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D-Cycloserine Facilitates Extinction of Conditioned Alcohol-Seeking Behaviour in Rats

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Abstract — Aims: The aim of the present study was to examine the influence of partial NMDA receptor agonist D-cycloserine (DCS) on the extinction of conditioned alcohol-seeking behaviour. **Methods:** For this purpose, rats were administered with DCS 60 min before each successive extinction session. Resumption of alcohol responding was carried out 1 day after the final extinction session. **Results:** We demonstrate that treatment of rats with 5 mg/kg of DCS was capable in facilitating extinction of conditioned alcohol-seeking behaviour, which subsequently reduced the resumption of extinguished operant responding. **Conclusions:** Our study suggests a good rationale for the development of new add-on medications for exposure-based psychotherapy so as to extinguish drug-conditioned appetitive memories in alcohol-dependent patients.

INTRODUCTION

Exposure to conditioned drug stimuli, i.e. extinction-based training, is used as a therapy in drug addicts to reduce drug seeking and relapse. It is suggested that extinction training inhibits conditioned responses through the learning of new contextual relationships (Bouton, 2002). Based on the knowledge that glutamatergic systems are critically involved in learning and re-learning processes, it was hypothesized that drugs that increase NMDA-receptor function might facilitate the extinction of learned conditioned responses. In fact, preclinical studies have shown that D-cycloserine (DCS)—an agonistic compound acting at the glycine-binding site of the NMDA receptor—facilitates extinction of learned fear (Walker *et al*., 2002; Mao *et al.*, 2006; Woods and Bouton, 2006; Norberg *et al*., 2008), a finding which has also been translated to humans, showing that DCS facilitates exposure-based psychotherapy as well (Davis *et al*., 2006).

These findings demonstrate a crucial role for NMDA receptors in the extinction of conditioned aversive memories. However, similar conclusion might be drawn from studies on extinction of conditioned reward-related memories, as DCS accelerates extinction of conditioned place preference induced by psychostimulants (Botreau *et al*., 2006; Sakurai *et al*., 2007; Tzschentke, 2007). The aim of the present study was to examine the influence of DCS on the extinction of conditioned alcohol-seeking behaviour under operant conditions and to study subsequently the resumption of alcohol-taking behaviour.

MATERIALS AND METHODS

All experimental procedures were approved by the Committee on Animal Care and Use and carried out in accordance with the local Animal Welfare Act and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

All experiments were carried out in eight operant chambers (MED Associates Inc., St Albans, VT, USA) enclosed in ventilated sound-attenuating cubicles. The chambers were equipped with a response lever on each side panel of the chamber. Responses at the appropriate lever activated a syringe pump that delivered an ~30-μl drop of fluid into a liquid receptacle next to it. A light stimulus (house light) was mounted above the right response lever and a loudspeaker (65 dB, 'beep') was positioned above the left response lever of the self-administration chamber. All animal training and extinction sessions were performed during the active dark phase.

Eight-week-old Wistar rats (Charles River, Sulfeld, Germany) were trained to self-administer 10% (v/v) ethanol in daily 30-min sessions using a fixed-ratio 1 (FR 1) schedule preceded by a saccharine-fading procedure. Throughout the training phase, responses at the opposite lever resulted in the delivery of a drop of water. The purpose of the conditioning phase was to train the animals to discriminate between the availability of ethanol (reward) and water (non-reward). Note that non-reward sessions were included in the study in order to see the selectivity of DCS towards the extinction of ethanol related stimuli. The conditioning phase started after the completion of the training phase. Discriminative stimuli predicting 10% ethanol or water availability was presented during each ethanol or water self-administration session (one 30-min session/day). During these sessions, the opposite lever was inactive (i.e. the presses on this lever did not have any consequences). An orange flavour extract served as the S+ for ethanol, whereas water availability was signalled by an anise extract (S−). These olfactory stimuli were generated by depositing six drops of the respective extract into the bedding of the operant chamber before each session. In addition, each lever press resulting in alcohol delivery was accompanied by a 5-s auditory stimulus ('beep', CS+), whereas a 5-s light stimulus (CS−) was presented with water delivery. The 5-s period served as a 'time-out', during which responses were recorded but not reinforced. Alcohol and water sessions were conducted in a random manner until the animals received a total of 10 alcohol and 10 water sessions. After completing the conditioning phase, the rats were subjected to daily 30-min extinction sessions for 12 consecutive days. Extinction sessions began by extending the

levers and the presentation of olfactory discriminative stimuli. In total, six S+ and six S− extinction sessions were performed in a random order. Responses at the previously active or inactive lever activated the syringe pump, without resulting in the delivery of either alcohol or water but with the presentation of response-contingent cues (CS+ or CS−).

