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Post-training and post-reactivation administration of amphetamine enhances morphine conditioned place preference

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Abstract

Amphetamine has been shown to enhance consolidation in a variety of memory paradigms. However, it is not known if amphetamine can modulate the consolidation of the types of context-reward associations involved in drug addiction, such as those formed in the conditioned place preference (CPP) task. Also, some types of memory exhibit a second period of lability following memory reactivation, and it is not known whether amphetamine administered during this period can modulate CPP. Our study investigated whether amphetamine can enhance morphine CPP when administered during the consolidation period or the post-reactivation period. Subjects were trained in the CPP task and injected with amphetamine or vehicle immediately or six hours after each training session. The day after the completion of training, they were tested. Amphetamine injected immediately but not six hours after training enhanced morphine CPP. In separate experiments, subjects were first trained in the CPP task. The day following the completion of training, subjects were given a memory reactivation session and injected with amphetamine or vehicle immediately or six hours after reactivation. Subjects were tested the next day. Amphetamine injected immediately but not six hours after memory reactivation enhanced morphine CPP. However, amphetamine injected without memory reactivation had no effect on the expression of morphine CPP. Our results suggest that amphetamine enhances the consolidation of morphine CPP and that morphine CPP exhibits a temporally limited period of post-reactivation lability during which the memory can be modulated.

Keywords

consolidation; reconsolidation; place conditioning; addiction; memory; learning; rat

Introduction

The consolidation period of a memory refers to a temporally limited period of lability immediately following acquisition during which a long-term memory is thought to be forming. Many pharmacologic agents have been shown to modulate consolidation in a variety of memory tasks (for reviews, see [20,21,31]). These agents modulate memory when administered immediately after training, but they have no effect when administered several hours later. Amphetamine (AMPH) is one agent that has been shown to enhance the consolidation of a variety of memory types, including spatial memory, active avoidance conditioning, and verbal declarative memory [18,25,37,50]. However, it is not known if AMPH can also modulate the consolidation of the learned associations between a context and reward that are formed in the conditioned place preference (CPP) task.

The CPP task tests an animal's ability to associate a drug reward with a unique environmental context. In this task, subjects are repeatedly exposed to pairings of a distinct environment with the administration of a drug. During a drug-free test, subjects are said to exhibit a conditioned place preference if they spend more time in the drug-associated environment than in an environment that has not been associated with the drug.

Although the CPP task has most commonly been used to determine the rewarding properties of drugs of abuse and natural stimuli (for review, see [52]), this task may also be used to investigate the mechanisms underlying context-reward associations [17]. Because there is no drug onboard during the test sessions, the expression of CPP must rely on an association between the drug reward and the environmental context of the drug-associated chamber.

Understanding the neural mechanisms of context-reward associations is important because of the influence that drug-associated environmental contexts and cues can exert on drug-seeking behavior. Both environmental contexts and specific environmental cues associated with a drug can consistently reinstate drug-seeking in abstinent rats (for reviews, see [44,46,47]). In humans, drug-associated stimuli increase self-reported levels of drug craving in addicts [6, 14,15,30] and can be an important factor in precipitating relapse in addicts [16,49].

Few studies have investigated the mechanisms underlying the consolidation of CPP. Protein kinases A and C are involved in the consolidation of cocaine CPP [5], and the amygdala, including muscarinic receptors within the amygdala, may play a role in the consolidation of amphetamine CPP [17,43]. However, there have been no studies investigating whether the consolidation of CPP can be positively modulated. Therefore, the first part of our study investigates whether amphetamine can enhance the consolidation of morphine CPP.

