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Effects of D-cycloserine on extinction of learned fear to an olfactory cue

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Abstract

D-cycloserine (DCS), a partial NMDA receptor agonist, facilitates extinction of learned fear in rats and has been used to treat anxiety disorders in clinical populations. However, research into the effects of DCS on extinction is still in its infancy, with visual cues being the primary fear-eliciting stimuli under investigation. In both human and animal subjects odors have been found to associate strongly with aversive events. Therefore, this study examined the generality of the effects of DCS on extinction by testing odor cues. Sprague–Dawley rats were conditioned and extinguished to an odor using varying parameters, injected with either saline or DCS (15 mg/kg) following extinction, and then tested for a freezing response 24 h later. Experiment 1 demonstrated that after 3 odor-shock pairings, rats did not display short-term extinction and DCS had no effect on long-term extinction. Experiment 2 demonstrated that after 3 odor-*noise* pairings, rats displayed significant short-term extinction and DCS significantly facilitated long-term extinction. Following 2 odor-shock pairings in Experiment 3, half the rats displayed short-term extinction ("extinguishers") and half did not ("non-extinguishers"). DCS facilitated long-term extinction in the "extinguishers" condition but not in the "non-extinguishers" condition. In Experiment 4, following 2 odor-shock pairings and an extra extinction session, DCS had a significant facilitatory effect on long-term extinction. Thus, extinction of freezing to an odor cue was facilitated by systemic injections of DCS, but only when some amount of within-session extinction occurred prior to injection.

Keywords: Rats; D-Cycloserine; Fear extinction; Odors; Freezing

1. Introduction

Anxiety disorders such as Phobia, post-traumatic stress disorder (PTSD), and Obsessive–Compulsive Disorder are often treated with exposure-based cognitive behavior therapy (Andrews, Grino, Hunt, & Page, 1994; Foa & Kozak, 1986; Thyer, Baum, & Reid, 1988; Zarate & Agras, 1994). This type of therapy involves exposing patients to a fearful, or anxiety-provoking, stimulus within a calm and safe environment. Gradually, over successive presentations of the stimulus within this environment, patients learn to respond without fear or anxiety. This type of treatment is procedurally very similar to 'extinction' training in animal models of emotional learning. For example, learned fear responses to a conditioned stimulus (CS; e.g., a light that had previously been paired with shock) can be reduced by repeatedly presenting the CS in the absence of shock. Eventually the animal learns that the CS no longer predicts the occurrence of shock and fear reactions decrease in amplitude and/or frequency.

Although very simple procedurally, extinction has proven to be an extremely difficult process to understand. Nonetheless, considerable effort has been directed at trying to identify techniques that render extinction—and potentially exposure therapy—more effective. One significant advance in this area has been the demonstration that D-cycloserine (DCS) can facilitate fear extinction. DCS was used because of its indirect agonist properties at *N*-methyl-D-aspartate receptors, which are critical for the neuronal plasticity required for emotional memory (see, Davis, Ressler, Rothbaum, & Richardson, 2006). Pre-clinical studies have demonstrated that DCS is a potent facilitator of fear

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extinction in rats (Ledgerwood, Richardson, & Cranney, 2003, 2004, 2005; Parnas, Weber, & Richardson, 2005; Walker, Ressler, Lu, & Davis, 2002; Yang & Lu, 2005). In addition, DCS has been found to enhance the effectiveness of exposure-based therapy in people treated for acrophobia (Ressler et al., 2004) and Social Anxiety Disorder (Hofmann et al., 2006). However, research on the effects of DCS on extinction is still in its infancy, and it is still not known whether DCS is effective at facilitating extinction to any, or all, types of feared stimuli, or whether its effectiveness is limited in some way.

The aim of the current study was to test the 'generality' of the effects of DCS on extinction. Both pre-clinically and clinically, DCS has primarily been used in conjunction with exposure to visual cues. However, fear-eliciting cues are not always visually-based. Odors, in particular, have been found to be especially effective in cuing emotional memory in non-clinical populations (Herz, 1998; Herz, Eliassen, Beland, & Souza, 2004), and can evoke strong trauma-related memories in people with PTSD (Vermetten & Bremner, 2003). Olfactory stimuli are also extremely salient and powerful cues for memory retrieval in rats. For example, conditioned fear associations using olfactory cues in rats are encoded more rapidly (Paschall & Davis, 2002), and require more trials to extinguish than do visual cues (Richardson, Tronson, Bailey, & Parnas, 2002). These findings suggest that odor cues may be processed by a different memory system. Indeed, in rats the neuroanatomical pathways subserving olfactory information are slightly different than for stimuli of other sensory modalities. For example, visual and auditory sensations are processed within the thalamus before being sent to the amygdala, which is the brain region crucial for recalling emotional associations. However, olfactory information is transmitted directly to the amygdala (Price, 1973), suggesting that odors have 'privileged' access to the amygdala and may prompt recall for emotional associations more quickly and easily. Therefore, because (1) odor-evoked memories can be powerful triggers for the emotional component of memory, and (2) the neuroanatomical substrates of odor-evoked emotional memories may differ to that for visually-evoked memories, the present study was designed to determine whether DCS facilitates extinction of conditioned fear to an odor CS in rats.

