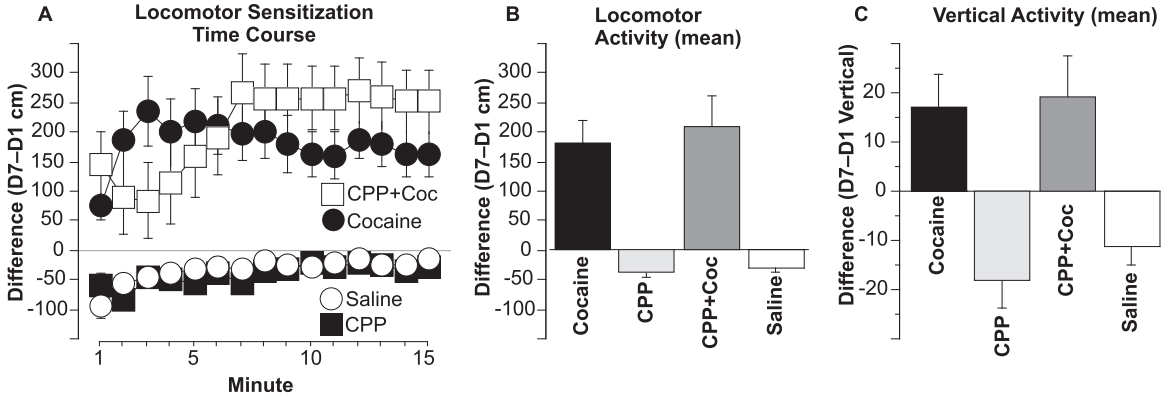

Fig. 3. Sensitization Difference Scores. Mice given Cocaine and CPP + Coc show sensitization of both locomotor and vertical activity. (A) Sensitization was measured as the difference in locomotor activity (Day 7–Day 1) on a min-by-min basis. Cocaine and CPP + Coc groups show substantial sensitization, while CPP and Saline control groups do not. (B) Sensitization was also quantified as the difference in average locomotor activity from Day 7 to Day 1. Cocaine and CPP + Coc groups show a similar increase in locomotor activity from Day 1 to Day 7, with no sensitization in control groups. (C) Mean vertical activity difference scores (Day 7–Day 1) revealed that Cocaine and CPP + Coc mice both exhibited substantial and similar sensitization compared to either CPP or Saline control mice. Each bar or point represents the mean ± 1 standard error.

simplify the statistical analysis, average locomotor activity data for day 7–day 1 is shown in Fig. 3B. There were significant group differences [$F(3,82) = 12.3$, $p < 0.0001$]. Cocaine and CPP + Coc mice did not differ in terms of overall sensitization of locomotor activity ($p = 0.69$) and were elevated with respect to both CPP and Saline groups (p values <0.001). CPP alone and Saline alone groups did not differ ($p = 0.45$). Thus, the difference score between day 7 and day 1 clearly revealed similar sensitization in the Cocaine and CPP + Coc groups, with no sensitization in either control group.

Similar results were found for average vertical activity sensitization difference scores (Fig. 3C; overall ANOVA, [$F(3,82) = 7.54$, $p < 0.001$]). Cocaine and CPP + Coc mice did not differ in terms of overall sensitization ($p = 0.87$); both groups exhibited sensitization compared to either CPP or Saline control mice (p values < 0.01). The two control groups showed hardly any change from day 1 to day 7 and did not differ in this respect ($p = 0.32$). Thus, despite having large effects on the acute cocaine response, CPP co-administration did not disrupt sensitization of locomotor or vertical activity.

3.1.4. Place preference

Sensitization (Figs. 1–3) was assessed during induction of place preference on the drug-paired side. In order to test place preference, mice were returned to the apparatus with access to both sides of the chamber for a 15 min preference test. Preference (Paired % time – Unpaired % time) is depicted in Fig. 4. There were significant group differences [$F(3,82) = 6.81$, $p < 0.001$]. Mice in the Cocaine group exhibited greater preference for the Paired side than all other groups (p values <0.03). They were the only group to show a significant place preference [one-sample two-tailed t-test against hypothesized $\mu = 0$, $t(24) = 4.14$, $p < 0.001$]. Place preference was abolished in the CPP + Coc group [$t(24) = 1.08$, $p = 0.3$] and there was also no significant preference in the CPP alone group [$t(17) = -1.34$, $p = 0.2$] or the Saline group [$t(17) = -1.51$, $p = 0.15$]. These groups also did not differ from one another ($p = 0.75$). Thus, CPP abolished place preference induced by cocaine.