In addition to the consolidation period, some types of memory also exhibit a temporally limited period of lability immediately following reactivation of the memory (for review, see [42]). This post-reactivation lability has been observed in a variety of memory types across several species including rodents [3,19,20,22,24,32,35,36,40,41], chicks [1], fish [11], crabs [38], and humans [23,53], and it has recently been observed in two different types of addiction-related learning [29,33]. Lee et al. [29] showed that zif268, an immediate early gene, is required in the basolateral amygdala after reexposure to a cocaine-associated conditioned stimulus in order for the stimulus to maintain its reinforcing properties. In addition, Miller and Marshall [33] recently showed that post-reactivation inhibition of MAP/ERK kinase (MEK) in the nucleus accumbens core disrupts the memory for a cocaine CPP. However, it is not known whether morphine CPP exhibits this type of post-reactivation lability, and there has been little investigation of whether any memory can be positively modulated (rather than impaired) during the post-reactivation period. To address this question, the second part of our study investigates whether amphetamine can enhance CPP when administered immediately after reactivation of the association.

Materials and Methods

Animals

All studies used male rats (weighing between 250-280 g at the beginning of the experiments) that received food and water ad libitum and were housed individually in polycarbonate cages in ventilation racks (Biozone, Fort Mill, SC, USA). Subjects were kept on a 12 hour light/dark cycle with lights on at 6 a.m., and all behavioral procedures were conducted during the light cycle. These procedures were approved by the Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center at the University of California, San Francisco, and are in accordance with "PHS Policy on Humane Care and Use of Laboratory Animals", Office of Laboratory Animal Welfare, National Institutes of Health, USA, revised 2002.

Apparatus

Each place preference apparatus (Med Associates, VA) contained three chambers separated by sliding doors. The two main chambers (28 × 21 × 21 cm) differed in color (black vs. white), lighting (white vs. red), and floor texture (metal bars vs. metal grid) and were connected by a middle chamber (12 × 21 × 21 cm) that was grey in color. The time spent in each chamber was automatically recorded by infrared beam breaks.

Drugs

Morphine (Sigma; St. Louis, MO) was dissolved in physiological sterile saline and injected subcutaneously (SC) at a dose of 10 mg/kg. This dose was chosen based on previous studies of morphine place conditioning [2]. d-Amphetamine (Sigma; St. Louis, MO) was dissolved in physiological sterile saline and injected into the intraperitoneal cavity (IP) at a dose of 1 mg/kg. This dose of amphetamine was chosen based on its ability to enhance memory consolidation in another behavioral paradigm [18].

Behavioral procedures

Experiment 1: What is the effect of AMPH on memory for morphine CPP?

Experiment 1a: Post-training injections of amphetamine: Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were first given a baseline session in which they were placed in the middle chamber for a 60 second adaptation period. The doors to the other chambers were then raised, and subjects were allowed to explore all three chambers for thirty minutes. The time spent in each chamber was recorded.

Training sessions were then conducted once a day for six consecutive days. On alternating days, rats were given injections of morphine or saline immediately before being placed in the middle chamber for a 30 second adaptation period and then confined for thirty minutes to the morphine-paired or saline-paired chamber, respectively. Rats received an injection of either amphetamine or vehicle immediately after each training session. Subjects were sorted into experimental groups semirandomly: groups were counterbalanced for drug-paired chamber, days receiving drug vs. saline, CPP box, and the order in which rats experienced behavioral sessions.

A test session identical to the baseline session was then conducted 24 hours after the last training session. A subset of subjects experienced four more test sessions, each separated by 24 hours.

Experiment 1b: Post-reactivation injections of amphetamine: Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were conditioned as described for Experiment 1a, except that 8 training sessions (4 morphine, 4 saline) were given. We increased the number of training sessions for this experiment in order to increase the probability that animals would develop a more robust memory which would be less likely to extinguish during the subsequent reactivation session. Twenty-four hours after the last training session, subjects were given their first 30 minute test, in which time spent in each side of the apparatus was measured. This test served as a reactivation of the memory for CPP. Subjects then received an injection of amphetamine or vehicle immediately after the memory reactivation. Rats were divided into experimental groups in the manner described for the first experiment.

Subjects were given a 30 minute test session 24 hours after the memory reactivation. A subset of subjects also experienced two subsequent test sessions separated by 24 hours.

Experiment 2: Is the effect of AMPH temporally limited?

