Brief Communication

D-Cycloserine enhances memory consolidation of hippocampus-dependent latent extinction

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Adult male Long-Evans rats were trained to run in a straight-alley maze for food reward and subsequently received hippocampus-dependent latent extinction training. Immediately following latent extinction, rats received peripheral injections of the NMDA receptor partial agonist D-cycloserine (DCS, 15 mg/kg), or saline. Twenty-four hours later, rats received four extinction “probe” trials. Relative to saline controls, latencies to reach the goal box on probe trials were significantly higher in rats that had received DCS. These findings indicate that memory consolidation underlying hippocampus-dependent latent extinction, a cognitive form of learning in which the previously rewarded response is not made during extinction training, can be enhanced by NMDA-receptor agonism.

Behavioral extinction is operationalized as a reduction of a previously acquired response, and this process can involve new learning (for review, see Bouton 2004). The initial acquisition of learned behavior involves multiple memory systems (for review, see Packard 2001) and we have recently expanded this view to the study of the new learning underlying behavioral extinction (Gabriele and Packard 2006). We used a task in which extinction of the same overt behavior could be achieved using either a “response” or “latent” extinction training procedure. In contrast to typical extinction training that involves performance of a previously reinforced response, during latent extinction training no overt response is made. For example, a food-rewarded approach response in a straight-alley maze can be extinguished by placing the animal in the goal box in the absence of reward (Seward and Levy 1949). Consistent with the hypothesis that multiple memory systems play a role in the learning that occurs during extinction, we observed that the learning underlying response and latent extinction of runway behavior is neuroanatomically dissociable. Specifically, neural inactivation of the hippocampus prevented latent extinction of maze runway behavior, but did not block response extinction of the same behavior (Gabriele and Packard 2006). Latent extinction is conducive to a cognitive learning process in which an expectancy (Tolman 1932) of food reward can be altered without performance of the previously acquired response (Seward and Levy 1949). According to the multiple memory systems hypothesis, the hippocampus is selectively involved in cognitive learning and memory (e.g., Hirsh 1974; Mishkin and Petri 1984), and the impairment of latent extinction produced by hippocampal inactivation is consistent with this view.

Although our previous study using a reversible lesion technique implicates the hippocampus in latent extinction, the findings do not provide any information concerning the neurochemical basis of this form of cognitive learning. Various neurotransmitter systems play a role in extinction (for review, see Mason 1983) and several recent studies have focused on the role of glutamatergic neurotransmission (for review, see Davis and Myers 2002). For example, administration of D-cycloserine (DCS), a partial agonist at the strychnine insensitive glycine-binding site of the NMDA receptor enhances extinction when administered either pre- or post-extinction (for review, see Richardson et al. 2004). Moreover, DCS administration has been suc-

cessfully used in recent clinical studies aimed at developing pharmacotherapeutic approaches to facilitating extinction of fear responses in anxiety disorders (Ressler et al. 2004; Hofmann et al. 2006). Whereas some extinction therapies used in clinical settings involve training in the presence of overt behavioral responses, other approaches are based on developing cognitive control over maladaptive behaviors, and this latter form of learning may not require overt responding during extinction training. To the extent that latent extinction in lower animals selectively involves cognitive learning and memory (Seward and Levy 1949; Gabriele and Packard 2006), elucidation of the neurochemical bases of this form of extinction learning may potentially inform therapeutic strategies used in the treatment of various psychopathologies. Therefore, the present studies examined whether post-extinction training injections of DCS are effective in enhancing memory consolidation underlying hippocampus-dependent latent extinction.

An elevated (34 inches) straight alley maze with a black Plexiglas floor and clear Plexiglas sides (70 inches long, 4.5 inches wide, and 8 inches tall) was used (Gabriele and Packard 2006). A food cup (1-inch diameter) was located at the goal end of the maze. The maze was located in a room containing several extramaze cues. The subjects were 26 adult male Long-Evans rats (275–300 g). Rats were individually housed on a 12:12 h light-dark cycle, with lights on from 8:00 a.m. to 8:00 p.m. All animals received water ad libitum. D-cycloserine (Sigma Pharmaceuticals) was dissolved in physiological saline and injected intraperitoneally at a dose of 15 mg/kg. This dose was selected based on previous studies examining the effectiveness of DCS on extinction behavior, in which 15 mg/kg was the dose most commonly found to be effective (Walker et al. 2002; Ledgerwood et al. 2003; Parnas et al. 2005; Lee et al. 2006). Control animals were injected with physiological saline.