Overall, there were three main findings: (1) CPP disrupted the acute locomotor and vertical activity response to cocaine; (2) when correcting for the disrupted acute response, CPP failed to disrupt sensitization of either behavior; and (3) CPP abolished the acquisition of place preference.

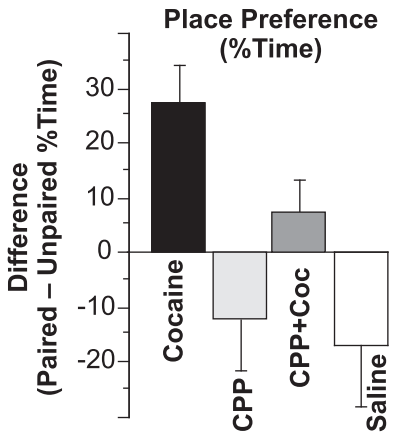

Fig. 4. Place Preference. CPP abolished place preference induced by cocaine. Preference was measured as the difference between the percent of time spent in the Paired and Unpaired sides of the place preference apparatus. Values greater than zero indicate a preference for the drug-paired side. Cocaine alone mice are the only group to show significant place preference. Each bar represents the mean ± 1 standard error.

3.2. Fear conditioning

Previous studies have suggested that 20 mg/kg of CPP is a highly amnesic dose. In order to verify this in our strain of mice, we examined Pavlovian fear conditioning, a standard model of associative memory.

3.2.1. Generalized activity

Mice were placed into the conditioning chambers and locomotor activity was assessed prior to any shock. As in the place preference chambers, CPP produced a small dose-dependent decrease in activity during the 2 min baseline period of training when measured by an automated computer scoring system (Fig. 5A, bottom). Group differences were found on an analysis of variance [ANOVA; $F(3, 65) = 9.73$, $p < 0.0001$]. Subjects administered a 5–20 mg/kg dose of CPP pre-training displayed significantly less activity during baseline than the saline control group (p values <0.02).

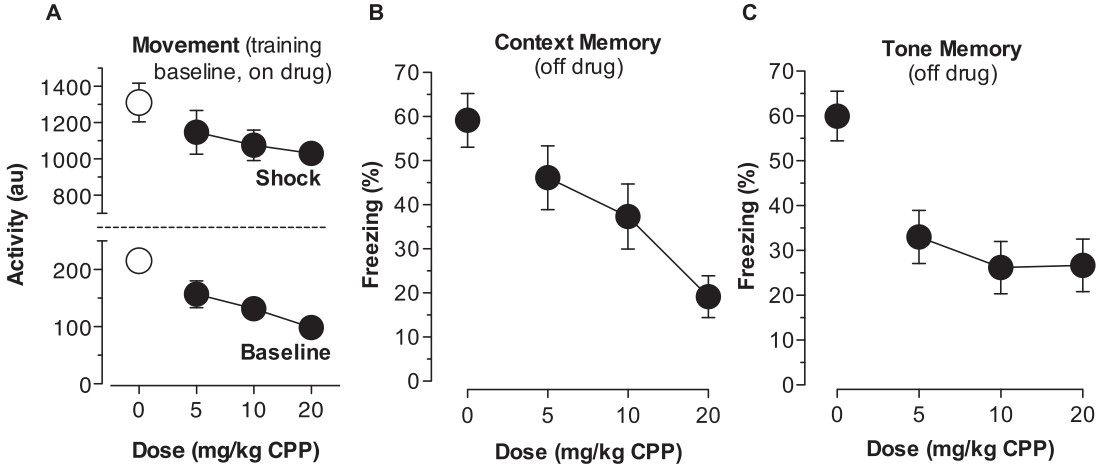

Fig. 5. Fear Conditioning. (A) Training Activity. Prior to any shock, during the 2 min baseline period, CPP produced a dose-dependent decrease in locomotor activity (bottom line). The first 2 s shock presentation elicited a large increase activity (top line). There were no significant group differences in shock reactivity, indicating that deficits seen on memory tests were not due to analgesia. (B) Context fear memory was assessed 7 days after training. Mice were tested off drug for 5 min in the conditioning context. Average freezing during the test is presented. Subjects that previously received 10 or 20 mg/kg CPP before training displayed less contextual fear than subjects given Saline or 5 mg/kg CPP. (C) Tone fear memory was assessed 24 h after the context fear test in an alternate context. Tone fear memory is defined as the average freezing to three tone presentations minus baseline freezing in the alternate context. Pre-training administration of any dose of CPP resulted in impaired tone fear memory. Each point represents the mean ± 1 standard error.

