Research report

Posttrial d-cycloserine enhances the emotional memory of an incentive downshift event☆

Jacob N. Norris 1, Leonardo A. Ortega, Mauricio R. Papini*

Department of Psychology, Texas Christian University, Fort Worth, TX 76129, USA

A R T I C L E   I N F O

Article history:
Received 20 March 2011
Received in revised form 28 April 2011
Accepted 2 May 2011
Available online 7 May 2011

Keywords:
Incentive downshift
d-Cycloserine
NMDARs
Frustration
Negative contrast
Emotional memory

A B S T R A C T

The present research was designed to determine whether an incentive downshift event induces an emotional memory that can be modulated by d-cycloserine (DCS), a partial agonist at the glycine site of N-methyl-D-aspartate receptor (NMDAR). DCS has been reported to have memory-enhancing properties in other training situations. Experiments 1 and 2 involved a consummatory successive negative contrast (cSNC) situation in which animals are exposed to an incentive downshift involving sucrose solutions of different concentrations. DCS administration (30 mg/kg, ip) immediately after the first 32- to 4% sucrose downshift trial (Experiment 1) retarded recovery of consummatory behavior, but immediately after the first 32- to 6% sucrose downshift trial (Experiment 2) did not affect recovery. There was no evidence that DCS affected consummatory behavior in the absence of an incentive downshift in a manner analogous to a conditioned taste aversion (Experiment 3). These results suggest that activation of NMDARs via the glycine modulatory site enhances the emotional memory triggered by exposure to an incentive downshift event.

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Consummatory successive negative contrast (cSNC) is the abrupt disruption of consummatory behavior after a downshift from a large to a small incentive, relative to the consummatory behavior of animals exposed only to the small incentive [1]. In a typical cSNC experiment, the incentives are 32% and 4% sucrose solutions, and the downshift occurs on Trial 11. The cSNC effect indicates that the change in behavior is not an adjustment to the new incentive conditions, but a reduction of consummatory behavior that may be the result of an increase in search behavior [2–4], escape from a frustrating situation [5,6], or release from response inhibition following a surprising devaluation of the incentive [7]. Assuming that the initial rejection of the devalued solution reflects an aversive internal state [8,9], hypothesized that emotional conditioning takes place during the incentive downshift event. The basis for this memory is postulated to be a Pavlovian association between the taste of the downshifted, 4% sucrose solution and the internal state of frustration induced by the downshift. As a result, tasting the 4% sucrose solution would elicit an internal aversive state that would tend to suppress consummatory behavior and also induce a behavioral switch from consumption to searching or escaping. The associative reactivation of this emotional memory is assumed to retard recovery from cSNC beyond the first downshift trial. Emotional memory reactivation is a source of consummatory suppression because animals tend to avoid stimuli associated with negative emotional content, such as the area where they have consumed the devalued sucrose solution (i.e., secondary frustration; [5,10]). The research reported here is concerned with the encoding of this aversive memory of the downshift.

The effects of drugs on memory consolidation can be best assessed by posttrial drug administration, that is, by administering the drug immediately after the relevant experience. Memory consolidation is a time-dependent process occurring after acquisition and rendering the memory trace more stable and more resistant to disruption [11]. Because training occurs before drug administration, behavior during training is not affected directly by the drug. Because behavioral testing of drug effects usually occurs a day after drug administration (as in the present experiments), and because many psychoactive drugs are metabolized within hours (e.g., in rats, the half-life of d-cycloserine, used in these experiments, was reported to be 60–70 min; [12,13]), it can be assumed that the drug has been metabolized by the time of testing. As a result, posttrial drug effects on behavior can be more safely attributed to the drug’s effect on memory consolidation, rather than to direct drug effects on motor, motivational, or attentional processes active during training and/or testing ([14–17]; but see Experiment 3, below.

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* Corresponding author. Tel.: +1 817 257 6084.
E-mail address: m.papini@tcu.edu (M.R. Papini).

1 Current address: Naval Health Research Center, 140 Sylvester Road, San Diego, CA 92106, USA.
for a potential alternative of the effects of posttraining drug administration).

