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Disruptive Effect of Amphetamines on Pavlovian to Instrumental Transfer

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Abstract

Reward seeking behavior can be powerfully modulated by exposure to a conditioned stimulus (CS) that was previously associated with that reward. This can be demonstrated in a Pavlovian-to-instrumental transfer (PIT) task where presentation of a CS (e.g., tone and light) previously paired with a rewarding unconditioned stimulus (US; e.g., food) leads to increases in a behavioral response, such as a lever press, that was also paired with the same US. The transfer effect can be enhanced in rats by exposing them repeatedly to amphetamine after they have undergone Pavlovian conditioning and instrumental training. However, it is not clear if amphetamine injections given immediately after Pavlovian conditioning, which are predicted to enhance memory consolidation for the CS-US association, would also enhance the transfer effect. We tested this hypothesis by giving male, Sprague-Dawley rats i.p. injections of saline or drug (0.5, 1.0, or 3.0 mg/kg amphetamine or methamphetamine) immediately following Pavlovian conditioning sessions. We found that amphetamine, but not methamphetamine, enhanced Pavlovian approach behavior. During a subsequent PIT test done under extinction conditions, we found that rats given either drug, particularly at the highest dose, exhibited deficits in PIT relative to saline-treated controls. These results suggest that treatment with amphetamines after Pavlovian conditioning sessions, when memory consolidation of the CS-US association is hypothesized to occur, inhibits the ability of the CS to subsequently elicit reward-seeking behavior.

Keywords

Pavlovian Instrumental Transfer; PIT; incentive salience; incentive motivation; amphetamine; methamphetamine; reward; addiction

1. Introduction

The process whereby cues repeatedly paired with drug taking experiences have the potential to elicit craving and drug-seeking behaviors has been suggested to play an important role in

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the development and maintenance of addiction [21]. It has been hypothesized that reward-related stimuli develop the ability to drive behavior because they acquire incentive-motivational properties [39] that subsequently lead an individual to direct significant attention towards drug-associated cues [19]. Imaging studies have demonstrated that, in abstinent abusers exposed to pictures of drug paraphernalia, craving is associated with activation of components of the limbic system and cortex, such as the amygdala and prefrontal cortex [6, 49]. Similar studies have found that exposure to these cues also leads to increases in neural activation in the cingulate cortex [20,32] and increases dopamine in the striatum in cocaine-addicted subjects [48,50]. In animal models of addictive behaviors, rodents and non-human primates have been shown to increase previously extinguished drug-seeking behaviors following exposure to cues that had been paired with the availability of drug infusions [14]. These behaviors are associated with robust activation of mesocorticolimbic circuitry [45].

The Pavlovian-to-instrumental transfer (PIT) paradigm provides a means to study how associative learning processes subsequently affect behavioral output directed at reward seeking [8,17,31]. In this paradigm, animals undergo several sessions of instrumental training (e.g., lever pressing for food pellets) followed by Pavlovian conditioning sessions wherein a conditioned stimulus (CS) is presented along with delivery of a food pellet (the unconditioned stimulus, or US). The ability of the CS to take on incentive salience and thereby activate reward-seeking behavior is then evaluated by measuring instrumental behavior in the context of repeated presentations of the CS. During this test for PIT, which takes place under extinction conditions (i.e., no food pellets are delivered), an observation of greater instrumental behavior during CS presentations compared to periods without the CS present is taken as evidence of a transfer effect. PIT is highly dependent upon activity within the nucleus accumbens, with both lesions of the accumbens and local blockade of dopamine receptors reducing transfer [12,23, 30]. Conversely, intra-accumbens infusion of an indirect dopamine agonist enhances PIT [52]. Other studies have also highlighted important roles for the amygdala [4,10,24], prefrontal cortex [26], dorsal striatum [9], and ventral tegmental area [11,35] in PIT.

