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A protein synthesis inhibitor, cycloheximide does not alter cue-induced reinstatement of saccharin seeking

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ABSTRACT

A large body of evidence indicates that reactivation of aversive memories leads to protein synthesis-dependent memory reconsolidation which can be disrupted by cycloheximide and other protein synthesis inhibitors. The aim of the present study was to investigate whether cycloheximide would alter reconsolidation of the associations involving discrete cues paired with a sweet reward in an appetitive instrumental task. Rats trained to lever press for 0.1% saccharin were repeatedly tested for cue-induced reinstatement of non-reinforced responding for saccharin. CHX (3 mg/kg, s.c.) or its vehicle was injected immediately after each reinstatement session. The protein synthesis inhibitor did not alter the ability of the saccharin-paired cues to reinstate saccharin seeking. The present results suggest that passive re-exposure to saccharin-paired discrete cues in the reinstatement procedure does not lead to any cycloheximide-sensitive reconsolidation of the original associations.

1. Introduction

It is a common observation that passive exposure of drug addicts to discrete drug-associated cues can evoke an intense craving and increase the risk of relapse to compulsive drug-seeking behavior (Drummond, 2000; Rohsenow et al., 2001; Sinha and Li, 2007). A similar picture emerged from studies on reactivity to food-associated cues in patients with eating disorders (Bulik et al., 1998; Jansen, 1998; Sobik et al., 2005). Therefore, understanding molecular mechanisms responsible for long-term associations between environmental cues and instrumental responses for drugs (or foods) may have broad implications for clinical practice. On a theoretical ground, amnestic agents, like protein synthesis inhibitors, could be considered as a new class of therapeutics for those psychiatric disorders in which addiction-like patterns of compulsive behavior are induced by discrete environmental cues (Jansen, 1998; Lee et al., 2006; Sinha and Li, 2007). More specifically, protein synthesis inhibitors could be considered as add-on medications to treatment approaches based on the cue exposure paradigm (Jansen, 1998; Rohsenow et al., 2001).

It has been suggested that a brief re-exposure to the original training context or discrete cues in the absence of an unconditioned stimulus or a reinforcer is associated with two opposing processes. One is the extinction of previously learned Pavlovian or instrumental conditioning and the other is termed reconsolidation (Bouton, 2002; Nader, 2003). During reconsolidation the original memory becomes labile and transiently sensitive to disruptive effects of amnestic agents (Nader, 2003; Dudai, 2004; Pedreira and Maldonado, 2003).

It has been repeatedly reported that acquisition, extinction, and reconsolidation of aversive Pavlovian conditioning depends on *de novo* protein synthesis in the brain (Lin et al., 2003; Nader, 2003; Dudai, 2004; Santini et al., 2004; Suzuki et al., 2004; Power et al., 2006). In contrast, little is known about the role of new protein synthesis in appetitive instrumental conditioning. Recently, we have shown that repeated post-session injections of a protein synthesis inhibitor, cycloheximide (CHX; Quinton and Kramarcy, 1977) blocked acquisition of cocaine self-administration but not extinction of non-reinforced responding for cocaine in the cocaine-associated context (Mierzejewski et al., 2006). In control experiments, the same dose of CHX (3 mg/kg) impaired acquisition of aversive Pavlovian conditioning and totally abolished acute c-Fos protein accumulation in the

Abbreviations: ANOVA, analysis of variance; CHX, cycloheximide; FR, fixed ratio; RT, random time.

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somatosensory cortex taken from rats exposed to foot-shock stress. Our findings can indicate that acquisition of cocaine self-administration, but not extinction of cocaine seeking in the cocaine-associated context, depends on new protein synthesis (Mierzejewski et al., 2006).

A possible role of *de novo* protein synthesis in reconsolidation of instrumental conditioning induced by a brief re-exposure to discrete environmental cues has not been studied as yet. Given the above, the aim of the present study was to assess whether repeated post-session CHX administration could alter the magnitude of cue-induced reinstatement of saccharin seeking. We used a simple model of appetitive instrumental conditioning in which responding on one of two levers led to a presentation of 0.1% saccharin accompanied by discrete visual and auditory cues. Rats which learned to work for the sweet reward were repeatedly tested for relapse to saccharin seeking in the within-session reinstatement procedure (for details, see Bienkowski et al., 2000; Maccioni et al., 2007). Saccharin seeking was reinstated by the non-contingent presentation of the saccharin-paired visual and auditory cues. CHX was administered immediately after each reinstatement session, i.e. after each re-exposure to the discrete cues. We hypothesized that post-session CHX administration would impair reconsolidation of the original associations involving the discrete cues and abolish the subsequent ability of these cues to reinstate saccharin seeking.

2. Methods

2.1. Subjects

Sixteen male Wistar rats (Medical Research Center, Polish Academy of Sciences, Warsaw, Poland) weighing 410–470 g at the beginning of the study were housed two per standard plastic cage in a room with controlled environmental conditions (temperature: 22±1 °C, ~60% humidity, and a 12-h light–dark cycle, lights on at 6 a.m.). Unless otherwise stated, standard lab chow (Labofeed H, WPiK, Kcynia, Poland) and tap water was available *ad libitum*. The animals were handled at least twice a week both before and after the start of behavioral procedures. The treatment of the rats in the present study was in full accordance with the ethical standards laid down in respective European (directive no. 86/609/EEC) and Polish regulations. All experimental procedures were reviewed and approved by the Ethics Committee for Animal Studies of the Warsaw Medical University.

