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Research article

The acquisition, extinction and spontaneous recovery of Pavlovian drug conditioning induced by post-trial dopaminergic stimulation/inhibition

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ABSTRACT

In contextual drug conditioning, the onset of the drug treatment is contiguous with the contextual cues. Evidence suggests that drug conditioning also can occur if there is a discontinuity between the onset of the drug effect and offset of the contextual cues. Here we examine whether post-trial contextual drug conditioning conforms to several Pavlovian conditioning tenets namely: acquisition, extinction and spontaneous recovery. Six groups of rats received apomorphine (0.05 or 2.0 mg/kg) and vehicle immediately or after a 15 min delay following a 5 min non-drug exposure to an open-field during three successive days (conditioning phase). The extinction phase occurred on days 4–8, in which all post-trial treatments were vehicle injections. After 2 days of non-testing, the final test was performed. The results showed that on the first test day, the activity levels of the 6 groups were statistically equivalent. On test day 2, there were marked differences in activity levels selectively between the two immediate post-trial apomorphine treatment groups. The immediate low dose apomorphine group displayed a reduction in activity and the immediate high dose group an increase in activity relative to their day 1 levels. The activity levels of both vehicle groups and both apomorphine delay groups remained equivalent to their day 1 activity levels. On test day 3, the differences in activity levels between the two immediate post-trial apomorphine groups increased but the activity levels of the vehicle groups and the 15 min delay post-trial apomorphine groups remained unchanged. In the extinction phase, the conditioned activity differences between the two immediate post-trial apomorphine groups were gradually eliminated. During the final test, the activity differences between the immediate post-trial apomorphine groups were partially restored, indicative of spontaneous recovery. These findings are consistent with several basic elements of Pavlovian conditioning and are supportive of drug induced trace conditioning.

1. Introduction

Conditioned drug effects are widely recognized features of a variety of centrally acting drugs and have been extensively studied using drugs with addictive potential. In preclinical experimentation, locomotor activation induced by psychostimulant drugs has been demonstrated in numerous reports to be a reliable treatment to induce conditioned drug effects (Beninger, 1983; Beninger and Herz, 1986; Borowsky and Kuhn, 1991; Mazurski and Beninger, 1991; Fontana et al., 1993; Heidbreder and Shippenberg, 1994; Mattingly et al., 1994; Carey and Gui, 1998; Bloise et al., 2007; Braga et al., 2009a; Braga et al., 2009b; de Matos et al., 2010; Dias et al., 2010; Filip et al., 2010).

Typically, in these studies, the psychostimulant drug is administered and the animal is subsequently placed into a habituated test environment so that the drug effect onset is associated with the test environment cues. In that, drugs such as amphetamine, cocaine and apomorphine have potent dopaminergic agonist activity, it is generally assumed that this dopamine activation evokes locomotor stimulation and incentivizes associated contextual stimuli such that the subsequent non-drug exposure to the context elicits dopamine like effects. Indeed, numerous studies have shown that increases/decreases in dopamine activity can have potent effects upon learning and memory by increasing/decreasing the reward and incentive value of associated stimuli (e.g. Beninger, 1991; Banasikowski and Beninger, 2012).

Pavlovian conditioning also can occur with a temporal CS/UCS discontinuity or gap between the termination of a conditioned stimulus (CS) and the onset of an unconditioned stimulus (UCS). In Pavlov's trace conditioning preparation, a punctate stimulus was used and the CS-UCS gap was brief, approximately a few seconds (Pavlov, 1927, 1928). In recent trace conditioning investigations using punctate stimuli as

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conditioned stimuli, the CS/UCS gap is of the order of 0.5 s and the central representation of the CS trace has been firmly connected to CNS activity in brain structures such as the hippocampus and frontal cortex (Siegel, 2014; Weiss et al., 1999). While trace conditioning has not been explored in drug conditioning, we have shown in several recent reports (de Mello Bastos et al., 2014; Santos et al., 2015) that administration of the dopaminergic drug apomorphine immediately after an animal has been removed from an open field can modify subsequent non-drug open field behavior. These findings are suggestive of drug induced trace conditioning.We have found that the direct acting D1/D2 agonist apomorphine is of particular interest for the study of the role of dopamine in psychostimulant drug conditioning and sensitization, in that this drug can have pronounced but opposite effects upon dopamine neurotransmission depending upon dose level. Using a low dose range in rats (< 0.1 mg/kg), apomorphine can induce a profound inhibition of movement presumably by preferential stimulation of dopamine auto-receptors and thereby inhibiting dopamine activity in the brain (Aghajanian and Bunney, 1973; Di Chiara et al., 1977; Missale et al., 1998). At higher dose levels (> 0.5 mg/kg), apomorphine increasingly stimulates dopamine post-synaptic receptors in addition to auto-receptors and is a potent behavioral stimulant generating hyper-locomotion (Mattingly et al., 1988a; Mattingly et al., 1988b; Rowlett et al., 1997).