Repeated exposure to psychostimulant drugs has also been reported to alter the transfer effect. For example, in rats that were given six daily injections of 3 mg/kg amphetamine (AMPH) following instrumental training and Pavlovian conditioning, PIT was enhanced during a test session given 10 days after the last drug injection [51]. These results were interpreted as being due to drug-induced enhancement of the incentive salience attributed to the CS, which the authors described as an increase in cue-triggered “wanting” of the food reward. AMPH has also been shown to enhance Pavlovian conditioning, as measured by an increase in approach behavior relative to saline-treated controls [3,44]. Because injections in these studies were given at the conclusion of conditioning sessions, these results are consistent with an effect of AMPH on memory consolidation of the CS-US association. This interpretation is supported by evidence that AMPH administration 2 hours after training does not increase approach behavior [44]. However, it is not clear from these studies if drug treatment during the memory consolidation process also enhances the salience of the CS, which would be measurable as an increase in instrumental behavior during CS presentations in a PIT paradigm. Thus, we investigated this in the current study by giving rats injections of psychostimulant drugs immediately after three of the six Pavlovian approach training sessions that followed instrumental training, but preceded PIT testing.

Another aim of this study was to compare the effects of AMPH and methamphetamine (METH) on Pavlovian approach and PIT. Although these drugs share nearly identical chemical structures and have very similar pharmacokinetic and pharmacodynamic properties [33], METH appears to have markedly higher abuse liability [7]. The reason for this is not clear, but one contributing factor may be a differential ability of these drugs to modulate associative learning processes. Although this hypothesis has not been tested directly, we showed

previously that AMPH and METH share similar potency for inducing hyperactivity and locomotor sensitization, but METH appeared more potent when presentation of a light and tone stimulus was paired with its effects [22]. Here, we more directly assessed the hypothesis that METH, compared to AMPH, has a greater ability to influence associative learning.

2. Methods

2.1 Subjects

A total of 84 male Sprague-Dawley rats, 2.5–4 months of age, were obtained from Harlan (Indianapolis, IN, USA) or were born in our animal facility from breeder rats originally obtained from Harlan. The number of purchased and bred rats was approximately equal across the control and treatment groups. The experimental procedures were performed in four different squads of rats (16–24/squad), with rats in each squad assigned randomly to a group and each group represented by at least two rats in each squad.

Rats were housed individually in translucent home cages either upon arrival from Harlan or starting at ~2 months of age. Before experiments began, rats were handled individually for 15 min on five separate occasions to reduce handling-induced stress. Water was available *ad libitum* throughout the study. Food was available *ad libitum* until 5 days prior to the start of the experiment, at which time food was restricted so that weight was reduced to ~85% of free-feeding weight. All rats were maintained at 85–90% of their free-feeding weight throughout the duration of the study. Additionally, rats were kept on a 12:12-h light/dark cycle, with experiments performed during the light cycle. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois and were consistent with the Principles of Laboratory Animal Care (NIH Publication no. 85-23).

2.2. Apparatus

Operant behavior was monitored using standard conditioning chambers (Coulbourn Instruments) located within sound attenuating cubicles that were equipped with ventilation fans. One wall of the chamber contained two retractable levers that were positioned on either side of a centrally located food trough. White cue lights were located above each lever and a tone emitting speaker (2.9 kHz Sonalert) was located directly above the food trough. Entries into the food trough were monitored by infrared detectors. A white house-light (4 W), which was illuminated during all training and test sessions, was located near the top of the chamber on the opposite wall from the food trough.

2.3 Instrumental Training

The PIT paradigm utilized here was adapted from previous studies [16,29,52]. All rats received a single 30 min session of magazine training to habituate them to the chambers and train them to eat from the food trough. Food pellets (45 mg, Bioserv; Frenchtown, NJ, USA) were delivered on a random time (RT) 30 sec schedule. Over the next ten days, rats underwent instrumental training for 60 min/day. Both levers were extended into the chamber during these sessions, but only one was active. Assignment of the active lever to the left or right side of the food trough was counterbalanced across rats. Responses on the active lever were reinforced with food pellet delivery using the following schedule: continuous reinforcement (sessions 1–2), random ratio (RR) 2 (session 3), continuous reinforcement (session 4), RR2 (sessions 5–6), RR5 (sessions 7–8), and RR10 (sessions 9–10). During these sessions, responses on the inactive lever were recorded but had no programmed consequences.