Behavioral procedures were similar to those of our previous study investigating the role of the hippocampus in latent extinction (Gabriele and Packard 2006). Prior to training, rats were reduced to 85% of ad lib body weight and maintained at this weight throughout training. Animals were habituated to the straight alley maze for 1 d in a single 2-min trial with no food available. Following maze habituation, rats received 15 Noyes food pellets (Formula P, 45 mg size) in their home cage. On day 1 of food-rewarded training, rats were placed in the start end of the maze and shaped to approach the food cup at the goal end of the maze by placing six pellets along the length of the alley and a single pellet in the food cup. On days 2–10 of food-rewarded

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maze training (six trials per day/30-sec intertrial interval), rats were placed in the start end and allowed to traverse the maze and consume one food pellet from the food cup. Upon reaching the food cup and consuming the pellet, rats were removed from the maze and placed in an opaque holding box adjacent to the maze for a 30-sec intertrial interval. If a rat failed to reach the food cup within 60 sec, it was removed for the intertrial interval. In each trial, the latency (seconds) to reach the food cup was recorded and used as a measure of task acquisition.

Twenty-four hours following the completion of acquisition training (i.e., day 11), rats were matched based on latencies to reach the food cup during the last 3 d of food-rewarded training to form two extinction groups—animals receiving DCS ($n = 9$) or saline ($n = 12$). During latent extinction training, rats were placed by the experimenter facing the empty food cup in the goal end of the maze and were confined for 60 sec by placement of a clear Plexiglas shield (8 inches from the end of the maze arm). Following confinement, rats were removed from the maze and placed in an opaque holding box located on a table adjacent to the maze for a 30-sec intertrial interval. Latent extinction training was administered in a single session (six trials) and rats received injections of saline or DCS immediately post-extinction training.

Twenty-four hours following the completion of extinction training (i.e., day 12), all rats were given an additional four extinction “probe” trials, in which they were placed in the start end of the maze, and latency to reach the empty food cup was recorded. These four trials allowed for an assessment of the effectiveness of the latent extinction procedure in saline and DCS treated rats. An additional group of animals ($n = 5$) received DCS injections (15 mg/kg) 2 h post-extinction training as a control group to control for any potential proactive non-mnemonic effects (e.g., sensory, motor, or motivational) of DCS on extinction, and to determine whether DCS influences memory consolidation in a time-dependent manner.

The acquisition of maze runway behavior in rats subsequently treated with either DCS or saline is shown in Figure 1. A two-way one-repeated measures ANOVA (Group X Trial) comparing the latencies to reach the food cup during acquisition revealed a nonsignificant interaction ($F_{(9,19)} = 0.224$, n.s.) and a nonsignificant main effect of Group ($F_{(1,19)} = 0.056$, n.s.). A significant Trial effect ($F_{(9,19)} = 47.961$, $P < 0.001$) revealed that the latency to reach the food cup during acquisition improved in both groups at a similar rate, and therefore, any drug-induced differences in extinction cannot be due to differential rates of acquisition between control and DCS-treated rats.

The effect of post-extinction training DCS on latent extinction is illustrated in Figure 2. In order to examine whether latent

Figure 1. Acquisition of maze runway behavior by rats that subsequently received saline or D-cycloserine following latent extinction. Mean $\pm$ standard error of the mean (SEM) of latency (in seconds) to reach the food cup over training days. For all drug conditions, there were no group differences in the initial acquisition of runway behavior.

Figure 2. The effect of peripheral saline or D-cycloserine on latent extinction probe trial behavior across all four probe trials. Mean $\pm$ standard error of the mean (SEM) of latency (in seconds) to reach the food cup during the extinction probe trials by treatment group. D-cycloserine administered immediately post-extinction training enhanced latent extinction.

extinction training produced significant extinction of runway behavior, a comparison of the mean latency to reach the food cup on the final day of acquisition training and the latencies across the four extinction probe trials was conducted. One-way ANOVAs comparing runway latencies revealed that animals receiving saline ($F_{(1,23)} = 14.537$, $P < 0.01$), or DCS ($F_{(1,17)} = 18.741$, $P < 0.01$) showed a significant latent extinction effect. In addition, a one-way ANOVA computed on the mean latency to reach the food cup across the four extinction probe trials revealed a significant Group difference ($F_{(1,20)} = 5.037$, $P < 0.05$), indicating that peripheral administration of DCS enhanced latent extinction relative to saline controls.

Peripheral injections of DCS administered 2 h post-extinction training did not enhance latent extinction (Fig. 2). A one-way ANOVA revealed a significant group difference between the mean runway latency across the four probe trials in rats that received DCS immediately post-extinction training and those that received DCS injections administered 2 h post-extinction training ($F_{(1,12)} = 5.545$, $P < 0.05$). Additionally, one-way ANOVA revealed no significant differences between the mean runway latency across the four probe trials in saline rats and those that received DCS injections administered 2 h post-extinction training ($F_{(1,16)} = 1.064$, n.s.). This finding suggests that DCS enhanced a time-dependent memory consolidation process underlying latent extinction (McGaugh 1989).

