

Research report

The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine

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Abstract

A central premise of a number of theories of addiction is that discrete environmental stimuli repeatedly paired with drugs of abuse acquire incentive salience as a result of Pavlovian learning. There is, however, no unequivocal evidence supporting this assumption. Thus, we employed a Pavlovian conditioning procedure known to imbue non-drug reinforcers with incentive salience and extended it to study the effects of intravenous cocaine. Specifically, we examined whether a cue paired with intravenous cocaine administration would come to elicit approach towards it (sign-tracking), even if no behavioral response were required to receive the cue or drug. We found that when a cue was paired with intravenous cocaine delivery (but not when it was unpaired) rats came to approach and investigate the cue, and did so with increasing rapidity. We conclude that Pavlovian learning can imbue drug-paired cues with incentive salience, making them attractive and “wanted” stimuli. Delineating the neurobiological mechanisms responsible for this process will be important for understanding and treating drug addiction.

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1. Introduction

A number of theories of addiction assume that when otherwise neutral environmental stimuli are repeatedly paired with the administration of a potentially addictive drug, such stimuli come to acquire incentive salience via Pavlovian learning [1–4]. There is, however, no experimental evidence showing that Pavlovian pairing of a discrete conditioned stimulus (CS; cue) and a drug unconditioned stimulus (US) results in the attribution of incentive salience to the CS. As put recently by Everitt and Robbins [5, p. 1482], “it might logically be thought that Pavlovian approach is involved in maladaptively attracting humans toward sources of addictive drug reinforcers . . . as emphasized in the incentive salience theory of addiction. However, . . . approach to a CS predictive of a drug . . . has [not] been clearly demonstrated in laboratory studies . . . although . . . [it is] readily seen in animals responding for natural rewards. It may be . . . that the behavioral

influence of CSs associated with drugs and natural reinforcers differ fundamentally in this regard.”

Indeed, in most studies examining the motivational properties of drug-associated cues, the CS and US have not been paired in a Pavlovian manner, where both the CS and US are presented *independent of any action*. Rather, during either training or testing, cues have been presented in the context of an instrumental [self-administration] task, where the cue and/or drug are presented only after an action, which can then be reinforced [5,6]. It is typically assumed that in such instrumental settings cues acquire incentive salience through simple Pavlovian processes, but this may not be a valid assumption. Furthermore, whether drug conditioned place preferences are solely due to Pavlovian learning is debatable [7,8].

One behavioral phenomenon that powerfully demonstrates the ability of Pavlovian conditioning to imbue cues with incentive salience is termed “autoshaping”, or more appropriately, sign-tracking [9,10]. In this situation, a discrete cue is presented just prior to the delivery of a reward, usually food or water, and following repeated pairings animals begin to approach, and oftentimes attempt to consume the cue [11]. It is important to emphasize that no behavioral response is required for the animal

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to receive the reward in this situation; the animal is not reinforced for approaching and interacting with the cue. The reward is delivered no matter what the animal does, but it nevertheless begins to approach and engage the cue, and does so even if approach leads to reward omission or moves the animal away from the reward [12,13]. The question we address here is whether a cue paired with intravenous cocaine delivery in a Pavlovian manner (i.e., not contingent upon an action) can become a “motivational magnet” [14], eliciting approach (sign-tracking), as do cues paired with natural rewards. If drugs do not support sign-tracking, as has been suggested [15], we would be forced to reconsider many assumptions about the psychological mechanisms by which drug-associated stimuli acquire motivational value and the ability to influence behavior [5].

2. Materials and methods

2.1. Subjects

Twenty-four male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 225–250 g were housed individually in clear square plastic cages and were given 1-week acclimatization before any experimental manipulation. The rooms were temperature- and humidity-controlled and maintained on a reverse 14-h light/10-h dark cycle (lights off at 7:00 a.m.), with food and water available ad libitum. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

2.2. Apparatus

Behavioral testing was conducted in standard operant chambers (Med Associates Inc., Georgia, VT) with an acrylic hinged loading door, stainless steel side panels, and an acrylic back panel (22 cm × 18 cm × 13 cm). The chambers were located in sound- and light-attenuating cabinets equipped with fans providing constant ventilation. A white noise generator provided low-level background noise, and a red house light provided illumination. A lever that could be extended and retracted was located on one side panel of the chamber. When extended, the lever was ~3 cm above the floor. There was also a stimulus light located behind the lever, which illuminated the lever only when it was extended. An infusion pump was located outside of each chamber.