2.4 Pavlovian Approach Training

Following instrumental training, all animals received six daily sessions of Pavlovian approach training in the same operant chambers but with the levers retracted. Each session consisted of ten, 30 sec presentations of the CS (2.9 kHz tone and illumination of both cue lights), with pellet delivery occurring on an RT 20 sec schedule during the final 20 sec of CS presentation. Individual CS presentations were separated by an interstimulus interval of 2–4 min. Immediately following sessions 1, 3, and 5, rats received an injection (i.p.) of 0.9 % saline (1 ml/kg), AMPH (0.5, 1.0, or 3.0 mg/kg), or METH (0.5, 1.0, or 3.0 mg/kg). This schedule and timing of drug administration was chosen based on previous work showing that AMPH given in this manner, but not 2–6 hours after training sessions, increases conditioned responding (i.e., trough-directed behavior) [3,44].

2.5 Instrumental Reminder Session and PIT Test

The day after the last session of Pavlovian training, rats received one 30 min instrumental training “reminder” session (RR 10). On the following day, they were placed in the test chambers with both levers extended. The CS was then presented three separate times with each presentation followed by a 2–4 min interstimulus interval. The session was administered under extinction conditions (i.e., no food pellet delivery).

2.6 Data Analysis

Data from all rats (10–13 rats/group; 84 rats total) were used in the analyses of Pavlovian approach behavior, which was evaluated by calculating the percentage of nosepoke responses into the food delivery trough that occurred during the 30-sec CS ($\text{CS responses/total responses} \times 100$) and trough entries that occurred during the 30-sec period immediately before CS onset ($\text{pre-CS responses/total responses} \times 100$). Data from saline injected rats were analyzed statistically using one-way repeated measures ANOVA (session). Data from AMPH- and METH treated rats were analyzed separately using two-way mixed factor ANOVA with session as the within-subjects factors and dose as the between-subjects factor. To analyze drug effects on the magnitude of conditioned behavior, the difference between the percentage of CS trough entries during session 6 and session 1 was calculated for each rat. Group differences in these difference scores were then analyzed statistically using one-way ANOVA with 7 levels of group (saline, 3 doses of AMPH, and 3 doses of METH). Significant main effects and interactions were further assessed by Holm-Sidak post-hoc tests.

Several rats did not learn to lever press during instrumental training, or failed to respond during the reminder session, and were therefore not included in the analysis of these operant sessions or in the analysis of the PIT test. Specifically, 2 rats were excluded from the 0.5 mg/kg AMPH, 1 mg/kg AMPH and METH, and 3 mg/kg METH groups, and 3 rats were excluded from the saline and 3 mg/kg AMPH groups. Thus, data from $n = 10$ rats/group were used for the analysis of behavior during instrumental training, the reminder session, and PIT testing.

Group differences in the rate of lever pressing during instrumental training and reminder sessions were examined using one-way ANOVA. Lever press and approach behavior during the PIT test was assessed by comparing responses during the CS and pre-CS time periods using two-way mixed factor ANOVA with time period as the within-subjects factor and group as the between-subjects factor. Drug effects were further assessed by analyzing the PIT ratio for each group. This ratio was calculated by dividing the number of lever presses during the CS by the total number of lever presses during both the CS and the pre-CS periods. These data were analyzed with one-way ANOVA. Significant main effects and interactions were further assessed by Holm-Sidak post-hoc tests.

2.7 Drugs

d-Amphetamine sulfate and *d*-methamphetamine HCl (Sigma-Aldrich; St. Louis, MO) were dissolved in sterile saline at concentrations of 0.5, 1.0, or 3.0 mg/ml and injected at a volume of 1 ml/kg. Dosages were calculated as the weight of the free base. Saline was injected at a volume of 1 ml/kg.

3. Results

Following ten days of instrumental training, rats exhibited high rates of lever pressing behavior during the final training session that ranged across groups from a minimum of 44.5 ± 3.44 to a maximum of 47.4 ± 4.42 responses/min. ANOVA revealed there were no significant group differences in these responses. Additionally, analysis of behavior during the reminder session that occurred prior to the PIT test revealed there were modest increases in lever pressing across groups (range: 51.0 ± 4.21 to 58.7 ± 5.18 responses/min), but there were no statistically significant treatment effects of injections given during the Pavlovian approach phase of training.