To test the effect of DCS, animals were divided into groups on the basis of their performance during the last four conditioning sessions. One group of animals was injected IP with vehicle (0.9% saline), while the other received 5 mg/kg of DCS (note: the dose of DCS was chosen based on our pilot studies, showing that higher doses of DCS, namely 15 mg/kg and 30 mg/kg, do not influence lever responding in the present experimental conditions) (data not shown). Drug administration was performed 60 min before each S+/CS+ and each S−/CS− extinction session.

As an addendum to extinction in order to study the influence of extinction on relapse-like behaviour, resumption of ethanol responding (Krank and Wall, 1990; Chiamulera *et al.*, 1995; Sanchis-Segura and Spanagel, 2006) was carried out 1 day after the final extinction session. In this test, S+ or S− condition was presented, in addition to ∼100 μl of ethanol or water given to the liquid receptacle. This served as an olfactory/gustatory cue initiating lever responding followed by the presentation of the CS+ or CS−.

Data obtained from last conditioning, extinction experiments were analysed by use of a three-way analysis of variance (ANOVA) with repeated measures [factors were: treatment group, lever (active versus inactive) and session]; resumption of lever-pressing behaviour was analysed by a two-way ANOVA [factors were treatment group and lever (active versus inactive)]. Whenever significant differences were found, *post hoc* Student–Newman–Keul's tests were performed. The chosen level of significance was $P < 0.05$.

RESULTS

Following the conditioning phase, lever-pressing behaviour was extinguished by presenting the S+/CS+ and the S−/CS− condition, which did not result in the delivery of either ethanol or water. Thus, at the end of the conditioning phase, rats exhibited 141 ± 14 rewarded lever presses (S+/CS+ condition) and 30 ± 3 non-rewarded lever presses (S−/CS− condition). To test the effect of DCS, animals were divided into groups on the basis of their performance during the last four conditioning sessions (factor treatment group: $P = 0.7$). In the vehicle treated animal group lever presses for the previously reinforced lever dropped down to 34 ± 5 (S+/CS+ condition) and to 19 ± 3 (S−/CS− condition) already on the first extinction day. The number of operant responses during S+/CS+ sessions progressively faded away across six extinction sessions [factor session: $F(5, 180) = 3.0$, $P = 0.01$]. This reduction was mainly seen in the vehicle-treated animal group (Fig. 1A). Thus, lever presses for the previously reinforced lever dropped down from 34 ± 5 to 21 ± 3 in this animal group. DCS treatment reduced lever responses already during the first extinction session, while additional five extinction sessions did not considerably modified operant responding. Thus, animals exhibited 21 ± 6 lever presses during the first extinction session which dropped to 15 ± 2 lever presses during the last extinction session (Fig. 1A). A three-way ANOVA displayed a significant difference in operant responding between vehicle- and DCS-treated animal groups [factor treatment group: $F(1, 36) = 15.4$, $P < 0.001$], showing that the treatment of rats with DCS was capable in facilitating extinction of conditioned alcohol-seeking behaviour. The detailed analysis of the lever responding revealed that lever pressing stopped within the first 15 min in both vehicle and DCS treatment groups, whereas DCS-treated animals exhibited lower number of lever presses during each 5-min bin. Lever responding during S−/CS− extinction sessions was only moderately modified by the administration of DCS (factor session: $P = 0.6$); however, a three-way ANOVA has shown the general effect of DCS on lever responding throughout six extinction sessions [factor treatment group: $F(1, 36) = 7.3$, $P < 0.01$] (Fig. 1B).

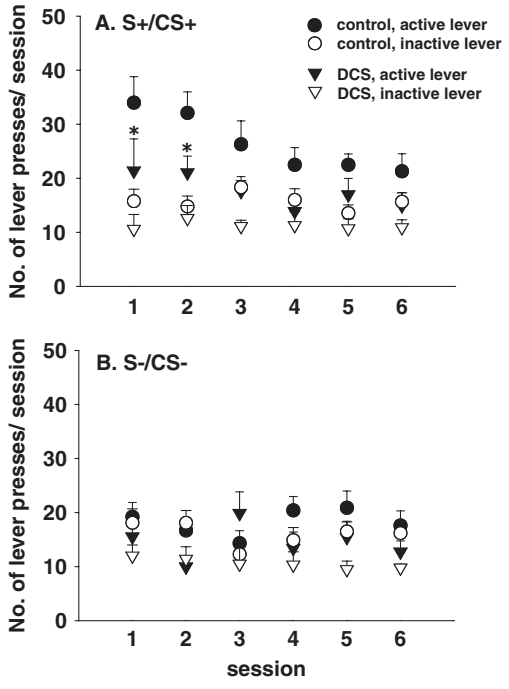

Fig. 1. The effect of vehicle ($n = 10$) and 5 mg/kg of D-cycloserine (DCS) ($n = 10$) on extinction of lever-pressing behaviour under both S+/CS+ (**A**) and S−/CS− (**B**) conditions. Drug administration was performed 60 min before each S+/CS+ and each S−/CS− extinction session. Data are shown as the average number of lever presses on alcohol-associated (S+/CS+ sessions) and water-associated (S−/CS− sessions) levers and on the inactive lever during each extinction session. Data are presented as means ± SEM. *Significant differences from the vehicle control group, $P < 0.05$.