Experiment 2a: Post-training injections of AMPH: Male CD rats (Charles River, Wilmington, MA, USA) were conditioned as described for Experiment 1a, except that 4 training sessions (2 morphine, 2 saline) were given. Because we found that animals from Charles River required fewer training sessions to develop a level of conditioning similar to the animals from Harlan (Blaiss and Janak, unpublished data), subjects in Experiment 2 were given fewer training sessions than in the analogous parts of Experiment 1. After each training session, all subjects received 2 injections. One group of animals received an injection of amphetamine immediately after each session and a vehicle injection 6 hrs after each session; the drugs were reversed for a second group of animals. Subjects were given a 30 minute test session 72 hours after the last training session. Rats were divided into experimental groups in the manner described for the first experiment, and any rat that had a difference score (see data analysis section) greater than 250 seconds during the baseline session was dropped from the study.

Experiment 2b: Post-reactivation injections of AMPH: Male CD rats (Charles River, Wilmington, MA, USA) were conditioned as described for Experiment 1a with 6 training sessions, and twenty-four hours after the last training session, subjects were given a memory reactivation test as described in Experiment 1b. After the memory reactivation, all rats received 2 injections. One group received an injection of amphetamine immediately after the memory reactivation and a vehicle injection 6 hrs after the memory reactivation; in a second group the drugs were reversed. Subjects were then given a 30 minute test session 24 hours after the memory reactivation.

Rats were divided into experimental groups in the manner described for the first experiment, and any rat that did not exhibit a CPP (difference score greater than zero) during the memory reactivation was also dropped from the study.

Experiment 3: Control – Is memory reactivation necessary for CPP

enhancement?—Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) were conditioned as described for Experiment 1b. Twenty-four hours after the last training session, subjects were transported to the behavioral testing room, given an injection of amphetamine or vehicle, and then returned to their home cages. On the next day, subjects were given one 30 minute test session. Rats were divided into experimental groups in the manner described for the first experiment.

Experiment 4: Control – Does AMPH also enhance the consolidation of morphine CPP when morphine is injected after (instead of before) training sessions?—Male CD rats (Charles River, Wilmington, MA, USA) were initially given a baseline session. Training sessions were then conducted once a day for 4 consecutive days. On alternating days, rats were given injections of morphine or saline immediately *after* being confined for thirty minutes to the morphine-paired or saline-paired chamber, respectively. Subjects were also injected with either vehicle or amphetamine immediately after every training session. A test session identical to the baseline session was then conducted 72 hours after the last training session. Rats were divided into experimental groups in the manner described for the first experiment, and any rat that had a difference score (see data analysis section) greater than 250 seconds during the baseline session was dropped from the study.

Data Analysis

During test sessions and the memory reactivation session, the time the subjects spent in each chamber was recorded by computer, and the difference in time spent in the drug-paired chamber and saline-paired chamber (difference score) was taken as a measure of place conditioning. To determine whether subjects exhibited an inherent preference during the baseline session, the

total time spent in the chamber to be paired with morphine was compared to the total time spent in the chamber to be paired with saline using paired *t*-tests. Data was analyzed in the same way (the time spent in the morphine-paired chamber compared to the time spent in the saline-paired chamber) to determine whether trained subjects exhibited a significant conditioned place preference. In order to determine whether there was an effect of Treatment during a single test session, we conducted 1-way ANOVAs with Treatment as a between-subjects factor. Data from subjects who received multiple test sessions were analyzed using 2-way repeated measures ANOVAs; ANOVAs were conducted with Treatment as a between-subjects factor and Test Session as a within-subjects factor.

