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Using conditioned suppression to investigate compulsive drug seeking in rats

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ABSTRACT

Background: Persistent drug seeking despite harmful consequences is a defining characteristic of addiction. Recent preclinical studies have demonstrated the occurrence of this hallmark feature of addictive behaviour in rodents. For example, it has been shown that the ability of an aversive conditioned stimulus (CS) to suppress cocaine seeking was diminished after an extended self-administration history. The present study aimed to optimize the experimental conditions to examine conditioned suppression of sucrose and cocaine seeking in rats, and its dependence on the longevity of self-administration experience.
Methods: We investigated whether conditioned suppression depends on the intensity and quantity of footshocks during conditioning. In addition, the effects of CS omission, extinction and reconditioning were investigated, as well as the influence of the CS interval sequence on conditioned suppression. We also compared conditioned suppression after a limited and extended sucrose or cocaine self-administration history.
Results: We found that conditioned suppression depended on the intensity rather than the quantity of footshocks, whereby a higher footshock intensity was necessary to induce suppression of cocaine seeking compared to sucrose seeking. Conditioned suppression was most pronounced when the test started with presentation of the aversive CS, and conditioned suppression could be extinguished and reacquired. In addition, conditioned suppression of cocaine, but not sucrose seeking was reduced after extended self-administration experience.
Conclusions: These data provide a detailed analysis of conditioned suppression of cocaine and sucrose seeking. Importantly, we confirm the usefulness of conditioned suppression to study persistent drug seeking after prolonged drug self-administration.

1. Introduction

Drug addiction is a chronic relapsing brain disorder, characterized by persistent drug-directed behaviour even with explicit knowledge of its negative consequences (American Psychiatric Association, 2000, 2013; Leshner, 1997; O'Brien and McLellan, 1996; Volkow and Li, 2004) . It has been estimated that 27 million people worldwide are addicted to illicit drugs, and 76 million suffer from alcohol use disorder (United Nations Office on Drugs and Crime, 2012; World Health Organization, 2004). Pharmacotherapies to treat addiction are limited in number and efficacy (Koob et al., 2009; O'Brien, 2008; Pierce et al., 2012; van den Brink, 2012). Therefore, a formidable challenge for addiction research is to unravel the neural mechanisms underlying addictive behaviour, in order to facilitate the development of novel treatments for this devastating disorder. In order to achieve this, it is essential to model the core features of addictive behaviour in animals.

Based on this idea, a number of animal studies have attempted to emulate aspects of genuine addiction-like behaviour, such as escalation of drug use, resistance to extinction, increased motivation for drugs and drug seeking despite adverse consequences (for reviews see Lesscher and Vanderschuren, 2012; Vanderschuren and Ahmed, 2013). Studies that examine continued drug use despite adverse consequences often employ punishment paradigms. For example,

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adulterating alcohol with the bitter tastant quinine is used as a punishment method in studies of alcohol addiction (Hopf et al., 2010; Lesscher et al., 2010; Wolffgramm, 1991). In other work, interoceptive aversion (e.g., histamine), post-ingestion illness (e.g., lithium chloride), or footshocks or shock-associated stimuli to punish drug seeking or taking are used (Deroche-Gamonet et al., 2004; Dickinson et al., 2002; Freeman et al., 2014; Kearns et al., 2002; Pelloux et al., 2007; Vanderschuren and Everitt, 2004). Recently, it has been shown that the ability of footshocks or shock-associated stimuli to suppress cocaine seeking is diminished after an extended cocaine self-administration history (Deroche-Gamonet et al., 2004; Jonkman et al., 2012; Pelloux et al., 2007; Vanderschuren and Everitt, 2004). Such persistent drug seeking behaviour after prolonged cocaine self-administration is thought to reflect the unflagging pursuit of drugs observed in human addicts (American Psychiatric Association, 2000, 2013; Leshner, 1997; O'Brien and McLellan, 1996; Volkow and Li, 2004). Therefore, we think that conditioned suppression of drug seeking is a useful way to study drug seeking behaviour despite adverse consequences. However, the conditions under which conditioned suppression of drug seeking can be observed remain incompletely understood.