2.3. Surgical procedures

Rats were anesthetized with ketamine hydrochloride (75 mg/kg i.p.; Fort Dodge Animal Health, Fort Dodge, IA, USA) and xylazine hydrochloride (7.5 mg/kg i.p.; Ben Venue Laboratories, Bedford, OH, USA), and catheters were implanted into the rat's jugular vein. Catheter construction and implantation were based on previously described procedures [16,17]. Briefly, a silicone catheter was inserted into the right external jugular vein, which was passed subcutaneously to exit the back of the animal, where it was connected to a pedestal constructed from a 22 gauge cannula connected to a piece of polyethylene mesh using dental cement. Following surgery, catheters were flushed daily with 0.1 ml sterile saline containing gentamicin (0.08 mg/ml) to prevent occlusions and microbial buildup in the catheter. Both before and after conditioning, catheters were screened for patency by manually injecting 0.1 ml of the short-acting barbiturate sodium thiopental (i.v.; 20 mg/ml in sterile water). Rats that became ataxic within 5 s were considered to have patent catheters. Following surgery but prior to conditioning, three animals did not have patent catheters and were excluded from the experiment. No catheters lost patency during conditioning.

2.4. Conditioning

Pilot studies revealed that animals approached and contacted the illuminated lever at a high rate during the first few training sessions, independent of drug

administration, presumably because of its novelty. Thus, in order to decrease baseline responding, animals were first habituated to the presentation of the illuminated lever and sound of the infusion pump. Habituation sessions were initiated by activating the house light and white noise generator, both of which remained on throughout the session. Habituation sessions consisted of 30 individual trials in which the illuminated lever was extended for 8 s and the infusion pump activated for 2.8 s. The inter-trial interval varied randomly with a mean interval of 120 s. An entire session lasted approximately 1 h. After 2 days of habituation, the animals underwent catheter surgery, as described above. Following a 5–6-day recovery period, animals were again habituated to the presentation of the lever for another 3 days.

Kearns and Weiss [15] reported earlier that intravenous cocaine does not support Pavlovian conditioned approach towards a lever. In their study trials were scheduled to occur randomly, with an average inter-trial interval of 90 s. In pilot studies we also failed to observe sign-tracking using relatively short inter-trial intervals. Thus, in the present study we lengthened the inter-trial interval. Although we cannot be sure, we reasoned that short inter-trial intervals may obfuscate the ability of rats to form an association between presentation of the CS and drug administration. Unlike the consumption of a single food pellet, drugs have relatively long-lasting direct effects, and the neurobiological/interoceptive effects of cocaine endure for longer than 90 s. If the effects of a previous injection were still being experienced at the time of the next CS–US pairing, it may be difficult for rats to associate these events. Indeed, Kearns and Weiss [15] noted that their animals were engaged in cocaine-induced stereotypy, which suggests that the effects of consecutive doses of cocaine accumulated, as would be expected given the pharmacokinetics of cocaine.

Therefore, following habituation, animals were randomly divided into two groups. Animals from both groups were brought to the test chambers and connected to infusion lines. Sessions began with the activation of the red house light and white noise generator. Animals were then given eight trials, with a randomly varying inter-trial interval (mean of 900 s; each session lasted ~120 min). For one group (paired; $n = 11$), each lever presentation (lasting 8 s) was paired with a non-contingent intravenous infusion of 0.3 mg/kg of cocaine (weight of the salt, dissolved in 0.9% saline). The infusion pump was activated upon insertion of the lever, because of the delay involved with any injection, and the injection itself took 2.8 s. The second group (unpaired; $n = 10$) received non-contingent infusions of 0.3 mg/kg cocaine that were explicitly not paired with the presentation of the lever (in this group cocaine was administered 2 min after retraction of lever). The dose of cocaine was chosen because we have found that it supports robust self-administration behavior. Testing was conducted daily for 22 days and the 1st, 8th, 15th, and 22nd sessions were video recorded using a digital recording system.