Results

Experiment 1: Amphetamine enhances memory for morphine CPP

Experiment 1a: Post-training injections of amphetamine enhance morphine

CPP—Rats were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 691.65 ± 23.69 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 645.48 ± 26.96 seconds; paired *t*-test, $t(27) = 1.13$, $P=.27$]. Then, to determine whether amphetamine can enhance the consolidation of morphine CPP, subjects were injected with either AMPH or vehicle immediately after every CPP training session. This included sessions in which rats were injected with morphine and confined to the morphine-associated chamber as well as sessions in which rats were injected with saline and confined to the saline-associated chamber. Subjects were then allowed to explore all chambers during a test session, and rats that received injections of AMPH immediately after training sessions showed an enhanced level of morphine CPP upon testing when compared to subjects that received vehicle. Specifically, the AMPH group had a greater mean difference score when compared to the vehicle group (Figure 1a; one-way ANOVA, $F(1,26)=5.94$, $P<.03$). As shown in Figure 1b, this effect of AMPH persisted across four subsequent tests for the subset of subjects that was repeatedly tested. While the vehicle group no longer showed a significant CPP by Test 3 [mean ($\pm$ SEM) time in the morphine chamber: 717.24 ± 55.86 seconds; mean ($\pm$ SEM) time in the saline chamber: 587.52 ± 86.19 seconds; paired *t*-test, $t(5) = 1.13$, $P=.31$], the AMPH group's place preference remained significant even on Test 5 [mean ($\pm$ SEM) time in the morphine chamber: 860.39 ± 88.03 seconds; mean ($\pm$ SEM) time in the saline chamber: 458.81 ± 75.02 seconds; paired *t*-test, $t(5) = 2.97$, $P<.031$]. A within-subject repeated measures ANOVA found a main effect of Treatment and Test Session across all five tests [Treatment: $F(1,10)=7.85$, $P<.02$; Test Session: $F(4,10)=2.83$, $P<.04$], but there was no significant interaction between Treatment and Test Session, suggesting that although the overall performance changed over time, the degree of difference between groups was not significantly different across the repeated tests.

Experiment 1b: Post-reactivation injections of amphetamine enhance morphine

CPP—Subjects were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 656.99 ± 19.48 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 676.78 ± 21.97 seconds; paired *t*-test, $t(27) = -.51$, $P=.61$]. To test whether post-reactivation administration of AMPH can enhance morphine CPP, rats were given CPP training sessions and a memory reactivation session. Figure 2a shows the level of morphine CPP seen during the memory reactivation, after subjects have been divided into treatment groups, but before amphetamine has been administered. There was no statistical difference between groups (AMPH group vs. vehicle group) during this session [one-way ANOVA; $F(1,26)=.67$, $P=.42$]. However, immediately after this memory reactivation session, rats were injected with either vehicle or AMPH, and during a test session 24 hours later, the AMPH group showed enhanced

morphine CPP when compared to the vehicle group [Figure 2b; one-way ANOVA; $F(1,26)=4.98$, $P<.04$]. In addition, for the subset of subjects that was repeatedly tested, the AMPH group exhibited enhanced CPP over two subsequent tests (Figure 2c); a within-subjects repeated measures ANOVA found a main effect of Treatment [$F(1,10)=5.15$; $P<.05$]. Similar to experiment 1a, there was no significant interaction between Treatment and Test Session, indicating that the degree of difference between groups was not significantly different across the repeated tests.

Experiment 2: The memory enhancing effect of amphetamine is temporally limited

Experiment 2a: Immediate, but not delayed, post-training injections of amphetamine enhance morphine CPP

—Rats were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 633.09 ± 19.29 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 619.86 ± 14.72 seconds; paired t -test, $t(29)=.46$, $P=.65$]. To investigate whether the memory enhancing effect of post-training injections of AMPH is temporally limited, we asked whether AMPH injected 6 hours after training can enhance the consolidation of morphine CPP in a manner similar to immediate post-training injections of AMPH. Because stress can modulate memory (for review, see [54]), we designed this study to control for the possibility that injection stress occurring at different times in the different groups could act as a confounding factor. Therefore, all rats were given injections both immediately and 6 hours after each training session. One group received an injection of AMPH immediately after training and a vehicle injection 6 hours after training; in the second group, the conditions were reversed. This serves to ensure that our results stem from an effect of the drug rather than any possible effects of injection stress. During a test session, the group that received AMPH immediately after training showed enhanced CPP when compared to the group that received AMPH 6 hrs after training [Figure 3a; one-way ANOVA; $F(1,28)=7.47$, $P<.02$].

