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Partial extinction of a conditioned context enhances preference for elements previously associated with cocaine but not with chocolate

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HIGHLIGHTS

- Exposition to single elements of compound CSs increases CPP for those elements.
- Increased CPP was evident only for cocaine but not chocolate conditioned CSs.
- The findings have implication for learning theory in regard to extinction learning.

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ABSTRACT

Drug-associated stimuli are crucial to reinstatement of drug-seeking after periods of abstinence, representing a central problem in treatment of addiction. The present study investigated the influence of partial extinction of the conditioned context on the expression of conditioned place preference (CPP). Mice of the inbred DBA/2J strain were conditioned with cocaine or chocolate in a context identified by multiple elements (A + B) and subsequently CPP expression was evaluated in a context containing only one element (A or B) or both (A + B). Cocaine- and chocolate-conditioned mice showed CPP in presence of the original compound stimulus. However, cocaine-conditioned mice did not show CPP when tested in A or B context, while chocolate-conditioned mice did show CPP to single element context. After conditioning mice were exposed to extinction training of the context A or B and then tested for CPP 1 and 9 days after the end of the extinction (days 9 and 18). Cocaine-conditioned mice showed CPP 9 days after extinction while chocolate-conditioned mice were relatively insensitive to the extinction procedure on day 1 after extinction, but they did not show CPP for the partial or the original compound 9 days after extinction. Cocaine-conditioned mice not submitted to the extinction training (simple passage of time) or submitted to a Sham-extinction procedure (saline injections and confinement in a new environment) did not show CPP on day 9 or 18. Cocaine-conditioned mice exposed to extinction training showed increased c-Fos expression in several brain areas in comparison to mice exposed to Sham-extinction. The extinction procedure did not specifically reduce behavioral sensitization. The results suggest that extinction training involving only elements of a drug-associated context can result in increased associative strength of those elements.

1. Introduction

Drug addiction is a chronic disease, and relapse in drug seeking after long periods of drug withdrawal is considered one of the main features of drug addiction [1]. Environmental stimuli associated with drug intake have the power to induce drug craving in humans [2,3] and to provoke reinstatement of drug-seeking behavior in preclinical models [4,5].

Extinction of drug-associated cues has been proposed as a mean of reducing the motivational properties of cues to prevent relapse [6,7]. However, clinical studies using extinction therapy have reported little success [8], mainly attributed to the context dependence of extinction learning [9]. Indeed the renewal phenomenon is a clear problem for cue-exposure addiction treatment and it appears to be a strong candidate for explaining why extinction-based treatments so often fail [10] and efforts to the generalizability of the extinction learning both by pharmacological and psychological treatment have been recently made [11–13].

Context refers to a configuration of many different stimuli (including smells, physical environments, interceptive drug states, mood or hormonal states and time of the day) providing the background setting of

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learning [14]. In animal research such compound of contextual stimuli is capable of modulating the extinction and reinstatement of drug-seeking [15,5]. Moreover, contextual stimuli associated with psychostimulant drugs provide a more effective CS and gain more associative control over behavior than discrete stimuli [16].

The conditioned place preference paradigm (CPP) has numerous advantages for the studying of the role of contextual stimuli in the reward process, though preference measures are not a direct model of "addictive" behaviors. Indeed, CPP response is based on a learned association between the contextual conditioned stimuli and the rewarding properties of the unconditioned stimulus (US), which result in animals spending more time in that context due to the reward evoking properties of those contextual stimuli. Unless specifically modified [17,18], a two-compartment CPP procedure is a context-conditioning, where multiple aspects of the environment (including visual, tactile, spatial, and olfactory features) in which drug is experienced enter into association with the drug. Thus, preference expressed in the absence of the drug represents both a conditioned "wanting" of the reward, but also a measure of the stamping-in of stimuli–reward associations. Moreover, preference for the conditioned compartment can be reinstated by addictive drugs following extinction [19].

We have previously reported that mice of the inbred strain DBA2/J (DBA) show high liability to prime-induced reinstatement of extinguished CPP after conditioning with a low cocaine dose and long withdrawal [20]. In the present study we further investigated the phenotype of DBA mice by manipulating the drug-associated contexts after the cocaine conditioning. Context was manipulated by extinction training of partial stimuli following the initial compound conditioning with tactile and visual stimuli. We also extended this investigation of the context role on expression of CPP when compound conditioning was with a natural reward. Finally, the neural substrate of CPP expression after context manipulation was investigated by quantification of c-Fos immunostaining.