Evidence consistent with the encoding of a downshift memory is provided by the effects of corticosterone administered immediately after the first downshift trial. Based on extensive research in a variety of learning paradigms, glucocorticoids can be safely assumed to enhance memory consolidation [18–21]. When administered in the cSNC situation, immediately after the first downshift trial, corticosterone retards recovery from cSNC [22]. Posttraining corticosterone has no effects on consummatory behavior under the following conditions [22,23]: (1) In the absence of a downshift event, as in unshifted controls or when administered after a single exposure to 4% sucrose; thus, corticosterone’s effects in the cSNC situation cannot be attributed to a conditioned taste aversion to 4% sucrose. (2) When corticosterone is administered 3 h after the first downshift trial, rather than immediately after, thus demonstrating the time-dependent nature of its effects. (3) When corticosterone is administered in an anticipatory contrast situation involving the same solutions used in the cSNC situation. In a procedure known to activate different brain mechanisms from those involved in cSNC [2]. Therefore, the selectivity of these corticosterone effects to the cSNC situation is consistent with the enhancement of the aversive memory of the downshift event.

Other drugs known from other learning paradigms to modulate memory consolidation and tested in the cSNC situation under similar conditions do not affect the course of recovery from the incentive downshift as corticosterone does. These include cholinergic drugs (e.g., atropine and phystostigmine), known to modulate consolidation of conditioned fear [24–26], but having no effect on cSNC [27], and opioids (naloxone, DPDPE, and naltrindole), known to modulate consolidation of conditioned fear [17,28,29], but having no detectable effect on recovery from cSNC [30]. Therefore, memory consolidation of the incentive downshift experience is modulated by some, but not all of the neurochemical systems known to be involved in other types of emotional memory.

The present experiments were designed to determine whether the N-methyl-d-aspartate receptor (NMDAR) is involved in memory consolidation in the cSNC situation. There is a well-established connection between glucocorticoids and NMDARs, although the relationship is complex. For example, glucocorticoids enhance NMDAR activation in hippocampal tissue extracted from neonatal rats [31]. Furthermore, Nair and Bonneau reported that acute restraint stress induced glucocorticoid elevation that, in turn, increased microglia proliferation (a proinflammatory response); this effect was blocked by administration of the NMDA receptor antagonist MK-801. Thus, we hypothesized that the enhancing effects of posttrial corticosterone on cSNC described above were mediated by activation of NMDARs. In these experiments, animals received administration of d-cycloserine (DCS) immediately after the first downshift trial following a 32- to 4% sucrose downshift (Experiment 1) or a 32- to 6% sucrose downshift (Experiment 2). Finally, Experiment 3 explored the possibility that DCS’s effects acted as an unconditioned stimulus supporting the development of a conditioned taste aversion to the 4% sucrose. DCS is a partial agonist at the glycine binding site (NR1B) of the NMDAR; the endogenous ligand, glycine, has greater affinity to this binding site than DCS, which is thus characterized as a partial agonist. As a result, DCS may act either as an agonist or an antagonist depending on the extracellular concentration of glycine [33]. DCS was selected because there is strong evidence from a variety of learning paradigms that this drug functions as a memory enhancer. For example, in the fear conditioning situation, DCS administration either before or after training sessions has been shown to facilitate extinction [34–37], reduced reinstatement following extinction [38], and increased generalization of extinction to nonextinguished stimuli [35]. In spatial learning situations, DCS improves performance in rats made spatially deficient either by hippocampal lesions [39] or scopolamine-induced amnesia [40,41]. In flavor learning situations, pretrial DCS administration enhanced LiCl-induced conditioned taste aversions [42] and also enhanced fructose-induced conditioned taste preferences [43]. DCS also reversed deficits on eyeblink conditioning in rabbits induced by exposure to inescapable shocks [44] and restored episodic-like memory in an object-recognition task with rats [45]. These references are but a subset of the growing literature on the effects of DCS on learning, all suggesting that, within some constraints (e.g., there is apparently an optimal dose for some of these effects; [46,47]), DCS enhances learning (i.e., the acquisition of new information).