3.2.2. Shock reactivity

A large increase in velocity, known as the "activity burst" or unconditioned response (UR), was elicited by the 2 s shock presentation. The average activity during the first 2 s shock is depicted in Fig. 5A (top). There were no significant overall differences in shock reactivity [$F(3,65) = 2.03$, $p = 0.12$].

3.2.3. Training

Subjects were in the conditioning context on the training day for a total of 10 min. Mice were given 3 tone-shock pairings during the first 5 min (on drug training data not graphed). There was a main effect of drug dose [$F(3,65) = 8.37$, $p < 0.0001$], with 0 mg/kg showing normal freezing (average of the 5 min, $15.1 \pm 12.8\%$) and 10 ($28.9 \pm 15.5\%$) or 20 mg/kg ($38.5 \pm 19.4\%$) showing increased freezing (p values <0.02) and 5 mg/kg producing no effect ($19.0 \pm 13.1\%$; $p = 0.48$). Mice were then left in the chambers for an additional 5 min as an immediate memory test (data not graphed). There were no significant differences in overall immediate memory [$F(3,65) = 2.15$, $p = 0.10$; means, 0 mg/kg: $65.8 \pm 27.1\%$, 5: $55.5 \pm 21.8\%$, 10: $63.5 \pm 17.2\%$, 20: $73.6 \pm 13.5\%$]. As these subjects were on drug, it is important to note that CPP's locomotor effects likely influenced the results. Any increase in freezing seen during training was likely due to the small depression of locomotor activity. Nonetheless, because CPP induces a small increase in freezing and hypoactivity during training, direct motor effects are unlikely to be related to the amnesia, reflected as decreased freezing, that is observed one week later, off drug (Fig. 5B and C).

3.2.4. Context fear

To examine if CPP given during training prevented the acquisition of fear conditioning memory, mice were returned to the conditioning chambers 7 days after training. Freezing was measured for 5 min, with all subjects off drug. The average of this test is shown in Fig. 5B. Dose-dependent differences in contextual fear were apparent [$F(3,65) = 8.56$, $p < 0.0001$]. Subjects that had previously received 10 or 20 mg/kg CPP before training displayed less contextual fear than saline controls (p values < 0.02), while 5 mg/kg failed to induce an amnesic effect on this test ($p = 0.15$).

3.2.5. Tone fear

Subjects were introduced to an alternate context 24 h after the context test, also off drug. Freezing was measured for 5 min, consisting of a 2 min baseline period followed by 3, 30 s tone presentations, each separated by 30 s. The tone was the same frequency and volume as that which had been paired with the shock during training. Baseline freezing (not depicted) in the alternate context was very low, but differed among the groups [$F(3,65) = 3.35$, $p < 0.03$]. Saline controls froze slightly more ($11.3 \pm 3.4\%$) than the groups that had previously received CPP (5 mg/kg, $6 \pm 2.8\%$; 10 mg/kg, $2.0 \pm 2.8\%$; 20 mg/kg, $2.6 \pm 1.1\%$; p values <0.05). Thus, tone fear was defined as the average of freezing to the three tones minus baseline freezing (Fig. 5C). Overall, pre-training administration of any dose of CPP resulted in impaired tone fear memory [$F(3,65) = 8.14$, $p < 0.0001$]. Subjects given 5, 10, or 20 mg/kg CPP prior to training froze significantly less than saline controls (p values <0.002). The same conclusions are reached if the raw tone freezing values are analyzed for any particular minute.

Overall, we were able to verify that 20 mg/kg CPP produced severe deficits in both contextual and tone fear memory on a standard model of associative learning.