2. Materials and methods

A total of 112 male mice of the inbred DBA/2JICo (DBA) strain (Charles River Laboratories, Italy) were purchased at 6 weeks of age and housed four to a cage on a 12-h light–dark cycle (lights on at 7:00 A.M.) for 2 weeks before behavioral testing. Experiments were carried out in accordance with the Italian national law (DL 116/92) on the use of animals in research.

2.1. Apparatus

All behavioral testing was performed in four identical boxes formed by two gray lateral polyvinyl chloride (PVC) chambers (15 × 15 × 20 cm) connected by a central alley (15 × 5 × 20 cm). Two sliding doors (4 × 4 cm) connected the alley to the chambers. This apparatus was originally designed to avoid biased preference for any of the chambers [21]. However, in the present study each chamber was differentiated by two compound stimuli: 2 different floor textures (transparent Plexiglas) constituted the A stimuli, and two three-dimensional patterns with triangular bases (5 × 5 × 20 cm) made of black PVC and arranged to shape one of the chamber walls (covering the same pavement surface and not impairing the exploration) constituted the B stimuli. Different AB combinations with the two chambers were counterbalanced across subjects. In all experiments conditioning training was made to the AB compound.

Behavioral data were collected and analyzed by "Etho Vision" (Noldus Information Technology, Wageningen, The Netherlands), a fully automated video tracking system. The acquired digital signal was then processed by the software to extract the "time spent" (in seconds) and the "distance moved" (in centimeters) in the three compartments of the apparatus.

2.2. Conditioned place preference

The behavioral procedure was previously described [20]. Briefly, on day 1 all mice were free to explore the entire apparatus for 20 min and time spent in the two chambers and the central corridor was recorded and analyzed (pretest). Subjects' assignment to groups in each experiment was counterbalanced such that no bias was shown (time spent in compartment by all mice: unpaired 460.4 ± 13.8; center 286.9 ± 6.8; paired 452.7 ± 11.5). On the following 8 days (conditioning phase), mice were injected and confined daily for 40 min alternatively in one of the two chambers. For each animal, during the conditioning phase, one of the patterns was consistently paired with a saline injection and the other one with a drug injection. Pairings were balanced so that for half of the animals in each experimental group, the drug was paired with one chamber and the other half with the other chamber. On days 1, 3, 5, and 7, all animals received injections of cocaine immediately before starting the conditioning session and on the other days received saline injections immediately before starting the conditioning session. In the present study a modification was added, so that during the training phase the two chambers were identified by a specific AB compound stimulus, consistently paired with a saline or cocaine injection, while in Experiment 2 the two chambers were paired with regular food or milk chocolate (Lindt). In all experiments conditioning training was always made to the AB compound. Testing was conducted on day 10 in drug-free state (or without food/chocolate in Experiment 2) and lasted 20 min similar to the pretest. However, for some mice one element of the conditioned AB compound was removed during the testing phase and never replaced in the following phases, so the animals were not re-exposed to the original AB compound stimulus.

After conditioning and the initial CPP test (TEST), mice were given extinction training by pairing saline (Experiments 1, 3, 4 and 5) or regular food (Experiment 2) with each of the two compartments, once per day over 8 days, similarly to the conditioning training. Extinction training started the day after the CPP test. Mice received extinction training in presence of the same A or B stimuli to which they were previously exposed in the CPP test, thus for example, animals tested for CPP in presence of the only A stimuli received extinction training in presence of the same stimuli. Preference for the originally conditioned chamber was re-evaluated by CPP testing the day after the last saline or food pairing (9 days after the first CPP test, day 9), and once again one week later (18 days after the first CPP test, day 18). In all experiments mice were left undisturbed in the colony room during the week between day 9 and day 18 testing. In Experiments 4 and 5 the extinction training was compared to Sham-extinction condition and mice were injected daily with saline for 8 days between the time points TEST and day 9 (see later description).

In Experiment 2 reinstatement of the original conditioned preference was investigated by an additional test the day after the day 18 test, by replacing into the chambers the original conditioned AB compound stimuli. Such test was not done in the previous experiment, as the increased preference at day 18 would have masked any reinstating effect of the original conditioned compound stimulus. The general timeline diagram of behavioral procedure is illustrated in Fig. 1.

2.3. Tissue preparation

Mice were killed by decapitation 40 min after the end of behavioral testing. Brains were immersion-fixed overnight in 4% paraformaldehyde (4 °C) and cryoprotected in 30% sucrose (4 °C) until they sank (~48 h), then they were frozen with dry ice and cut into 40 μm transversal sections with a sliding microtome. Two adjacent series of sections were collected starting from −1.46 to 1.96 from bregma according to Mouse Brain Atlas [22]. One series was immunostained for Fos protein while the other one was Nissl-stained.