Because of the memory enhancing effects reported for both corticosterone and DCS, and because of the connection between glucocorticoid levels and NMDAR activation, we predicted that DCS would have effects in the cSNC situation similar to those described above for corticosterone. Furthermore, we assumed that DCS would not affect well-established memories, such as the memory of the preshift solution. Thus, if DCS retards recovery from cSNC, this result would be interpreted as an enhancement of the emotional memory of the downshift.

1. Experiment 1

It was hypothesized that posttrial DCS administration would have the same effect on cSNC than corticosterone did in previous experiments [22,23]. Therefore, Experiment 1 tested the hypothesis that DCS administration prolongs the cSNC effect. A 30 mg/kg dose was chosen based on prior research showing that this dose was effective with rats trained in other tasks [40,47,48].

2. Method

2.1. Subjects

The subjects were 41 male Long-Evans rats bred at the TCU vivarium from an original group purchased from Harlan Laboratories (Indianapolis, IN), approximately 90 days old at the start of the experiment and experimentally naive. The average ad libitum weight of the entire sample was 405 g (range: 338–484 g). Rats were housed in a vivarium under a 12:12-h light, dark cycle (lights on at 07:00 h) and were deprived of food to an 81–84% of the free-food weight. Water was continuously available in each individual cage. Animals were trained during the light phase of the daily cycle.

2.2. Apparatus

Training was conducted in 4 conditioning boxes (MED Associates, St. Albans, VT) constructed of aluminum and Plexiglas, and measuring 29.4 cm × 28.9 cm × 24.7 cm (L × W × H). The floor was made of steel rods 0.5 cm in diameter and 1.2 cm apart running perpendicular to the feeder wall. A bedding tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine. Against the feeder wall was an elliptical hole 1 cm × 2 cm (W × H), 3.5 cm from the floor. A sipper tube, 1 cm in diameter, was inserted through this hole. When fully inserted, the sipper tube was flush against the wall. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube, and detected contact with the sipper tube via a circuit involving the steel rods in the floor. Each conditioning box was placed in a sound-attenuating chamber that contained a house light, a speaker to deliver white noise, and a fan for ventilation. Together, the speaker and fan produced noise with an intensity of 80.1 dB (SPL, Scale C).

2.3. Procedure

Training lasted 15 trials, administered 1 trial per day. Each rat was randomly assigned to one of the conditioning boxes and always trained in that box. The order of training of the 4-rat squads varied across days. After each trial, conditioning boxes were cleaned with a damp paper towel, feces removed, and bedding material replaced as needed. During trials, the houselight, white noise, and fan were on continuously. The 15 trials were divided into a preshift phase (Trials 1–10) and a postshift phase (Trials 11–15). Prior to Trial 1, rats were matched by ad libitum weight and then randomly assigned to the downshifted or unshifted condition. After Trial 10, rats exposed to 32% sucrose were matched in terms of overall preshift performance and randomly assigned to one of the two different drug conditions, Saline or 30 mg/kg DCS. The unshifted controls were assigned likewise. This drug dose is frequently reported as effective in prior research (e.g., [37]).
For the two 32-to-4 groups [32/Sal, 32/DCS], the 10 preshift trials involved access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water); the 5 postshift trials involved access to a 4% solution (w/w, 4 g of sugar for every 96 g of distilled water). The two 4-to-4 groups (4/Sal, 4/DCS) received the 4% sucrose solution in all 15 trials. Each trial started with a variable interval of 30 s (range: 15–45 s). At the end of this interval, the sipper tube was automatically presented. The first recorded contact with the sipper tube initiated a 5-min interval during which the sucrose solution was freely available. At the end of this interval, the sipper tube was retracted and a final variable interval of 30 s (range: 15–45 s) ended the trial. The dependent variable was the cumulative amount of time in contact with the sipper tube, measured in 0.05-s units and labeled goal-tracking time. Goal-tracking times were recorded for each entire 5-min trial and also in 5-s bins to analyze within-trial performance. Goal-tracking times were subjected to conventional analysis of variance (ANOVA). The alpha value was set to \( p < 0.05 \) for all statistical tests.