Experiment 2b: Immediate, but not delayed, post-reactivation injections of amphetamine enhance morphine CPP

—Subjects were first given a baseline session to ensure that they showed no natural preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 621.35 ± 17.23 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 637.50 ± 19.88 seconds; paired t -test, $t(21) = -.56$, $P=.59$]. To test whether the enhancing effect of post-reactivation injections of AMPH is temporally limited, rats were then given CPP training sessions and a memory reactivation session. Figure 4a shows the level of morphine CPP seen during the memory reactivation, after subjects have been divided into treatment groups, but before amphetamine has been administered. There was no statistical difference between groups (AMPH -- immediate injections vs. AMPH -- delayed injections) during this session [one-way ANOVA; $F(1,20)=.22$, $P=.65$]. All rats were given injections both immediately and 6 hours after this memory reactivation session. One group received an injection of AMPH immediately after reactivation and a vehicle injection 6 hours after reactivation; in the second group, the conditions were reversed. As in Experiment 2a, this design ensures that our results stem from an effect of the drug rather than any possible effects of injection stress. During the test session 24 hours later, the group that received AMPH immediately after memory reactivation showed enhanced morphine CPP when compared to the group that received AMPH 6 hrs after memory reactivation [Figure 4b; one-way ANOVA; $F(1,20)=6.58$, $P<.02$].

Experiment 3: After the memory is formed, reactivation is necessary for amphetamine to enhance morphine CPP

In this control study, we investigated whether the enhancing effect of AMPH seen in experiments 1b and 2b required reactivation of the memory. Subjects showed no natural

preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 679.56 ± 34.48 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 649.64 ± 31.17 seconds; paired *t*-test, $t(15)=.49$, $P=.63$]. Rats were given CPP training sessions, but unlike the subjects in experiments 1b and 2b, they did not experience a memory reactivation session. Instead of a memory reactivation session, they were simply transported to the behavioral testing room, given an injection of AMPH or vehicle, and returned to their home cages. During the test the next day, there was no statistical difference between the groups' difference scores [mean ($\pm$ SEM) difference score for AMPH group ($n=8$): 465.25 ± 90.24 seconds; mean ($\pm$ SEM) difference score for vehicle group ($n=8$): 414.10 ± 57.24 seconds; one-way ANOVA; Treatment: $F(1,14)=.229$, $P=.64$], suggesting that memory reactivation is necessary for AMPH to enhance morphine CPP.

Experiment 4: Amphetamine does not enhance morphine CPP consolidation when morphine is injected after (instead of before) training sessions

In Experiments 1a and 2a, AMPH was administered after each training session. If it is possible for rats to develop a morphine CPP without having morphine present during the actual training session, then it also might be possible that our results in Experiments 1a and 2a were due to a drug-drug interaction between morphine and amphetamine that enhances the rewarding properties of morphine (see Discussion). Therefore, in this control study, we investigated whether amphetamine could enhance the consolidation of morphine CPP when morphine or saline was injected after training sessions (i.e. after confinement to the appropriate chamber) rather than before training sessions. During the baseline session, rats showed no natural preference for either of the conditioning chambers [mean ($\pm$ SEM) time in the chamber to be paired with morphine: 636.74 ± 35.74 seconds; mean ($\pm$ SEM) time in the chamber to be paired with saline: 631.00 ± 26.29 seconds; paired *t*-test, $t(15)=0.15$, $P=.88$]. During alternating training sessions, all subjects were given injections of morphine or saline immediately *after* being confined to the morphine-paired or saline-paired chamber, respectively. Subjects were injected with either vehicle or AMPH immediately after each training session. Upon testing, there was no difference between the vehicle group and the AMPH group [mean ($\pm$ SEM) difference score for vehicle group ($n=8$): 164.23 ± 153.33 seconds; mean ($\pm$ SEM) difference score for the AMPH group ($n=8$): 133.71 ± 131.44 seconds; one-way ANOVA; $F(1,14)=.02$, $P=.88$]. In fact, neither group exhibited a statistically significant level of CPP during the test session (see Table 1).