As shown in Fig. 1, all groups developed Pavlovian approach behavior over the course of training. For rats given saline injections after sessions 1, 3, and 5, a one-way ANOVA on trough entries during CS presentations revealed a significant main effect of session ($F_{5, 60} = 9.10, p < 0.001$). For rats given AMPH, a two-way ANOVA on these data revealed a significant main effect of session ($F_{5, 170} = 30.6, p < 0.001$); the main effect of dose and the session $\times$ dose interaction were not significant. For rats given METH, there was also a main effect of session ($F_{5, 155} = 24.7, p < 0.001$), a non-significant main effect of dose, and a non-significant session $\times$ dose interaction.

Analysis of the magnitude of conditioned behavior during the last compared to the first training session revealed a significant main effect of treatment group ($F_{6, 77} = 3.43, p < 0.01$). This was due to a significantly more trough entries during the CS for rats given 1 or 3 mg/kg AMPH following sessions 1, 3 and 5, compared to those given saline after these training sessions (Fig. 2). The lowest dose of AMPH and all three doses of METH were not significantly different from saline-injected controls.

During the transfer test, behavior was significantly modulated by the presentation of the CS (Fig. 3). Mixed factor ANOVA of the rate of lever pressing revealed a significant main effect of time period ($F_{1, 64} = 19.4, p < 0.001$) and a significant group $\times$ time period interaction ($F_{6, 64} = 4.00, p < 0.01$). As shown in Fig. 3A, rats in the saline group exhibited PIT, as evidenced by significantly more lever pressing during the CS compared to the pre-CS time period. This transfer effect was blocked in all the drug treatment groups, however. At most doses, there was instead a decrease in lever pressing during presentation of the CS. This decrease was statistically significant in rats given 3.0 mg/kg AMPH or 1.0 and 3.0 mg/kg METH. ANOVA of treatment effects on the PIT ratio, which was calculated by dividing the number of lever presses during the CS by the total number of lever presses during the CS and those during the pre-CS baseline, revealed a significant main effect of group ($F_{6, 63} = 4.83, p < 0.001$). Subsequent post-hoc tests indicated that AMPH doses of 0.5 and 3.0 mg/kg and METH doses of 1.0 and 3.0 mg/kg significantly reduced PIT ratios compared to saline-treated controls (Fig. 3B). These effects on lever pressing behavior did not appear to be related to significant changes in trough entries during the PIT test, as all groups increased trough entries during the three presentation of the CS (Fig. 4). ANOVA on these data revealed a significant main effect of time period for the number of trough entries/trial ($F_{1, 63} = 47.1, p < 0.001$) and the percentage of the total entries ($F_{1, 63} = 78.3, p < 0.001$). With both measures, there was a non-significant main effect of group and a non-significant group $\times$ time period interaction.

4. Discussion

In the experiments described here, we utilized a PIT paradigm to examine the ability of two psychostimulants, AMPH and METH, to alter the incentive-motivational value of a CS and, in turn, influence its ability to induce reward-seeking behavior. We found that both saline- and drug-treated groups increased conditioned responding (i.e., trough entries) during the Pavlovian approach phase of training. Moreover, administration of 1.0 and 3.0 mg/kg AMPH immediately after training sessions significantly increased the magnitude of this conditioned behavior. METH at all doses tested here was ineffective at altering Pavlovian approach behavior. However, both drugs reduced the transfer effect. At the 0.5 and 3.0 mg/kg doses of AMPH and the 1.0 and 3.0 mg/kg doses of METH, there were statistically significant reductions in lever pressing during CS presentations relative to the immediately preceding pre-CS periods. This effect was not due to a general disruption in lever pressing as we did not observe group differences in response rate during the instrumental reminder session that occurred between Pavlovian conditioning sessions and PIT testing. The reduced transfer also did not appear to be a reflection of response competition between instrumental and approach behavior, which has been shown to be a factor in previous studies of PIT [13,15]. Here, we found that trough entries increased when the CS was presented during the PIT test, but there were no significant group differences in the expression of this conditioned behavior.