DCS or saline was administered immediately following the first downshift trial (Trial 11). Saline animals received an equal-volume injection of isotonic saline. Drug concentrations were adjusted to a 1 ml/kg volume. At the end of the trial, animals were placed back into the transport rack and moved into the holding room where they received the appropriate injection. This took approximately 30–90 s. The following groups were included: 32/Sal (\( n = 11 \)), 32/DCS (\( n = 10 \)), 4/Sal (\( n = 10 \)), and 4/DCS (\( n = 10 \)). It was assumed that DCS had no direct effects on Trials 12–15, as DCS’s levels in brain and plasma are in the order of 60–70 min [12,13]. DCS was purchased from Sigma-Aldrich Chemicals (Saint Louis, MO). It was freshly dissolved in isotonic saline solution (30 mg/ml).

3. Results

The results are shown in Fig. 1. A Drug \( \times \) Sucrose \( \times \) Preshift Trial analysis revealed a significant change across trials, \( F(9, 333) = 81.95, p < 0.001 \), a main effect of sucrose, \( F(1, 37) = 10.81, p < 0.01 \), and a significant trial by sucrose interaction, \( F(9, 333) = 3.18, p < 0.01 \), but not significant main effect of drug or of drug-related interactions, \( F_S < 1 \). A Drug \( \times \) Sucrose \( \times \) Postshift Trial analysis indicated a significant main effect of trial, \( F(4, 148) = 16.6, p < 0.001 \), a significant trial by sucrose interaction, \( F(4, 148) = 7.14, p < 0.001 \), and a trial by sucrose by drug interaction, \( F(4, 148) = 2.57, p < 0.001 \). All other effects were not significant, \( F_S < 1 \).

To clarify the source of the three-way interaction, one-way ANOVAs for Trials 11–15 were calculated for pairs of groups involving the same drug condition, but different behavioral treatment: 32/DCS vs. 4/DCS and 32/Sal vs. 4/Sal. For the saline groups, the cSNC effect was significant on Trial 11, \( F(1, 19) = 8.34, p < 0.01 \), marginal for Trial 12, \( F(1, 19) = 4.23, p = 0.054 \), and not significant for Trials 13–15, \( F_S < 1.49, p > 0.23 \). However, the cSNC effect was extended for DCS-treated groups throughout the entire postshift phase, Trials 11–15. Group 32/DCS contacted the sipper tube significantly less than Group 4/DCS on Trials 11–15, \( F_S(1, 18) > 4.86, p < 0.05 \).

Further pairwise comparisons were computed, also for Trials 11–15, between groups with the same behavioral condition, but different drug condition: 32/DCS vs. 32/Sal and 4/DCS vs. 4/Sal. Thus, Group 32/DCS contacted the sipper tube significantly less than Group 32/Sal on Trial 13, \( F(1, 19) = 10.90, p < 0.005 \), marginally on Trial 12, \( F(1, 19) = 3.79, p = 0.067 \), and not significantly on Trials 11, 14, and 15, \( F_S(1, 19) < 1.32, p > 0.26 \). However, DCS had no effect on the unshifted controls. The comparisons between Groups 4/DCS and 4/Sal were all not significant for Trials 11–15, \( F_S < 2.05, p > 0.16 \).

Previous research indicated that consummatory suppression is not evident during the initial 100 s of postshift trials, but it emerges fully during the last 100 s of such trials [49]. Consequently, the within-trial results for the initial and last 100 s of Trials 10–15 are presented in Fig. 2. In both cases, a Drug (DCS, Sal) \( \times \) Sucrose (32%, 4%) \( \times \) Trial (11–15) analysis yielded a triple interaction that fell short of significance: \( F(4, 124) = 2.10, p = 0.085 \) for the first 100 s, and \( F(4, 124) = 1.55, p = 0.191 \) for the last 100 s. Given the results for the entire session (Fig. 1) and the graphical depiction of the within-trial data (Fig. 2), a second analysis was calculated only for Trials 11–13. The results were as follows,
For the first 100 s (Fig. 2, top panel), the Drug × Sucrose × Trial (11–13) analysis indicated a significant triple interaction, F(2, 62) = 3.61, p < 0.04, as well as a significant contrast effect, with downshifted scoring below unshifted controls, F(1, 31) = 9.24, p < 0.01, and significant change across trials, F(2, 62) = 3.21, p < 0.05. All other effects were not significant, Fs < 2.43, ps > 0.08. Pairwise analyses for groups matched by drug condition showed that Groups 32/Sal vs. 4/Sal did not differ on Trials 11–13, Fs(1, 16) < 2.26, ps > 0.15, whereas Groups 32/DCS vs. 4/DCS also failed to differ on Trial 11, before DCS administration, F(1, 15) = 3.08, p > 0.09, but the cSNC was significant for Trials 12–13, Fs(1, 15) > 14.61, ps < 0.003, after DCS administration. Comparisons for groups matched in terms of the behavioral treatment showed that Group 32/Sal contacted the sipper tube significantly more than 32/DCS on Trial 13, F(1, 15) = 7.49, p < 0.02. On Trials 11 the difference was not significant, F < 1, and on Trial 12 was also not significant, marginal, F(1, 15) = 3.88, p = 0.068. However, DCS had no detectable effect on the unshifted controls, as indicated by a comparison between Groups 4/Sal vs. 4/DCS for Trials 11–13, Fs < 1.