To enhance our understanding of persistent drug seeking, the first aim of the present study was to provide an extensive parametric characterization of conditioned suppression of cocaine and sucrose seeking. To this end, we examined the effect of different footshock intensities and quantities during the acquisition of the conditioned stimulus (CS)-footshock association on conditioned suppression of cocaine and sucrose seeking. In addition, we studied the role of the CS-sequence presentation, CS extinction and re-acquisition. The second aim was to use the optimal experimental parameters to examine the extent to which the aversive CS suppressed seeking behaviour in rats with a limited or extended sucrose or cocaine self-administration history, respectively.

2. Methods

2.1. Animals

Male Wistar rats (Charles River, Sulzfeld, Germany) weighing 260–280 g at the time of arrival were individually housed in Macrolon cages (40 × 25 × 18 cm; $l \times w \times h$) in climate-controlled rooms (temperature 20–21 °C, 55 ± 15% relative humidity) under a reversed 12 h light-dark cycle (lights on at 19.00 h). Animals were allowed to habituate to the housing conditions for at least 9 days before surgery. Rats received 20 g chow (SDS) per day, which is sufficient to maintain body weight and growth. Water was available ad libitum. Self-administration sessions were carried out between 9 am and 6 pm, for 5–7 days a week. Experiments were approved by the Animal Ethics Committee of Utrecht University, and were conducted in agreement with Dutch legislation (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

2.2. Apparatus

All subjects were trained and tested in operant conditioning chambers (29.5 × 24 × 25 cm; $l \times w \times h$; Med Associates, Georgia, VT, USA). The chambers were placed in light- and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8 cm wide retractable levers, placed 11.7 cm apart and 6.0 cm from the grid floor. The assignment of the left and right lever as seeking and taking lever (see below) was counterbalanced across rats. A cue light (28 V, 100 mA) was present above each lever and a house light (28 V, 100 mA) was located on the opposite wall. A liquid dipper (0.04 ml) delivered sucrose solution to a recessed magazine situated between the levers. Cocaine infusions were controlled by a syringe pump placed on top of the cubicles. During the cocaine self-administration sessions, polyethylene tubing ran from the syringe placed in the syringe pump via a swivel to the cannula on the subjects' back; in the operant chamber tubing was shielded with a metal spring. Experimental events and data recording were controlled by procedures written in MedState Notation using MED-PC for Windows.

2.3. Surgery

Rats allocated to cocaine self-administration experiments were anaesthetised with ketamine hydrochloride (Narketan, 75 mg/kg, i.m.) and medetomidine hydrochloride (Cepetor, 0.4 mg/kg, s.c.) and supplemented with ketamine as needed. A single intravenous catheter was implanted into the right jugular vein aimed at the left vena cava. Catheters (Camcaths, Cambridge, UK) consisted of a 22 g cannula attached to silastic tubing (0.012 ID) and fixed to nylon mesh. The mesh end of the catheter was sutured subcutaneously (s.c.) on the dorsum. Carprofen (50 mg/kg, s.c.) was administrated once before and twice after surgery. Gentamycin (5 mg/kg, s.c.) was administered before surgery and for 5 days post-surgery. Animals were allowed 7–9 days to recover from surgery.