2.5. Scoring

The video records were scored by visual observation by someone blind to treatment condition. An approach was scored when the nose of the rat came within ~1 cm of the lever during the 8 s period it was extended. The number of approaches per session was determined by counting the number of trials out of the eight CS presentations in which the animal approached the lever. In addition, the latency for the rat to approach the lever was recorded for each trial.

2.6. Statistics

Two questions were addressed statistically. First, to examine whether the number of approaches or latency changed across sessions, one-way mixed model ANOVA with day included in the model was performed on the paired and unpaired groups separately. Mixed model ANOVA is especially appropriate for analyzing data with repeated measures, when correlations among the measurements are likely, and allows for greater flexibility in modeling time effects than other repeated measures analyses [18]. A Satterthwaite approximation for the denominator degrees of freedom was used, producing decimal places in these values. Second, to investigate whether the paired group approached the illuminated lever on more trials or with a faster latency than the unpaired group, mixed model ANOVA was used with group and day included in the model. Planned t -tests for each of the four days of testing were used to examine if the groups differed on specific days of training.

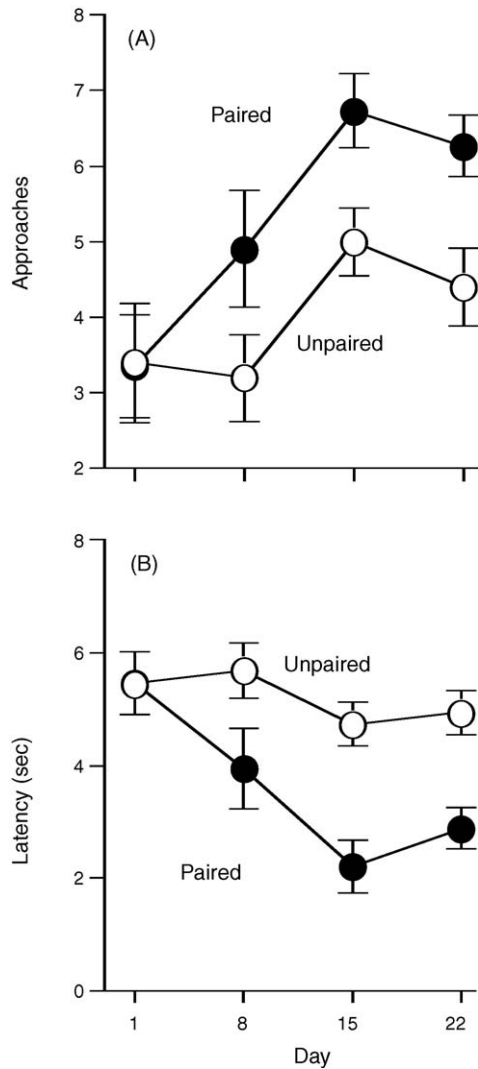


Fig. 1. Pavlovian conditioned approach (sign-tracking) to a cue (lever presentation) paired with intravenous cocaine administration. When the cue was paired with cocaine animals approached the cue more frequently (A) and more rapidly (B) than when cue and drug presentation were explicitly unpaired. The maximum approaches possible and the longest latency possible both equal 8. Data represent means \pm S.E.M.

3. Results

3.1. Approaches per session

The number of approaches per session is shown in Fig. 1A. The maximum number of approaches possible per session is eight, as there were eight lever presentations per session. One-way mixed model ANOVA revealed that in the paired group there was a significant increase in the number of approaches per session over days of training (main effect of day; $F_{(3,28,38)} = 6.09$; $p = 0.002$). There was a trend for the unpaired group to approach more often later in training, however, this did not reach statistical significance (main effect of day; $F_{(3,23,43)} = 2.49$; $p = 0.09$). Overall, the paired group approached the lever on significantly more trials than the unpaired group (main effect of

group; $F_{(1,23,84)} = 6.3$; $p = 0.02$). Although there was not a significant interaction between day and group (group \times day; $F_{(3,52,34)} = 1.28$; $p = 0.29$), the paired and unpaired groups did not differ on days 1 and 8, but did on days 15 and 22 (days 1 and 8 p 's > 0.05 ; days 15 and 22 p 's < 0.05).