Fig. 2(bottom panel) also shows the results for the last 100 s of Trials 10–15. The Drug × Sucrose × Trial (11–13) analysis provided a significant triple interaction, F(2, 62) = 3.71, p < 0.04, as well as a significant contrast effect, F(1, 31) = 31.88, p < 0.001, and significant change across trials, F(2, 62) = 4.97, p < 0.02. All other effects were not significant, Fs < 3.16, ps > 0.08. Pairwise analyses for groups matched by drug condition showed that Groups 32/Sal vs. 4/Sal differed significantly on Trials 11–12, Fs(1, 16) > 8.01, ps > 0.02, but not on Trial 13, F < 1, whereas Groups 32/DCS vs. 4/DCS exhibited significant differences in all three postshift trials, Fs(1, 15) > 9.59, ps > 0.006. Comparisons for groups matched for behavioral treatment showed that Group 32/Sal contacted the sipper tube significantly more than 32/DCS on Trial 13, F(1, 15) = 8.92, p < 0.01. On Trials 11 the difference was not significant, but marginal, F(1, 15) = 4.07, p = 0.062, but on Trial 12 it was not significant, F < 1. DCS had no detectable effect on the unshifted controls on Trials 11–13, Fs(1, 15) < 1.37, ps > 0.25.

In summary, DCS administration after the first downshift trial (Trial 11) enhanced suppression of consummatory behavior in subsequent trials. As shown by within-trial analyses, such suppression was detectable during the initial 100 s of subsequent trials (Trials 12–13), as well as during the last 100 s of the same trials. The fact that DCS-treated animals show consummatory suppression earlier than saline-treated animals is consistent with facilitated retrieval of the emotional memory of the incentive downshift event experienced a day earlier. Such facilitated retrieval seems to have modulated behavior throughout the trial, as assessed by facilitated cSNC effect during the last 100 s, relative to saline groups. This result is thus consistent with DCS-induced facilitation of memory retrieval.

3.1. Experiment 2

In the present experiment, the same DCS dose and administration used previously occurred after a 32-to-6% sucrose downshift. The 32-to-6% downshift was used for two reasons. First, a reduction in incentive disparity attenuates cSNC [50], thus reducing the opportunity for floor effects in DCS-treated animals. Second, Ruetti et al. [23] showed that posttrial administration of corticosterone enhanced cSNC following a 32-to-4% sucrose downshift, but not an 8-to-4% sucrose downshift, concluding that corticosterone’s enhancing effects were possible only when an animal experienced a large incentive disparity. Similarly, Daniel et al. [30] showed that naloxone administered prior to the first downshift trial reduced consummatory behavior after a 32-to-6% sucrose downshift, but not after a 16-to-6% sucrose downshift. Likewise, using a less severe downshift will help determine whether the effects of DCS depend on a minimum incentive disparity.

4. Method

4.1. Subjects and apparatus

The subjects were 37 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naive. The average ad libitum weight was 423 g (range: 352–526 g). Housing, training procedure, apparatus, drug preparation, and route of administration were similar to Experiment 1.