4. Discussion

The present study examined the effect of the competitive NMDAR antagonist CPP on the induction of behavioral sensitization and conditioned place preference to cocaine. There were three principal findings, discussed in turn: (1) co-administration of CPP and cocaine altered the acute response to cocaine, suggesting a direct pharmacodynamic interaction between the two drugs; (2) NMDAR antagonism had no effect on behavioral sensitization; and (3) NMDAR antagonism blocked conditioned place preference.

(1) Acute or chronic administration of CPP alone produced little to no effect on locomotor activity, but significantly altered the locomotor response to cocaine (Fig. 1B). After a 5 min enhancement of cocaine-induced horizontal activity, a dramatic reduction was observed. By the seventh day of drug administration, this pattern was absent (Fig. 1C). In contrast to locomotor activity, CPP produced a dramatic acute and chronic reduction in vertical activity when given alone or with cocaine (Fig. 2). These findings are in agreement with previous studies reporting that the competitive NMDAR antagonists CPP [55], CGS19755 [55] and AP5 [53] attenuate or completely block the acute behavioral responses to cocaine. If NMDAR antagonists interfere with a psychostimulant's primary pharmacological actions, necessary for both acute and sensitized responses, then blockade of the induction of sensitization by NMDAR antagonists cannot simply be attributed to a blockade of neuroplasticity.

The nature of the interaction between CPP and cocaine is puzzling, but may be pharmacodynamic. First, CPP may interact with some element of dopamine (DA) neurotransmission. CPP and AP5 have both been shown to increase DA synthesis and efflux in the striatum as measured by in vivo microdialysis [66]. Second, some of cocaine's effects could be through NMDARs, either directly or indirectly. Indeed, acute cocaine exposure causes a delayed increase in NMDAR-mediated synaptic currents in DA neurons by stimulating DA D5 receptors, which leads to an increase in NR2B-containing NMDARs [67]. Several studies have now reported that cocaine modulates the NMDAR population, noting sustained increases in NR1 and/or NR2B densities in the striatum, VTA, amygdala, and hippocampus, following both acute and chronic cocaine exposure [67,68]. Likewise, cocaine increases available NMDA and PCP binding sites [69,70]. Some evidence suggests this interaction between cocaine and NMDARs is responsible, in part, for cocaine-induced convulsions; NMDAR antagonists are able to protect against cocaine-induced toxicity [55]. Finally, we should not forget cocaine's canonical local anesthetic effect at voltage gated sodium channels [71]. It is obvious that inhibition of sodium conductance can alter NMDAR function [72,73]. Thus, although the exact cause of the interaction observed here cannot be fully explained, there is considerable evidence for bidirectional interactions between NMDAR antagonists and cocaine.

So far we have focused primarily on studies using cocaine, rather than amphetamine, because of their distinct pharmacodynamics. For example, amphetamine does not share cocaine's local anesthetic effect, and selective DA antagonists differentially affect the induction of sensitization to cocaine and amphetamine [74,75]. Despite these differences, however, the behavioral findings with amphetamine are consistent with those of cocaine. Competitive, non-competitive, and glycine-site NMDAR antagonists interfere with both the acute and sensitized responses to amphetamine [45,46,27,47,48,52].

(2) CPP clearly had no effect on behavioral sensitization when the difference in either locomotor or vertical activity between day 7 and day 1 is taken as a measure of cocaine sensitization (Fig. 3). It has been accepted that NMDARs are critical for the development of cocaine-induced sensitization. This notion is primarily based on evidence that co-administration of MK801 with cocaine completely blocks [15–23] or reduces [16,33,49,76,77] cocaine-induced sensitization. However, this conclusion is still highly controversial and the role of NMDARs in addiction remains unclear [78]. The problems with using MK801, including its non-specific side effects and paradoxical ability to induce sensitization to itself, have been reviewed

extensively [34–36]. It has been repeatedly suggested that alternative antagonists, particularly competitive antagonists, be used in place of MK801.

In addressing this controversy, Wolf [79] argued that many labs have now shown that several classes of NMDAR antagonists block the induction of sensitization without producing the troubling side-effects associated with MK801. For example, her lab [52] found that CGS19755 produced a "clean block" of sensitization by preventing any increase in amphetamine-induced activity across training days and during the sensitization challenge test. However, in both this and a prior study [27], the acute response to amphetamine was dramatically increased when CGS19755 was co-administered (compare [52], their Figs. 1A and 2A, day 1 response for amphetamine only and amphetamine with CGS19755 and [27], their Fig. 3). We believe that most or all competitive NMDAR antagonists alter the acute response to psychostimulants, and that using a difference score to calculate sensitization can control for this interference. This, however, results in little evidence that NMDARs are involved in sensitization (Fig. 3).