2.2. Apparatus

Instrumental responding for 0.1% (w/v) saccharin (saccharin sodium salt hydrate; Sigma-Aldrich, Poznan, Poland) was studied in eight identical chambers (Coulbourn, Inc., Allentown, PA, USA). The chambers consisted of stainless-steel test cages enclosed within sound-attenuating cubicles with fans for ventilation and background white noise (for details, see Bienkowski et al., 2000). A white house light was centered near the top of the front wall of the cage. The test cage was also equipped with two response levers separated by a liquid delivery system (the liquid dipper; E14-05, Coulbourn). The liquid dipper consisted of an arm ending in a 0.1-ml stainless-steel cup, an electric engine to raise the arm, and a 100-ml fluid reservoir located outside the test cage. The liquid dipper and the response levers were mounted on the front wall of the cage, 4 cm above a stainless-steel grid floor. Only one lever ("active") activated the liquid dipper. Presses on the other lever ("inactive") were recorded but not reinforced. The location of the "active" lever (left or right) was randomized across all the subjects. Programming of every experimental session as well as data recording was made using the L2T2 Software package (Coulbourn) running on an IBM-compatible PC.

2.3. Saccharin self-administration

The rats were trained to lever press for saccharin according to the procedure described previously by Bienkowski et al. (2000). The start of each experimental session was signaled by turning the house light on. The dipper presented 0.1% saccharin in a volume of 0.1-ml for 5 s and provided a set of discrete saccharin-associated cues, including a brief audible click and white stimulus light (4 W) located inside the liquid delivery system. The stimulus light was on during the entire 5-s dipper activation. After that time, the liquid dipper and its stimulus light were switched off. Self-administration sessions lasted 30 min and one session was given each day between 1 and 5 p.m. During the first 3 days of training, the rats were deprived of water for 22 h/day and shaped to lever press for saccharin according to a fixed ratio 1 (FR1) schedule of reinforcement. As soon as lever pressing was established, tap water became freely available in the home cages. Thus, except for the initial phase of training, the rats were never deprived of food or water. The animals were allowed to stabilize their instrumental responding for saccharin over the next 12 days (a self-administration phase). The mean (±S.E.) number of responses on the "active" lever in the last three days of saccharin self-administration was 94.5±10.5 responses/30 min. On average, the rats received 753 pairing of the discrete cues with the saccharin reward in the self-administration phase. In the whole study, the mean number of "inactive" lever presses was marginal (<2 responses/session) and was not analyzed further.

On the final day of the self-administration phase, the animals were randomly assigned to one of two experimental groups: a saline- and CHX-treated group (N=8 rats per group). The two groups did not differ in terms of saccharin self-administration ($p>0.6$). On the next day, reinstatement sessions started.

2.4. Effects of CHX on cue-induced reinstatement of saccharin seeking

The within-session procedure (Bienkowski et al., 2000; Shaham et al., 2003; Maccioni et al., 2007) was used to study relapse to saccharin seeking induced by the discrete saccharin-paired cues. The experimental session lasted 70 min, consisting of a 60-min extinction phase and a 10-min reinstatement phase. In the extinction phase, the liquid delivery system was off and lever pressing had no consequences. As expected, extinction of non-reinforced lever pressing for saccharin was rapid and responding during the last 30 min of the extinction phase (min 31–60) was close to zero (Fig. 1).

In the reinstatement phase (min 61–70), a set of discrete cues was repeatedly delivered (15×7.5 s) according to a random time 15 s schedule (RT15 s). The set of discrete cues included an audible click associated with elevation of the liquid dipper arm and illumination of the stimulus light. Saccharin was not available and the reservoir was filled with tap water. To avoid superstitious behavior, each next stimulus presentation was postponed until 3 s had elapsed without any lever pressing. Following fifteen non-contingent presentations of the discrete cues, extinction conditions were maintained until the end of the reinstatement phase (for details, see Bienkowski et al., 2000; Maccioni et al., 2007).

There were five reinstatement sessions conducted every fifth day. CHX (3 mg/kg; Sigma-Aldrich, Poznan, Poland) or its vehicle (0.9% NaCl; Kabi Fresenius, Kutno, Poland) was injected s.c. in a volume of 1 ml/kg immediately after reinstatement sessions 1–4. The protocol of repeated injections of CHX employed in the present study followed that used in our study on cocaine self-administration (Mierzejewski et al., 2006). One rat treated with CHX died for unknown reasons after the first injection and thus the CHX-treated group consisted of seven animals.

2.5. Control experiments — effects of CHX on locomotor activity and saccharin consumption

CHX produces non-specific adverse effects (Davis and Squire, 1984), which can interfere with instrumental behavior. Thus, saccharin consumption in a two-bottle choice test and open field locomotor activity was assessed on the day of the last reinstatement session, i.e. five days after the last (fourth) injection of CHX.