Contrary to the relatively selective effect of DCS treatment for the S+/CS+ condition during the extinction sessions, the following resumption of lever-pressing behaviour was significantly reduced under both S+/CS+ and S−/CS− conditions [factor treatment group: $F(1, 36) = 4.7$, $P < 0.05$ and $F(1, 36) = 8.3$, $P < 0.01$, for the S+/CS+ and S−/CS− condition, respectively] (Fig. 2).

DISCUSSION

In the present study, we show that a low dose of DCS facilitates extinction of alcohol-seeking behaviour. Furthermore,

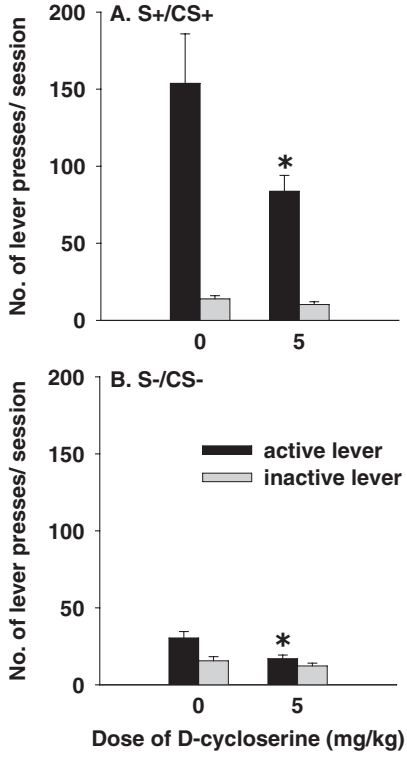

Fig. 2. Resumption of lever-pressing behaviour under both S+/CS+ (**A**) and S−/CS− (**B**) conditions. Vehicle ($n = 10$) and 5 mg/kg of D-cycloserine (DCS) ($n = 10$) administration was performed 60 min before each S+/CS+ and each S−/CS− extinction session. Resumption testing was performed in drug-free animals. Data are shown as the average number of lever presses on alcohol-associated (S+/CS+ session) and water-associated (S−/CS− session) levers and on the inactive lever for each session. Data are presented as means ± S.E.M. *Significant differences from the vehicle control group, $P < 0.05$.

subsequent relapse-like drinking behaviour accompanied with the S+/CS+ presentation in conjunction with a small oral ethanol-priming dose is also significantly reduced by administration of DCS. However, following the presentation of the S−/CS− lever pressing was also reduced when compared to the vehicle-treated animals. This showing that DCS treatment is affecting the ability of an animal to resume extinguished operant responding unselectively probably by enhancing learning of new contextual relationships during extinction sessions. In accordance, DCS was found effective in facilitation of other learning tasks (Quartermain *et al*., 1994; Land and Riccio, 1999; Lelong *et al.,* 2001).

The low dose of DCS, such as the one used in the present study, was also tested by Bertotto *et al.* (2006) on the extinction process in animals previously subjected to alcohol withdrawal, showing that this dose is ineffective in alcohol-naïve animals; however, it affects fear extinction in alcohol-abstinent rats. This could be explained by the well-known hypersensitivity of the glutamatergic system and particularly NMDA receptor in alcohol-withdrawn animals. Thus, it could also explain our findings, showing that the low doses of DCS might be more beneficial in the present experimental paradigm. Furthermore, in line with the clinical studies, our results demonstrate that the number of DCS administration sessions does not improve the treatment outcome (Norberg *et al*., 2008). In contrast, lessening of DCS effects was seen when the drug was administered repeatedly.

In conclusion, our study suggests that DCS might be useful as an add-on medication for extinction training on cue reactivity in alcohol-dependent patients (Conklin and Tiffany, 2002). Most importantly, DCS has already been approved for use in humans as an antibiotic to treat tuberculosis and therefore would be relatively safe to use in alcohol-dependent patients. However, the clinical significance of our present findings is limited as we do not know whether DCS exhibits generalized extinction. That is, once lever-pressing behaviour in rats that were given DCS in conjunction with one particular alcohol-associated cue has been extinguished, will these animals also exhibit less alcohol-seeking behaviour to other non-extinguished appetitive cues? This will be an important question to answer in the future as usually alcohol-dependent patients show cue responsivity and associated craving to a whole variety of conditioned cues. It should be mentioned, however, that generalized extinction has been demonstrated in fear-conditioning studies (Ledgerwood *et al*., 2005)—a finding that may also translate to the extinction of appetitive memories.

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