4.2. Procedure

The only difference with respect to the previous experiment was the use of 6% sucrose as the downshifted solution (w/w, prepared by mixing 6 g of sucrose for every 94 g of distilled water). The following groups were included: 32/Sal (n = 9), 32/DCS (n = 9), 6/Sal (n = 9), and 6/DCS (n = 10). All other procedural aspects were as described in the previous experiment.

5. Results

The results are shown in Fig. 3. A Drug × Sucrose × Preshift Trial (1–10) analysis revealed significantly higher goal-tracking scores with 32% sucrose than 6% sucrose, F(1, 33) = 4.83, p < 0.04, and a significant increase across trials, F(9, 297) = 83.64, p < 0.001; the main effect for drug and the interactions were all not significant, F(9, 297) < 1.56, ps > 0.12. A Drug × Sucrose × Preshift Trial (11–15) analysis yielded significant main effects for sucrose, F(1, 33) = 6.67, p < 0.02, trial, F(4, 132) = 16.45, p < 0.001, and the trial by sucrose interaction, F(4, 132) = 18.4, p < 0.001. All other effects were not significant, Fs < 1, including the triple interaction.

The results of the within-trial analyses are presented in Fig. 4. Whether in the first or last 100 s, there was no hint of a DCS effect in these data. Drug × Sucrose × Trial (11–15) analyses, whether for the first or last 100 s, provided no evidence of a triple interaction, F < 1. The contrast effect was significant for both the first and last 100 s sets, in terms of a sucrose by trial interaction, F(4, 132) = 6.70, p < 0.001. The main effect of sucrose as also significant for the first 100 s data set, F(1, 33) = 11.43, p < 0.003, but not for the first 100 s data set, F < 1. Changes across trials were significant for both analyses, F(4, 132) > 3.47, ps < 0.02. Other factors were not significant, Fs < 1. The same results were obtained with a similar analysis restricted to Trials 11–13, as had been done in Experiment 1.

The results of Experiment 2 failed to uncover any effects of DCS after a 32-to-6% sucrose downshift. One implication of these results...
is that a minimum disparity between preshift and postrial sucrose concentrations is required for DCS administered immediately after the first downshift trial to affect recovery from cSNC. Notice that the failure to detect an effect of postrial DCS administration occurred despite the presence of a significant cSNC effect after a 32-to-6% sucrose downshift.

5.1. Experiment 3

Experiment 1 showed that DCS administered immediately after the first downshift trial prolonged the cSNC effect in subsequent trials. Whereas these results provide support for the hypothesis that activation of NMDA receptors using DCS enhances cSNC by facilitating the consolidation and subsequent retrieval of the emotional memory of the incentive downshift event, one alternative remains to be evaluated. Nunnink et al. [37] reported that pairing DCS at 30 mg/kg with access to saccharine reduced preference against water in subsequent two-bottle tests, compared to a saline control. Although the experiment lacked an unpaired control, it is plausible that the effect of DCS on cSNC may be the result of a conditioned taste aversion, rather than of emotional memory consolidation. This analysis is described in detail in similar experiments involving other drugs administered after the first downshift trial in the cSNC preparation [23,30,51]. Briefly, the 4% sucrose solution is relatively novel for downshifted animals and may act as an efficient conditioned stimulus for the effects induced by DCS administration, which would be the unconditioned stimulus. This factor would be less prominent in unshifted controls because of their extensive pre-exposure to 4% sucrose during preshift trials (i.e., latent inhibition reduces conditioned taste aversions [52]).

Experiment 2 provided data inconsistent with the conditioned taste aversion hypothesis because DCS had no measurable effect on the subsequent consumption of 6% sucrose. However, because of the different concentrations used in Experiments 1 and 2 (4% vs. 6% sucrose) the role of this factor cannot be safely excluded. For example, the lesser disparity between 6 and 32% sucrose, compared to 4 and 32% sucrose, may have rendered the 6% solution relatively less novel and, therefore, relatively less likely to enter into an association with the consequences of DCS administration. Experiment 3 evaluated the conditioned taste aversion hypothesis by pairing 4% sucrose to DCS in the absence of an incentive downshift, but in a situation that was otherwise the same as that used in Experiment 1. Moreover, unlike in Nunnink et al.’s [42] experiment, the present study included a nonassociative control group that received exposure to both 4% sucrose and DCS, but in an unpaired fashion. If the effects of DCS observed in previous experiments are mediated by a conditioned taste aversion, rather than memory consolidation, then DCS should result in a similar suppression of consummatory behavior in the absence of the downshift event.