To the best of our knowledge, seven studies have used competitive NMDAR antagonists on the induction of cocaine sensitization [17,19,20,41,49,53,80]. Every one of these studies claim to find a blockade or attenuation of cocaine-induced sensitization when a competitive NMDAR antagonist is co-administered, but all also show either acute interactions between the NMDAR antagonist and cocaine or do not report acute response data. First, CPP [19,80] and CGS19755 [20] have been reported to block cocaine-induced sensitization, but only data from the sensitization challenge tests were reported in these studies. As such, the results are difficult to interpret given that competitive NMDAR antagonists dramatically interfere with the acute response to cocaine (Figs. 1 and 2) [53,55]. Similarly, Haracz et al. [49] found that d-CPP-ene attenuates cocaine-induced sensitization, but they also do not report acute response data. It is possible that intact sensitization was masked in these studies by a reduced behavioral response to cocaine. Druhan and Wilent [41] co-administered CPP into the ventricles with cocaine (i.p.) and also found a blockade of the induction of sensitization to cocaine. However, they present their induction data as an average of all training sessions; it is clear that CPP reduced the response to cocaine during training (their Fig. 5), but it is impossible to discern acute effects during the first injection. Further, sensitization was reduced in mice co-administered CPP and cocaine because control mice showed an elevated response to CPP, rather than Paired mice showing a reduction in activity when pre-treated with CPP (their Fig. 7).

The remaining two studies report an altered acute response to cocaine when competitive NMDAR antagonists were co-administered. Kalivas and Alesdatter [17] demonstrated that CPP given intra-VTA with cocaine (i.p.) blocked one-shot sensitization to cocaine (their Fig. 6), but CPP appeared to reduce the acute horizontal activity induced by cocaine (their Fig. 5). Additionally, Licata et al. [53] found that AP5, given intra-VTA, co-administered with cocaine (i.p.), appeared to reduce acute distance traveled and attenuated the sensitized locomotor response to cocaine (their Fig. 2). Therefore, we believe a number of studies could have possibly taken a direct reduction of overall response as a reduction in sensitization.

(3) NMDAR antagonism by CPP abolished the induction of cocaine-induced conditioned place preference and Pavlovian fear conditioning (Figs. 4 and 5). This is consistent with conditioned place preference being a form of associative learning and is not controversial; NMDARs are critical for the formation of most or all associative memories [13,81,82]. Our findings support and extend previous literature indicating that non-competitive [31,33,83,84], competitive [85], and glycine-site [86,87] NMDAR antagonists block cocaine-induced place preference.

The present results suggest that addiction may recruit multiple types of memories, and therefore, multiple types of plasticity. Anagnostaras and colleagues [11,12] have previously suggested that sensitization recruits both associative and non-associative forms of plasticity. In this view, sensitization is likely sustained by non-associative learning, the mechanisms of which only partially overlap with those underlying traditional associative learning. Indeed, some forms of non-associative learning do not require the mechanisms typically implicated in associative learning. For example, habituation of the Hering–Breuer apnea reflex in the primary vagal pathway, a classic model of non-associative memory, recruits NMDAR-independent plasticity [88]. Likewise, mossy fiber long-term potentiation is NMDAR-independent and often non-Hebbian [89]; it can be induced without any post-synaptic activity or calcium influx at all [90].

Therefore, we propose that addiction is better characterized as inducing both associative and non-associative forms of memory that together contribute to compulsive drug taking behavior. Associations between drug-induced affective states and contextual cues could trigger craving and some goal-directed behavior toward drugs, like that seen in the conditioned place preference paradigm. However, the transition from recreational to pathological and compulsive drug seeking may involve non-associative processes, such as sensitization, whereby the neural substrate mediating the unconditioned response to the drug is directly augmented [8–10]. The long-term neural changes induced by psychostimulants, that lead to chronic relapse, likely involve synaptic modifications not seen during ordinary associative learning.

Acknowledgements

This work was supported by NIDA (DA020041 to SGA) and Hellman Fellowship (SGA). We thank Tristan Shuman for helpful comments on an earlier version of this manuscript.

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