Discussion

Our study found that amphetamine injected immediately, but not 6 hrs, after training enhances morphine CPP, indicating that amphetamine enhances the consolidation of morphine CPP. In addition, we have shown that amphetamine injected immediately, but not 6 hrs, after memory reactivation also enhances morphine CPP, suggesting that morphine CPP exhibits a temporally limited period of post-reactivation lability during which the memory can be modulated. These findings demonstrate that the consolidation of morphine CPP can be modulated in a manner similar to other types of memory, provide evidence that memories can be positively modulated after reactivation, and suggest the possibility of a novel line of inquiry into the treatment of addiction.

Amphetamine enhances the consolidation of morphine CPP

In Experiment 1a, we showed that AMPH injected immediately after training enhances the expression of morphine CPP. In addition, memory for CPP was enhanced not only during the first test but during five tests across five consecutive days. The effect of AMPH is therefore not transitory; there is a persistent effect of the drug. Also, in order to have an effect, AMPH must be injected within a discrete time window after training. In Experiment 2a, we showed

that AMPH enhances the expression of morphine CPP only when injected immediately, but not 6 hours, after training. Therefore, the results from Experiments 1a and 2a together suggest that amphetamine enhances the consolidation of the association between morphine and the drug-paired chamber.

An alternative explanation is that amphetamine is not enhancing the memory for morphine CPP but is instead enhancing the hedonic or rewarding properties of morphine. However, two aspects of the study design argue against this possibility. First, amphetamine was administered after every training session, including those sessions where subjects were injected with saline and exposed to the saline-associated chamber. Second, amphetamine was administered after (rather than before) each training session. Therefore, the hedonic effect of morphine could not have been altered during the training session when subjects had the opportunity to experience both the hedonic effect of morphine and the morphine-associated environment and to associate them. However, this argument rests on the assumption that morphine must be present during the actual training session in order for an animal to develop a morphine CPP. We tested that assumption directly in Experiment 4. In that experiment, we showed that when morphine is injected *after* training sessions (so that subjects never experience the pharmacological effects of morphine until *after* they have been exposed to the drug-paired chamber), amphetamine does not enhance morphine CPP, and in fact, these rats do not even develop a morphine CPP. In addition, Gaiardi, et al. [13] have shown that concomitant administration of AMPH (at doses of .5, 1, and 2 mg/kg) does not affect the level of morphine CPP (at morphine doses of 5 and 20 mg/kg), suggesting that there is no synergistic interaction between the two drugs that affects CPP. Finally, our results in Experiments 1b and 2b show that amphetamine can enhance morphine CPP when administered after a test session with no drug onboard, indicating that amphetamine can enhance memory for morphine CPP independent of a pharmacological interaction with morphine. Therefore, the combination of the above arguments suggests that it is highly unlikely that our results in Experiments 1a and 2a are due to an enhancement of the rewarding properties of morphine.

Post-reactivation administration of amphetamine enhances morphine CPP

In Experiment 1b, we show that AMPH injected immediately after memory reactivation enhances morphine CPP. This enhancement of morphine CPP also persisted across multiple test sessions, suggesting that the long-term memory for the association was enhanced and that the results cannot be explained by a transitory effect of AMPH on memory retrieval. In Experiment 2b, we show that the enhancing effect of AMPH is temporally limited; AMPH enhances morphine CPP when injected immediately, but not 6 hours, after memory reactivation. We then conducted a control study (Experiment 3) in which subjects were injected with amphetamine 24 hrs before the CPP test, without memory reactivation. In these subjects, amphetamine had no effect on CPP. This indicates that amphetamine's enhancement of CPP when administered post-reactivation is not a general effect of the drug, but rather requires reactivation of the memory. The combined results of these experiments suggest that after reactivation of the memory for the morphine CPP, there is a temporally limited period of post-reactivation lability during which the memory can be modulated. Although other types of memory have also been shown to exhibit a period of post-reactivation lability (see Introduction), our results are a rare example of positive modulation of a memory during this period.