6. Method

6.1. Subjects and apparatus

The subjects were 36 male, experimentally naïve Long-Evans hooded rats, approximately 90 days old at the start of the experiment. The average ad libitum weight for the entire sample was 397 g (range: 305–519 g). Rats were maintained as described in Experiment 1. The training apparatus and drug preparation were also the same described previously.

6.2. Procedure

Training lasted 3 daily trials, scheduled to be identical to trials in the cSNC experiments reported above. Subjects received 4% sucrose solution in every trial. Prior to Trial 1, rats were matched by ad libitum weight and then randomly assigned to one of three conditions: Paired (n = 12), Unpaired (n = 12), or Saline (n = 12). All groups received two injections; the first injection was administered immediately at the end of Trial 1 and the second injection was administered 3 h after the end of Trial 1. The first injection was saline for Groups Unpaired and Saline, and DCS (30 mg/kg, ip) for Group Paired. The second injection was saline for Groups Paired and Saline, but DCS (30 mg/kg, ip) for Group Unpaired. Thus, groups were matched in terms of the number and temporal distribution of injections; moreover, Groups Paired and Unpaired were also matched in terms of exposure to DCS.

7. Results

The results are shown in Fig. 5. A Group × Trial (1–3) analysis showed a significant increase of behavior across trials, F(2, 66)=67.77, p < 0.001, but groups were not significantly different, F(2, 33)=1.14, p >0.30, as was the interaction effect, F <1. One-way ANOVAs for each trial yielded not significant group effect for Trials 1–3, F(2, 35)<1.57, ps >0.22. The not significant difference obtained on Trial 1 indicates that subjects were behaviorally
matched before the treatment. These results provide no evidence that the effects of DCS reported in Experiment 1 were the result a conditioned taste aversion.

8. General discussion

The current studies demonstrated that the partial NMDAR agonist DCS retarded recovery from cSNC when administered immediately following the first trial involving a 32-to-4% sucrose incentive downshift. DCS failed to induce conditioned taste aversion, suggesting that its suppressive effects on consummatory behavior were not the result of an acquired rejection of the relatively novel 4% sucrose solution in downshifted animals. Furthermore, DCS had no detectable effect on the consummatory behavior of nonshifted rats. These results suggest that the effects of DCS on cSNC were not caused by performance factors (i.e., perceptual, motivational, or motor effects). Therefore, the effects of DCS were restricted to animals that had some experience with an incentive downshift event—an interpretation consistent with a mnemonic effect of DCS. These results were interpreted to support the hypothesis that NMDA receptor activation via the glycine-modulatory site enhances the consolidation, hence facilitating retrieval of the emotional aversive memory induced by the unexpected incentive downshift event.

The effects of posttrial DCS administration were interpreted as acting on mnemonic processes that presumably occur after DCS administration, but conclude prior to next day's trial. It is implausible that DCS is still active after its administration [12,13]. Behavioral evidence from the present experiments (e.g., unshifted groups in both experiments) and from fear conditioning experiments [48] suggests that the dose used here (30 mg/kg, ip) does not have measurable behavioral effects 24 h after its administration.

When DCS was selected for study (see Section 1 for rationale), it was assumed that DCS facilitated memory consolidation based mainly on results from the fear conditioning situation. Recent evidence suggests that the effects of DCS on fear extinction reflect the erasure of the original fear acquisition, rather than an enhancement of fear extinction learning [53]. Mao et al. [53] reported that DCS facilitated the reversal of postsynaptic changes caused as a result of fear extinction, as assessed in terms of a decreased surface expression of GluR1, a subunit of AMPA(α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid) receptors, a type of receptor known to mediate conditioning [54]. Thus, DCS may act to facilitate NMDA-mediated endocytosis of AMPA receptors, erasing learning.