In order to extend our observations of apomorphine induced post-trial effects, we undertook to evaluate whether these post-trial effects of apomorphine are consistent with Pavlovian conditioned drug effects. As a first step in this process, it is important to determine if the immediate post-trial apomorphine effects we have previously observed undergo extinction when the post-trial treatments are withdrawn and, in addition, following extinction if a non-exposure interval to the test cues results in a partial spontaneous recovery of the response. These determinations are of substantial importance in that extinction and spontaneous recovery are typical features of Pavlovian conditioning. In the present study, we used a brief 5 min exposure to a novel open-field environment as the contextual CS and subsequently administered 2.0 mg/kg apomorphine or 0.05 mg/kg apomorphine immediately post-trial to induce the conditioned response effects. We used the low and high dose treatments in order to generate opposite conditioned response effects. The rational for this strategy was to eliminate issues related to the post-trial injection procedure per-se in that our prediction based on the opposite behavioral impacts of these two dose level post-trial treatments was that the conditioned effects would be bidirectional. By using low and high doses of apomorphine, the conditioning expectation was that the conditioned responses would be in line with the behavioral response induced by the two dose levels of apomorphine (0.05 behavioral inhibition and 2.0 behavioral stimulation). Therefore, the conditioned responses induced by the low and high dose apomorphine post-trial treatments would be opposite (response inhibition/response stimulation). In addition, we wanted to determine if the conditioned stimulatory and inhibitory responses underwent similar patterns of acquisition, extinction and spontaneous recovery. We also included apomorphine groups given the same post-trial treatments but after a 15 min delay in order to control for drug exposure per-se and vehicle groups given vehicle treatments either immediately or 15 min post-trial in order to be able to assess changes in the baseline behavioral responses over the course of the experiment. The present report details the findings of this investigation.

2. Methods

2.1. Subjects

Male Wistar albino rats provided from the animal facility at CECAL/FIOCRUZ (Rio de Janeiro, Brazil), initially weighing 200–250 g, were housed in individual plastic cages (25 × 18 × 17 cm) until the end of the experiments. Food and water were freely available at all times. The vivarium was maintained at a constant temperature (22 + 2 °C), and a 12/12 h light/dark cycle (lights on at 07:00 h and off at 19:00 h). All experiments were carried out between 14:00 and 18:00 h. In order to mitigate possible stress factors linked to handling and injections, for 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. All experiments were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus and behavioral measurements

The behavioral measurements were conducted in a black open field chamber (60 × 60 × 45 cm). A closed-circuit camera (IKEGAMI, model ICD-49) mounted 60 cm above the arena was used to record behavioral data. Locomotion, measured as distance traveled (m), was automatically analyzed using EthoVision (Noldus, The Netherlands). The complete test procedure was conducted automatically without the presence of the experimenter in the test room. All behavioral testing was conducted under dim red light to avoid the possible aversive quality of white light and to enhance the contrast between the white subject and the dark background of the test chamber. Masking noise was provided by a fan located in the experimental room that was turned on immediately prior to placing the animal in the experimental arena and turned off upon removal of the animal from the experimental arena (i.e., test chamber).

2.3. Drugs

Apomorphine-HCl (Sigma, St. Louis, MO, USA) dissolved in 0.1% ascorbate/saline (2.0 mg/ml), was injected subcutaneously in the nape of the neck at a dose of 2.0 and 0.05 mg/kg. A 0.1% ascorbate/saline solution was used as vehicle for the apomorphine experiments. All doses were administered in a volume of 1.0 ml/kg body weight. Drug solutions were freshly prepared before each experiment.

2.4. Experimental procedure

The experiments were conducted following a modified protocol from Santos et al. (2015). The post-trial drug/vehicle injections were administered either immediately or after a 15 min delay. For both the immediate and delay post-trial groups, all rats received pre-trial vehicle injections immediately before being placed into the experimental arena for 5 min. This pre-test vehicle injection procedure was done in order to insure uniform handling prior to placement into the arena and to leave open the later possibility of introducing pre-test drug treatments. In addition, it allowed for an assessment of the role of injection linked drug conditioning in that the delay and immediate post-trial apomorphine groups would have received equivalent drug injections and therefore any conditioning linked specifically to the injection procedure itself would be the same for both groups. For the immediate post-trial groups, injections were made straight after removal from the test environment following completion of the test session (I-POST). For the immediate post-trial treatments, the rats were equally subdivided into three groups in which one group received vehicle (VEH-I-POST n = 7), a second group received apomorphine 0.05 mg/kg (APO 0.05-I-POST; n = 7) and a third group received apomorphine 2.0 mg/kg (APO2.0-I-POST; n = 7). The conditioning induction phase was conducted on three successive days (test days 1–3). On the following day the extinction phase was initiated during which all animals received vehicle injections post-trial. There were 5 extinction sessions of 5 min duration with one session conducted per day (test days 4–8). After completion of this extinction phase, the animals were not tested again until test day 11. On test day 11, all groups were tested for 30 min. The purpose of this test day was two-fold. One objective was to assess whether a spontaneous recovery of conditioning would occur following a non-test interval. The second objective was to assess whether the possible spontaneous recovery of conditioning was selective to the