3.2. Latency

The latency to approach the lever is shown in Fig. 1B. One-way mixed model ANOVA revealed that in the paired (but not unpaired) group there was a significant decrease in approach latency across days (paired: main effect of day; $F_{(3,28,88)} = 6.85$; $p < 0.001$; unpaired: $F_{(3,24,36)} = 1.08$; $p = 0.38$). Overall, the paired group approached the lever with a shorter latency than the unpaired group (main effect of group; $F_{(1,25,74)} = 13.09$; $p < 0.001$). Finally, there was a trend for a group and day interaction ($F_{(3,59,94)} = 2.49$; $p = 0.07$), and t -tests revealed that the two groups did not differ on days 1 ($t_{(19)} = 0.18$; $p = 0.986$) or 8 ($t_{(19)} = 1.98$; $p = 0.06$), but the paired group had a shorter latency to approach on days 15 ($t_{(19)} = 4.09$; $p < 0.001$) and 22 ($t_{(19)} = 3.88$; $p < 0.001$).

3.3. Qualitative nature of the approach response

The form of the conditioned response (CR) was determined by examination of the video records. In the paired group, approaches to the lever were characterized first by orientation to the lever and then, moving close to the lever, sniffing and seemingly inspecting it. However, the rats seldom directly contacted the lever with sufficient force to record a lever press. The number of lever contacts for both groups across the 22 days of training was very low (animals averaged 1.7 lever presses per session on day 22). If we had relied solely on lever presses as a measure of sign-tracking, as is often done when insertion of a lever serves as the predictive stimulus, we would have been misled.

In addition, the behavioral response to lever presentations on trials in which no approach was scored also differed between the paired and unpaired groups by the end of training. In the paired group, lever presentations almost invariably resulted in a startling or orienting response upon presentation of the illuminated lever, even if they did not approach to within 1 cm of the lever (93.8% of lever presentations in which an approach was not scored). This was seldom observed in the unpaired group (7.7% of the lever presentations).

4. Discussion

The phenomenon of sign-tracking, whereby a cue paired with the delivery of a non-drug reinforcer comes to elicit Pavlovian conditioned approach towards it, powerfully demonstrates that such cues are attributed with incentive salience. Here we asked whether a cue (presentation of an illuminated lever) paired with intravenous cocaine administration would acquire incentive salience, as indicated by approach, even if no behavioral response were required to receive drug or the cue. We found that when a cue was paired with intravenous cocaine delivery (but not when it was unpaired) rats came to approach it, and

did so with increasing rapidity. We established, therefore, that a drug-associated cue can acquire incentive salience leading to Pavlovian conditioned approach behavior.

We believe this is the first report demonstrating approach (sign-tracking) to a discrete Pavlovian CS paired with non-contingent intravenous drug delivery. There are reports that an orally consumed ethanol/saccharine [19,20] or amphetamine/saccharine [21] solution can produce sign-tracking, but in these cases it is unclear whether it is the drug or the sweet solution that engenders approach. Drug administration does support the development of a conditioned place preference (CPP), which is often interpreted as Pavlovian conditioned approach behavior. However, it is not clear that a CPP is only attributable to Pavlovian conditioning [7,8,22]. For example, on testing, whenever an animal enters a place previously paired with drug administration it may experience a conditioned positive affective state. It may then be reinforced for instrumental actions that keep it in that place, or bring it back to that place, because such actions produce the positive affective state. By this interpretation CPP does not provide evidence for Pavlovian-mediated incentive salience, but instrumental reinforcement. Of course, in CPP multiple psychological processes could be operative at the same time [22]. Similarly, it is well established that drug-associated cues can serve as conditioned reinforcers, supporting the acquisition of new actions [23], and reinstating operant responding following extinction [6,24]. But again, in these situations it is not clear the extent to which these behaviors are due to the incentive value of the cue, or whether the cue is acting as a positive reinforcer of instrumental actions. Does the cue elicit the *next* response as a Pavlovian incentive, “acting as a persistent goad to response generation” [2, p. 263], or does it reinforce (“stamp-in”) the *prior* response? Thus, we believe this is the first unequivocal demonstration that Pavlovian conditioning can imbue a drug-associated cue with incentive salience, eliciting approach towards it.

