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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.

Keywords: Pavlovian conditioning; Drug addiction; Conditioned stimuli; Autoshaping; Sign-tracking; Motivation

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, Ford 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 thiopenthal (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.