Another possible interpretation of these experiments is that AMPH is blocking the consolidation of an extinction memory rather than enhancing the memory of the original association. Our memory reactivation session is inherently an extinction session, and by test 1, rats that received a vehicle injection after reactivation no longer showed a significant difference between the time spent in the morphine-paired chamber and the saline-paired

chamber (see Table 1). On the other hand, during the same test, rats that received an AMPH injection immediately after reactivation still showed a significant difference between the time spent in the morphine- and saline-paired chambers. Recent studies of fear conditioning and conditioned taste aversion have shown that the same post-reactivation manipulation can either modulate the original learning or new extinction learning, depending on relatively small changes in the behavioral protocol [11,38], and it is possible that future studies using different behavioral protocols could determine for certain whether our results are due to a modulation of the original memory or the new extinction memory. However, because AMPH has been shown consistently to enhance a wide variety of memory types across multiple species (see Introduction) but has rarely been shown to block any type of memory in any species, the most parsimonious interpretation of our data is that post-reactivation injections of AMPH enhance the original memory.

It should also be noted that during the memory reactivation (before the injection of amphetamine) in Experiment 1b, the group that was to receive amphetamine immediately after reactivation exhibited a slightly higher mean difference score than the group that was to receive vehicle immediately after reactivation. Although this difference is not statistically significant (see Results), it could still be argued that the difference between groups during the tests is simply an amplification of this initial slight difference. However, there is no correlation between the level of CPP exhibited during the memory reactivation and during the following test ($n=28$; $R=.303$; $P>.1$). This suggests that our results are due to the effect of amphetamine rather than any inherent difference between groups.

Possible Mechanisms

Further studies are needed to determine the mechanisms by which post-training and post-reactivation injections of amphetamine act to enhance morphine CPP. Studies investigating the mechanisms underlying the memory enhancing effects of amphetamine in other behavioral paradigms have implicated both the norepinephrine and dopamine systems [4,28], including multiple types of dopamine receptors [12,39]. Noradrenergic and dopaminergic signaling have also been shown to modulate consolidation in a variety of learning and memory paradigms ranging from the Morris water maze to the conditioned taste aversion task [7,21,26,27,34,45]. Although the role of these neurotransmitter systems in reconsolidation has not been as extensively characterized, the D1 receptor antagonist SCH23390 has been shown to impair the reconsolidation of an inhibitory avoidance task in day-old chicks [48], and the β -adrenergic receptor antagonist propranolol has been shown to impair the reconsolidation of auditory fear conditioning, inhibitory avoidance, and a spatial memory task in rats [8,41]. It is possible that amphetamine is acting through these neurotransmitter systems to produce our results.

Although AMPH had the same effect during the consolidation and post-reactivation period, the degree of similarity in the mechanisms underlying AMPH's effect during these two phases is currently unclear. In other memory paradigms, the nature of the post-reactivation lability, the mechanisms underlying it, and its degree of similarity to the consolidation period are only beginning to be investigated. Some molecules that are necessary during the consolidation period also appear to be necessary during the post-reactivation period for continued maintenance of the memory [3,20,22,36,40,41]; other molecules necessary during the consolidation period do not appear to be necessary during the post-reactivation period [51].

Implications and Conclusions

Our results have strong implications for both basic science and clinical studies of drug addiction. Associations formed between a drug reward and the environment (similar to those formed during conditioned place preference) can impact addicts even after years of abstinence. Further investigation into the post-reactivation lability of conditioned place preference could

help to elucidate how these memories are shaped and why they persist for such a long time. Also, Miller and Marshall [33] recently showed that memories for cocaine CPP can be modulated after their initial formation, and our results confirm that the same general principle holds true for the associations involved in morphine CPP. If similar types of associations can be negatively as well as positively modulated in humans, then a novel avenue of treatment for cue-associated relapse in human addicts could be possible. If drug-environment associations can be negatively modulated in addicts after their initial formation, it may be possible to decrease the chance that exposure to drug-related environments and environmental cues might trigger relapse.

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Figure 1.