It is interesting that the form of the conditioned response in the present study was quite different from that seen when food or water have been used as the US. In Pavlovian conditioning the topography of the CR usually resembles the motor patterns elicited by the US itself [11]. For example, when food is used rats not only approach a lever CS, but they contact it, biting and gnawing it, seemingly trying to “eat” it, and when water is used rats lick the lever [25,26]. Here, rats approached the lever, sniffed at it, seemingly investigated it, but they rarely contacted it, and therefore, very few lever presses were recorded. This may be because with i.v. cocaine no actions are required to “consume” the US, as is usually the case with food or water. This idea is supported by a study in which water was delivered directly into a pigeon’s mouth via an intra-oral cannula, obviating the need for a consummatory behavioral response [27]. In this situation pigeons learned to approach a light paired with water delivery, but they did not contact the light nearly as frequently as when they were required to actively drink the water. Similarly, when lever presentation was paired with intracranial stimulation at sites that support electrical self-stimulation behavior, rats directed, “their behavior toward CS+ much more than their recorded contacts would suggest. On most presentations

the subjects moved very close to CS+, but sometimes contacted it only with their whiskers as they sniffed or explored near it.” [28, p. 1011]. Thus, the exact form of the CR depends on the requirements of the US, and whether it needs to be contacted to be consumed. In the case of USs that do not require a consummatory response, as in the case of i.v. drug administration or intracranial stimulation, the CR seems to consist primarily of approach and investigatory behavior.

In conclusion, a discrete stimulus that signals an intravenous injection of cocaine comes to elicit approach towards it (sign-tracking). This is consistent with the notion that Pavlovian learning can imbue drug-paired cues with incentive salience, such that they become “motivational magnets”, making them attractive and “wanted” [1,2,14]. In abstinence, such stimuli may be potent initiators of relapse. A number of theories of addiction have assumed that cues acquire incentive salience via Pavlovian learning, but the demonstration here that this actually occurs is not just important for theoretical reasons. Most of our knowledge concerning the neurobiology of drug-related incentive motivation and reward comes from studies that employ self-administration (instrumental) procedures, not Pavlovian procedures. In the former both the CS (cue) and US (drug) are presented contingent upon an action, but in the latter presentation of neither CS nor US are contingent upon an action. Although stimuli associated with drug self-administration certainly acquire incentive motivational properties it should not be assumed that this is reducible to simple Pavlovian conditioning. For example, in an instrumental setting an animal may come to believe its action causes delivery of both the cue and the reward, whereas in the Pavlovian situation it may come to believe that the cue causes delivery of the reward, two situations that could be quite different both psychologically and neurobiologically. Indeed, there is reason to believe that the cues paired with drug delivery in a Pavlovian manner have different effects than cues paired with drug delivery in an instrumental setting. Thomas et al. [29,30] reported that the presentation of a purely Pavlovian CS induces a different pattern of gene expression than presentation of a CS acquired in an instrumental task, and lesions have dissociable effects on behavior maintained by purely Pavlovian CSs versus CSs acquired in instrumental settings [31,32]. This is not just of esoteric theoretical interest, because in the “real world” of addicts environmental stimuli that acquire incentive value and induce relapse are typically present *prior* to consumption of a drug, and they are not elicited contingent upon the action that produces drug delivery. Thus, in exploring the neurobiology of addiction and relapse it will be critical to understand the psychological and neurobiological mechanisms by which Pavlovian conditioning confers incentive salience upon drug-associated stimuli, and whether these are the same or different as in a self-administration setting. Otherwise we are liable to develop a distorted view of the neurobiological and psychological processes underlying addiction and relapse in addicts.

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