This memory-erasure hypothesis can be applied to the cSNC situation as follows. With posttrial administration (Experiment 1), it could be argued that DCS erased the emotional memory of the downshift event acquired during the first downshift trial. This would predict a cSNC effect on the second downshift trial (Trial 12) that would be essentially identical to that of the first downshift trial (Trial 11). Instead, Experiment 1 showed that DCS-treated animals exhibited greater early suppression on the second and third downshift trials (Trials 12–13) than saline-treated animals, an effect contradicting the memory erasure hypothesis. Moreover, this hypothesis cannot adequately explain the effects of DCS on maze learning, conditioned place preference, and conditioned taste aversion reported by others [42,43,47].

The effects of DCS on cSNC reported here were assumed to be mediated by an increase in the activity of NMDA receptors, which would lead to the strengthening of the emotional memory of the downshift event. However, available evidence suggests that DCS could act in some cases as an antagonist. For example, Hoyt et al. [33] have shown that DCS’s binding to the NMDA receptor is only approximately 40–50% as effective as glycine. Thus, under an appropriate combination of DCS dose and extracellular glycine levels, DCS could actually reduce NMDA-receptor effectiveness. Along these lines, Saul’skaya and Solov’eva [55] reported that glycine levels in the nucleus accumbens are transiently elevated following forced changes in feeding behavior in rats. This is especially relevant to the current studies for two reasons. First, the nucleus accumbens shows increased c-Fos transcription (a transcription factor for immediate early gene expression correlated with synaptic changes such as growth and protein kinase activity) and reduced dopamine efflux following 32-to-4% sucrose downshift [56,57]. Second, increased glycine levels in Saul’skaya and Solov’eva’s [55] study were observed selectively when animals were exposed to both feeding and a fear-inducing stimulus previously paired with shock-induced pain. Thus, it is plausible that the forced rearrangement of feeding behavior caused by incentive downshift in the cSNC situation acted to increase glycine levels, thus converting DCS into an antagonist at the glycine site. Despite these potential interactions of DCS and extracellular glycine levels, the present results do not support the hypothesis that DCS acted as an antagonist. Experiments 1 and 2 showed that the effectiveness of DCS scaled to the magnitude of the downshift. DCS interfered with recovery from cSNC following a 32-to-4% sucrose downshift, but had no detectable effect following a 32- to 6% sucrose downshift. If 30 mg/kg DCS acted as an antagonist, then the effects of posttrial DCS administration following these two levels of incentive downshift should have yielded similar results. However, if DCS at 30 mg/kg acted as an agonist, then its effects should be directly related to the size of the disparity between preshift and postshift incentives. Therefore, the results reported here indicate that DCS functioned as an agonist in the cSNC preparation.

The present results are consistent with the hypothesis that DCS enhanced the consolidation of the emotional memory of the incentive downshift event. This is so because DCS retarded recovery from cSNC, a consequence consistent with the strengthening of an emotional memory. Because posttrial administration of corticosterone also retarded recovery from cSNC [22,23], it is tempting to argue that these two effects are related. The memory enhancing effects of stress hormones like corticosterone, epinephrine, and norepinephrine are well documented [15,17]. These mnemonic effects of stress hormones are mediated by glutamatergic receptors in the brain. For example, norepinephrine levels enhance contextual fear conditioning in mice by phosphorylating GluR1 subunits of the AMPA receptor [54]. Similar interactions occur between glucocorticoid administration and activation of NMDA receptors. For example, restraint stress enhances allostynia (increased pain
sensitivity to nonpainful stimuli) induced by neuropathic pain and this effect is mediated by glucocorticoid release. Corticotoc Stere analysis often has similar effects to those of restraint stress and its effects on allodynia are eliminated by coadministration of memantine, an NMDA receptor antagonist [58]. Because the effects of corticosterone in other situations are mediated by glutamatergic activity [32], we hypothesize that the retardation of recovery from cSCN induced by corticosterone and DCS reflects effects at two different stages of the same cascade leading to enhanced consolidation of the emotional memory of the downshift.

Acknowledgements

The research reported here was partially supported by SERC grant # 80301, TCU School of Science and Engineering. The authors thank C. Torres, A. E. Mustaca, and M. F. Lopez-Seal for valuable comments on an earlier version of the manuscript.

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