Effect of immediate post-training injections of amphetamine.

a) Place preference during Test 1 (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score (time spent in morphine-associated chamber minus time spent in saline-associated chamber) $\pm$ SEM. (* $P < .03$)

b) Place preference during Tests 1-5 (vehicle group, n=6; AMPH group, n=6), expressed as a mean difference score $\pm$ SEM. A 2-way ANOVA found a main effect of Treatment across all tests ($P < .04$).

Figure 2.

Effect of amphetamine injected immediately after memory reactivation.

a) Place preference during Memory Reactivation (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score $\pm$ SEM.

b) Place preference during Test 1 (vehicle group, n=14; AMPH group, n=14), expressed as a mean difference score $\pm$ SEM. (* $P < .04$)

e) Place preference during Tests 1-3 (vehicle group, n=6; AMPH group, n=6), expressed as a mean difference score $\pm$ SEM. A 2-way ANOVA found a main effect of Treatment across all tests ($P < .05$).

Figure 3.

Effect of amphetamine injected immediately vs. 6 hours after training.

a) Place preference during the Test session (AMPH-immediately group, n=15; AMPH-6 hrs group, n=15), expressed as a mean difference score $\pm$ SEM. (* $P < .02$)

Figure 4.

Effect of amphetamine injected immediately vs. 6 hours after memory reactivation.

a) Place preference during Memory Reactivation (AMPH-immediately group, $n=11$; AMPH-6 hrs group, $n=11$), expressed as a mean difference score $\pm$ SEM.

b) Place preference during Test 1 (AMPH-immediately group, $n=11$; AMPH-6 hrs group, $n=11$), expressed as a mean difference score $\pm$ SEM. (* $P<.02$)

Table 1

The mean amount of time in seconds ($\pm$ SEM) spent in the morphine-paired and saline-paired chambers by each group for each experiment. The times spent in the morphine-paired versus the saline-paired chambers were compared using paired *t*-tests, and the resulting P-values are listed in the last column.

Experiment	Test Session	Group	Mean ($\pm$ SEM) time in morphine chamber (sec)	Mean ($\pm$ SEM) time in saline chamber (sec)	P-value
1a	Test 1	Veh	722.76 ($\pm$ 30.24)	587.92 ($\pm$ 25.61)	<.02
		Amph	768.11 ($\pm$ 29.97)	451.71 ($\pm$ 36.80)	<.001
1b	Memory Reactivation	Veh	693.03 ($\pm$ 36.31)	481.86 ($\pm$ 60.72)	<.008
	Test 1	Amph	724.1 ($\pm$ 38.04)	427.33 ($\pm$ 31.98)	<.001
		Veh	606.07 ($\pm$ 45.09)	480.28 ($\pm$ 45.55)	0.07
		Amph	739.69 ($\pm$ 50.61)	404.17 ($\pm$ 32.19)	<.001
2a	Test 1	Amph - imm	870.37 ($\pm$ 52.36)	420.27 ($\pm$ 40.33)	<.001
		Amph - 6 hrs	718.42 ($\pm$ 31.00)	579.39 ($\pm$ 43.85)	0.095
2b	Memory Reactivation	Amph - imm	833.73 ($\pm$ 37.20)	398.52 ($\pm$ 32.46)	<.001
	Test 1	Amph - 6 hrs	831.1 ($\pm$ 51.35)	442.72 ($\pm$ 46.39)	<.001
		Amph - imm	917.68 ($\pm$ 56.87)	385.67 ($\pm$ 41.23)	<.001
		Amph - 6 hrs	731.43 ($\pm$ 51.50)	559.2 ($\pm$ 55.26)	0.098
3	Test 1	Veh	772.02 ($\pm$ 59.67)	357.92 ($\pm$ 36.04)	<.001
		Amph	831.48 ($\pm$ 60.37)	366.23 ($\pm$ 37.16)	<.001
4	Test 1	Veh	732.72 ($\pm$ 80.74)	568.49 ($\pm$ 73.57)	.32
		Amph	673.99 ($\pm$ 66.29)	540.28 ($\pm$ 68.09)	.34