The results of this study suggest that administration of AMPH and METH during memory consolidation of the CS-US association (i.e., immediately after Pavlovian conditioning sessions) interfered with the ability of the CS to induce a motivational state wherein the CS drives the execution of the instrumental action. Thus, rather than increasing the incentive motivational properties of the CS, the AMPH and METH treatment procedure we used here appeared to reduce cue-triggered “wanting” of the food reward. These effects on PIT seem to be different from those reported previously when AMPH exposure occurred following the completion of both Pavlovian conditioning and instrumental training [51]. In this study, rats were given daily injections of saline or 3 mg/kg AMPH for 6 days and underwent PIT testing 10 days following their last injection. The authors found that AMPH-exposed rats exhibited increased transfer relative to saline-exposed controls, which was argued to be reflective of an increase in cue-triggered wanting. Our results may not be at odds with this previous study, however, if a potential influence of response competition is considered. As noted above, the absence of group differences in Pavlovian approach behavior during the PIT test argues against an influence of competition between instrumental and approach behavior, but we cannot rule out an influence of other competing behaviors such as “sign-tracking”. If rats had increased behaviors directed towards the light CS, this likely would have decreased the expression of behaviors directed towards the trough (“goal-tracking”) and the lever. Inconsistent with this interpretation, however, is a recent report showing that pre-exposure to five daily injections of 2 mg/kg AMPH increased goal-tracking, rather than sign-tracking, in rats trained in a Pavlovian conditioning paradigm that was similar to the one used here [43]. Nevertheless, the results of the current experiment and those of Wyvell and Berridge [51] demonstrate that the timing of drug exposure relative to when CS-US associations are developed is critically important for how that exposure can modulate the eventual influence that the CS has on behavior.

The effects of drug administration on Pavlovian approach that we observed are consistent with previous studies showing that post-training session treatments with 1.0 or 2.0 mg/kg AMPH increase approach behavior compared to saline-treated controls [3,44]. Interestingly, we found that METH did not have this same effect. Thus, AMPH appears to be more potent at modulating associative learning processes, which is somewhat counter to the known difference in abuse liability for these two drugs. Though METH is generally considered to be the more potent form of AMPH [18,36], there is sparse evidence for this in the relatively small number of studies that directly compare the behavioral effects of low to moderate doses of these drugs. For

example, drug discrimination studies have shown that across a fairly wide dose range, neither rats [27] nor humans [28] are able to discriminate between the two drugs. Additionally, rats and rhesus monkeys that have been trained to self-administer AMPH and METH will take approximately equal amounts of the drugs [2,53]. Even when tested in an open-field chamber, animals given a single dose of 0.5, 1, 2, or 4 mg/kg AMPH or METH exhibit similar increases in locomotor activity [22,34,42]. The differences in potency that have been observed between these two drugs were primarily found at higher doses following binge-like exposure. For example, when 2.5 or 4 mg/kg AMPH and equimolar doses of METH were first given via 15 once-daily injections and later in “binge” treatments (once every 2 h) METH was shown to produce a prolonged “post-stereotypy” response compared to AMPH [40]. These high dose effects may be more relevant for the abuse liability of these drugs, which does appear to be greater with METH compared to AMPH [7].

The neural systems that mediate the PIT effect are diverse (reviewed in [25]), but a key role has been identified for dopamine and its receptors in the nucleus accumbens [12,23,30,52]. One contributing factor to the reductions in PIT we observed here may be a relative deficit in dopaminergic functioning in drug-treated rats. Even though there were only a total of three drug administrations, it is possible that the time course of repeated, intermittent administration of psychostimulant utilized here resulted in sensitization. Evidence for this lies in the fact that relatively few administrations, as few as a single dose of 5 mg/kg AMPH, are sufficient to elicit long-term behavioral and neurochemical sensitization [47]. Additionally, in the several days following AMPH sensitization there is evidence of decreased dopamine transmission within the dorsal striatum and, to a lesser extent, in the nucleus accumbens. As time passes, dopamine and its metabolites eventually increase and exceed their original baseline levels in both these brain regions [37]. In the present study, PIT testing occurred only 72 h after the final psychostimulant administration. It is possible that there was a relative depletion of dopamine in the accumbens due to the repeated AMPH- or METH-administration, particularly at the highest doses where we observed the greatest decreases in PIT. This relative depletion could interfere with PIT since dopamine transmission within the accumbens is critical for expression of PIT [30,38,52]. Although this hypothesis needs to be directly tested in future studies, it should be noted that the doses and the injection procedure used here resulted in considerably less AMPH exposure compared to the escalating, binge-like procedure of injecting 1–10 mg/kg AMPH over several weeks that has been shown to induce dopamine depletion after three days of withdrawal [37]. In fact, a study that used a similar dosing procedure as ours [41] reported no change in baseline levels of dopamine in the nucleus accumbens following 1–5 days of withdrawal from 2.5 or 3 mg/kg AMPH.