The present findings indicate that post-training administration DCS enhances memory consolidation underlying latent extinction of runway behavior in a straight-alley maze. The results are consistent with evidence that DCS facilitates extinction in other learning tasks, including those involving Pavlovian fear conditioning (e.g., Walker et al. 2002; Ledgerwood et al. 2003; Richardson et al. 2004) and conditioned place preference behavior (Boutreau et al. 2006). During latent extinction training, the previously reinforced response (in this case an approach response in a maze runway) is not overtly performed. Therefore, in addition to tasks involving “traditional” response extinction training, the present findings extend the learning situations in which DCS can influence extinction behavior by providing evidence that the drug can also enhance nonresponse or latent extinction.

The phenomenon of latent extinction is clearly problematic for stimulus-response (S-R) learning theories (e.g., Hull 1943, 1952) that emphasize the importance of inhibitory processes that develop during extinction training as a direct consequence of performing the previously reinforced approach response. The idea that extinction of fractional anticipatory responses provides an adequate S-R learning mechanism underlying latent extinction (e.g., Moltz 1955, 1957) has also met with empirical difficulty (Treisman 1960; Gabriele and Packard 2006). Alternatively,

it is possible that confinement to the goal-box during latent extinction training may lead to extinction of a Pavlovian stimulus-reinforcer association between the goal box and food reward. Weakening of such an association may serve to reduce "sign-tracking" (e.g., Hearst and Jenkins 1974) to the goal box, and thereby extinguish the instrumental approach response exhibited in the maze runway. However, this alternative is inconsistent with evidence that lesions of hippocampus impair latent runway extinction (Gabriele and Packard 2006) but do not impair acquisition of sign-tracking (Bussey et al. 2000; Parkinson et al. 2000; Ito et al. 2005). To our knowledge, the effects of hippocampal lesions on extinction of sign-tracking behavior have not been investigated. However, sign-tracking is essentially a form of discrete cue learning, and hippocampal lesions do not impair extinction of instrumental behaviors in tasks involving discrete cues (e.g., Kaplan 1968; Nadel 1968; Thomas and McCleary 1974). In contrast, in view of evidence that rats can learn the topographical relationships among extra-maze stimuli and that the hippocampus (e.g., O'Keefe et al. 1975; O'Keefe and Conway 1980) mediates this form of learning, it is possible that latent extinction involves the acquisition of "cognitive" (Tolman 1932) information, indicating that the spatial context is no longer associated with food reward. Consistent with this suggestion, latent extinction is particularly robust in open mazes providing visual access to extra-maze cues (Dyal 1962).

During memory retrieval, memories may become labile and undergo a reconsolidation process (Nader et al. 2000). According to the reconsolidation hypothesis, post-extinction training DCS injections may potentially enhance extinction by impairing memory for the original learning rather than enhancing memory for the new learning that can occur during extinction. However, in the present case, this hypothesis appears inconsistent with findings that post-training DCS administration also enhances memory consolidation during the new learning that occurs during initial task acquisition (Monahan et al. 1989; Hughes 2004). Additionally, following conditioned emotional response training, DCS enhances extinction, but does not impair renewal, indicating that the original association is still intact (Woods and Bouton 2006). In the current study, DCS was ineffective when injected 2 h post-training, indicating that the drug facilitates memory consolidation in a time-dependent manner and not via an influence on non-mnemonic (e.g., sensory, motor, or motivational) factors (McGaugh 1989).

Glutamatergic NMDA receptors have been implicated in memory consolidation during initial learning in several tasks. For example, post-training administration of DCS enhances memory consolidation during task acquisition (Monahan et al. 1989; Hughes 2004). In addition, post-training administration of the NMDA receptor antagonists MK-801 (Packard and Teather 1997a) or AP5 (Packard and Teather 1997b) impairs hippocampus-dependent spatial memory, and peripheral pretraining injections of DCS facilitate the acquisition of hippocampus-dependent trace eye-blink conditioning in rabbits (Thompson et al. 1992). Taken together with our previous findings indicating that latent extinction is mediated by the hippocampus (Gabriele and Packard 2006), it is possible that this brain structure is a critical site of action for the enhancing effects of peripheral administration of DCS on latent extinction. Moreover, the present findings provide further evidence that memory consolidation during initial task acquisition and the new learning that occurs during extinction can similarly involve NMDA receptor function (e.g., Falls et al. 1992).

Finally, pre-extinction training administration of DCS has been used successfully in conjunction with exposure therapy in the treatment of acrophobia (Ressler et al. 2004) and social anxiety disorder (Hofmann et al. 2006). The ability of DCS to enhance

cognitive memory processes such as those putatively involved in latent extinction suggest that the drug may potentially have efficacy when used in conjunction with relatively "nonbehavioral" therapeutic strategies such as cognitive restructuring.

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