2.4. Behavioural procedures

2.4.1. Cocaine self-administration. Rats were trained to lever press for cocaine under a heterogeneous seeking–taking (ST) chain schedule of reinforcement (Olmstead et al., 2000; Vanderschuren and Everitt, 2004; Veeneman et al., 2012) with a random interval (RI) of 120 s on the seeking link (ST(RI-120)). Self-administration training started with the acquisition of the taking response under a fixed-ratio 1 (FR-1) schedule of reinforcement. During acquisition sessions, only the taking lever was present. Pressing this lever resulted in the infusion of 0.25 mg cocaine in 0.1 ml saline delivered over 5.6 s, the illumination of the cue light above the taking lever for 5.6 s, the retraction of the lever and the switching off of the house light. After a 20 s time-out period, the taking lever was reintroduced and the house light illuminated, signalling the start of a new cycle. Once animals had acquired cocaine self-administration, they were gradually introduced to the ST chain schedule, starting with a schedule with a RI requirement of 2 s on the seeking link. ST(RI)-sessions started with the introduction of the seeking lever and the illumination of the house light. The first press on the seeking lever initiated the RI and pressing this lever was without consequences until the RI had elapsed. When the RI had elapsed, pressing the seeking lever resulted in retraction of the seeking lever and insertion of the taking lever. Next, responding on the taking lever (under the FR-1 schedule of reinforcement) resulted in an infusion with cocaine, illumination of the cue light, retraction of the taking lever and the switching off of the house-light. This was followed by a 10 min time-out period to minimize the influence of cocaine-induced psychomotor effects on responding for the next infusion. After the time-out period, a new cycle started with the reintroduction of the seeking lever and the illumination of the house-light. When the rats had acquired the task under a RI of 2 s, the RI was progressively increased between sessions until animals had acquired the task under an RI of 120 s. The program automatically ended after 2 h or if animals had obtained 10 rewards, whichever occurred first. After each self-administration session, intravenous catheters were flushed with a gentamycin–heparin-saline solution to maintain the patency of the catheters. Priming infusions of cocaine to stimulate self-administration were never given.

2.4.2. Sucrose self-administration. Rats were trained to lever press for sucrose under a ST(RI-120) schedule of reinforcement. This procedure was similar to the ST(RI-120) with cocaine as the reward, with the following exceptions. After a response on the taking lever, 0.2 ml of a 20% sucrose solution was delivered by presenting the dipper five times for 5 s at a rate of one presentation per second. In addition, the session was terminated when 30 min had passed or when a maximum of 30 rewards had been obtained during the FR-1 training, or after 10 rewards during the RI training sessions.

2.4.3. Acquisition of the CS-shock association. Once stable responding under the ST(RI-120) schedule was achieved (i.e. when the mean number of seeking responses per minute of the last three training sessions of an individual rat did not exceed a difference of 10% of the overall mean of those three sessions), rats were assigned to groups that either underwent conditioning with CS-footshock pairings (CS-shock group) or underwent control conditioning (control group). Assignment to the groups was based on the mean seeking responses per minute and seeking latency of the three last training sessions prior to conditioning, so that CS-shock and control groups had equal mean response rates and seeking latencies.

Acquisition of the CS-shock association was established in operant chambers different from those where the rats had received training for self-administration of sucrose or cocaine. To facilitate CS-shock, rather than context-shock association, the animals were pre-exposed to the shock boxes for 30 min for 2 days. The CS-shock conditioning session comprised a lead-in period of 5 min followed by two periods of 10 min with a 85 dB, 2900 Hz tone (with an intertrial-interval of 10 min) during which 10 unpredictable, scrambled footshocks (1 s duration) were delivered (i.e., 20 shocks in total). The tone-shock association session ended with a lead-out period of 5 min. Rats in the control group were subjected to the same procedure but without the delivery of footshocks.

2.4.4. Conditioned suppression of sucrose- and cocaine-seeking behaviour. After conditioning, rats received 4 additional ST(RI-120) training sessions. Subsequently, a test session for conditioned suppression of sucrose- or cocaine-seeking behaviour was performed. This conditioned suppression test was conducted in the same operant chambers where rats received self-administration training. After a lead-in period of 2 min, the seeking lever was inserted for 14 min with the house light illuminated. Two-minute intervals in which the tone CS was presented (CS-ON interval) were alternated with two-minute intervals where the tone CS was absent (CS-OFF interval).