2. Methods and materials

2.1. Subjects

Experimentally-naive, male Sprague–Dawley rats (450–700 g) obtained from the breeding colony maintained by the School of Psychology at the University of New South Wales were used. The rats were housed in groups of eight in plastic boxes (67 cm long × 40 cm wide × 22 cm high) in a colony room with a natural light cycle. Food and water were continuously available. All experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales and adhered to the ethical guidelines described in The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Prior to experimentation, all rats were handled for approximately 5 min on 3 consecutive days.

2.2. Apparatus

In all experiments conditioning occurred in one context and extinction training and testing occurred in a different context. All experimental cages were located within a sound- and light-attenuating wood cabinet where a ventilation fan provided a 60-dB ambient noise level in each chamber. Conditioning occurred in one of 4 identical cages (20 cm long × 12 cm wide × 12 cm high). The front wall, rear wall, and ceiling of each cage were made of Plexiglas, and the side walls and floor consisted of stainless steel bars, with 1.3 cm between each bar. Each cage was suspended 13 cm above the floor of the wood cabinet and a tray of bedding was placed under each cage, which was changed between each rat. During conditioning, the doors to the wood cabinets remained open and the room was illuminated by a red light.

The context used for extinction training and testing in Experiments 1 and 2 differed slightly from that used in Experiments 3 and 4. In Experiments 1 and 2, extinction training and testing occurred in one of 2 identical cages (30 cm long × 22.5 cm wide × 30 cm high) with a clear Perspex front, black and white striped side and rear walls, and a wood hinged lid. The floor consisted of stainless steel bars, with 1.0 cm between each bar, and was suspended 8.5 cm above the floor of the wood cabinet. In Experiments 3 and 4, extinction training and testing occurred in one of 2 identical clear Perspex cages (29 cm long × 28 cm wide × 29 cm high). The floor consisted of stainless steel bars, with 1.5 cm between each bar, and was suspended 10 cm above the floor of the wood cabinets.

A white light bulb in each cabinet provided illumination at all times during extinction and testing. Between sessions, these chambers were wiped with 0.5% eucalyptus solution and the bedding below the grid floor was changed. Extinction and test sessions were recorded for later assessment.

2.3. Procedure

In all experiments the CS was a grape odor (0.1 ml in a plastic jar; #182380019 from Wild Flavours, Heidelberg) and the method of presentation involved the odor jar being placed under the experimental cage for the required time period and then being removed and capped with an air-tight lid. The unconditioned stimulus (US) varied for each experiment.

The procedures for Experiments 1 and 2 were identical except that a different US was used. In Experiment 1 the US was a 1 s, 0.6 mA footshock and in Experiment 2 the US was a 100 ms, 120 dB white noise stimulus (rise-fall time <5 ms). In each experiment a 2 × 2 factorial design was employed, where the first factor was drug condition (DCS or saline) and the second factor was condition (extinction or no extinction). This produced 4 groups: extinction + DCS (E-DCS), extinction + saline (E-Sal), no-extinction + DCS, and no-extinction + saline; the latter two groups were subsequently pooled into a single group (NE-Pooled).

On Day 1, all rats were pre-exposed to the conditioning context for 20 min. Three hours later they were returned to the conditioning cages and received 3 pairings of the CS and US. That is, 120 s after being placed in the conditioning cages, the odor-jar was placed beneath the cage. Ten seconds later, the US was presented and then the odor-jar was removed. The 3 CS–US pairings occurred 120 s apart and rats were removed from the conditioning cages 120 s after the last pairing. On Day 2, rats allocated to the extinction groups were placed in the extinction cages. After 2 min in the cage, rats were presented with the jar containing the odor 6 times (each exposure was 2-min long, with a 2-min ITI). The rats were removed from the extinction cages 120 s after the last extinction trial, and half were injected with saline (E-Sal) and half were injected with DCS (E-DCS). Rats that did not receive extinction training were removed from their home cage and injected with either saline or DCS (NE-pooled). After injection all rats were returned to their home cage. On Day 3, all rats were tested for odor-elicited freezing. That is, 2 min after being placed in the cage, the odor was presented for 3 min.

The procedure for Experiments 3 and 4 was identical to Experiments 1 and 2 except that rats received 2 CS–US pairings and the US was a 0.5 s, 0.6 mA footshock. After extinction training in Experiment 3 rats were