The mechanisms underlying the PIT interference effect we observed in AMPH- and METH-treated rats are not identifiable in the current study. Several theories have been offered to account for the general phenomenon of PIT, with each accounting for some but not all of the findings with this paradigm [25]. Two of these theories are the behavioral chaining account [46] and the associative-cybernetic model [1]. In the former, emphasis is placed on the ability of the previously conditioned CS to initiate the first step in the response chain that was learned during instrumental conditioning. In this conceptualization, presentation of the CS leads to trough entries that in turn activate a “trough entry-lever press” chain. Thus, according to the behavioral chaining account, the interference effect observed here may be due to drug-induced enhancement of the CS-US (i.e., tone-food pellet) association that inhibited the ability of the CS to activate the response chain. However, the finding that both AMPH and METH treatment reduced transfer, but only AMPH treatment increased Pavlovian approach, is inconsistent with this hypothesis. In the associate-cybernetic model, emphasis is placed on the representations that develop from stimulus-outcome (S-O) and response-outcome (R-O) conditioning. In the non-selective form of PIT used in the present study, a single outcome is represented by both the CS and the lever press response. The transfer effect is thus considered an expression of an

S-O-R chain that develops: activation of the outcome representation by presentation of the stimulus leads to expression of the response. According to this account, the effects of AMPH and METH on transfer may be due to drug-induced interference in the development of the S-O-R chain and/or activation of a single outcome representation by the CS. Our results do appear to be consistent with this hypothesis, but this account will have to be tested empirically in future studies.

In summary, the results presented here suggest that the incentive value of the CS is modified during the PIT test when psychostimulants are administered following Pavlovian approach training. In light of previous reports showing that pre-exposure to drugs of abuse such as AMPH can alter Pavlovian approach [3,44] and PIT [51], and because drug-associated cues play an important role in relapse [19], the PIT paradigm clearly has important implications for studies of cue-controlled behavior and its neural basis [5,25]. Our findings are consistent with the concept of drugs of abuse altering the PIT response, but further research is needed to clarify the processes that lead to drug-induced enhancement, compared to reduction, of reward-seeking behavior.

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Figure 1.

The effects of saline (**A**), amphetamine (**B**) or methamphetamine (**C**) on the development of Pavlovian approach behavior ($n = 10\text{--}13$ rats/group). Data are presented as a proportion of the total number of trough entries during the training session. For each group, mean trough entries during the 30-sec conditioned stimulus (CS) or the 30-sec period just prior to the CS (pre-CS) are plotted. Injections were administered immediately post-training on days 1, 3, and 5.

Figure 2.

The effects of saline, amphetamine (AMPH), or methamphetamine (METH) on the change in Pavlovian approach behavior across sessions ($n = 10\text{--}13$ rats/group). Data are presented as mean difference scores for CS trough entries on the last and first training sessions. CS trough entries were measured as the proportion of the total number of trough entries during each training session (as in Fig. 1). ** $p < 0.01$, compared to saline

Figure 3.

Activity on the PIT testing day ($n = 10/\text{group}$). **A.** The rate of lever pressing (number/trial) for the 30 sec pre-CS and CS periods. $*p < 0.05$ and $**p < 0.01$, compared to pre-CS time period in the same group. **B.** The transfer effect expressed as a ratio of lever-press responses during the CS compared to responses during both the CS and the pre-CS periods. $**p < 0.01$ and $***p < 0.001$, compared to saline-treated rats.

Figure 4. Trough entries during the PIT test ($n = 10/\text{group}$). Data are presented as the number of entries/trial (A) and as a proportion of the total number of trough entries during the training session (B) for the pre-CS and CS periods.