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Memory interfering effects of chlordiazepoxide on consummatory successive negative contrast

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ABSTRACT

Long–Evans rats downshifted from 32% to 4% sucrose solution exhibit lower consummatory behavior during downshift trials than rats exposed only to 4% sucrose. In Experiment 1, this effect, called consummatory successive negative contrast (cSNC), was attenuated by administration of the benzodiazepine anxiolytic chlordiazepoxide (CDP, 5 mg/kg, ip) before the second downshift trial (Trial 12), but was not affected when CDP was administered before the first downshift trial (Trial 11). In Experiment 2, CDP administered after Trial 11 actually enhanced the cSNC effect on Trial 12. This posttrial effect of CDP was reduced by delayed administration (Experiment 3). This CDP effect was not present in the absence of incentive downshift (Experiments 4–5), or when animals were tested with the preshift incentive (Experiment 6) or after complete recovery from cSNC (Experiment 7). The posttrial CDP effect was observed after an 8-day interval between Trials 11 and 12 (Experiment 8) and when administered after Trial 12, rather than Trial 11 (Experiment 9). Experiment 10 extended the effect to Wistar rats. Because CDP is a memory interfering drug, it was hypothesized that its posttrial administration interferes with the consolidation of the memory of the downshifted incentive, thus prolonging the mismatch between expected (32% sucrose) and obtained (4% sucrose) incentives that leads to the cSNC effect.

1. Introduction

In a typical experiment on consummatory successive negative contrast (cSNC), two groups of food-deprived rats receive access to either 32% or 4% sucrose solution during 10 daily trials, followed by access to 4% sucrose during subsequent trials. cSNC involves the transient suppression of consummatory behavior in the group exposed to an incentive downshift from 32% to 4% sucrose, relative to the 4-to-4% sucrose, unshifted controls (Flaherty, 1996). An intriguing property of cSNC is the apparent selectivity with which consummatory performance can be affected by the benzodiazepine anxiolytic chlordiazepoxide (CDP) on Trials 11 and 12—the first and second downshift trials (Flaherty et al., 1986, 1990). Whereas CDP significantly reduces cSNC on the second downshift trial, it has no apparent effects on the first downshift trial. A similar trial selectivity was observed with other anxiolytics (Flaherty, 1996). Additional studies demonstrated that CDP can have a contrast-reducing effect on the first downshift trial provided that trial is longer than the typical 5 min (Flaherty et al., 1986) or that rats are exposed to repeated cycles of incentive downshift (Flaherty et al., 1996).

Flaherty (1996) considered several hypotheses that could explain this trial selectivity of CDP, but none of them includes a direct reference to a memory process. He favored the idea that CDP reduces the negative emotion induced by incentive downshift, which would peak on the second downshift trial. To explain CDP's lack of action on the first downshift trial, Flaherty (1996) argued that the initial reaction to the downshift involves search behavior, rather than emotional activation. Unlike Flaherty's (1996) account, the present view incorporates memory processes to account for the cSNC effect. We suggest that the dependence of these CDP effects on experience with the downshifted solution, as illustrated by experiments with trials longer than the typical 5 min and with repeated downshifts (see above), suggests a memory-related mechanism (Bentosela et al., 2006; Norris et al., 2011). In the cSNC situation, there are at least three relevant memory sources: (1) the memory of the preshift incentive, formed during the initial trials of exposure to 32% sucrose; (2) the memory of the emotional response to the downshift event, formed during and after the first downshift trial (usually Trial 11); and (3) the memory of the downshifted solution, formed during subsequent downshift trials. Because (1) and (3) are incentive memories (i.e., environmental events), they were called "allocentric" (the prefix "allo" implies external to the organism), but because (2) is an emotional memory (i.e., internal event) it was called "egocentric" (the prefix "ego" implies internal to the organism; Papini, 2003). Therefore, during downshift trials, animals are assumed to encode two different memories: The egocentric memory of the negative emotional experience and the allocentric memory update of the new, less valued incentive. With posttrial administration, drugs that enhance egocentric memory or interfere with allocentric memory should promote consummatory suppression, whereas drugs that interfere with egocentric memory or enhance allocentric memory should promote the recovery

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of consummatory behavior. Posttrial drug administration is routinely used to modulate memory consolidation (McGaugh, 2000). The drug was not present when the memory is acquired (during the downshift event) and it is excreted before the next trial is administered, 24 h later. In rats, a single oral dose of CDP (10 mg/kg) has a half-life of 4–6 h (Koechlin and D'Arconte, 1963). Therefore, CDP could only influence consummatory behavior if it had memory effects, either on memory consolidation or via conditioned taste aversion.

Generally speaking, drugs affecting memory are either memory enhancing or memory interfering drugs (Amadio et al., 2004; Hirshman, 2004; McGaugh and Izquierdo, 2000). Thus, the working hypothesis is that if the administration of a memory enhancing drug after Trial 11 in the cSNC situation increases the cSNC effect, it can only act by potentiating the egocentric emotional memory of the downshift (i.e., enhancing allocentric memory should lead to attenuated cSNC because the expected incentive would tend to match the obtained incentive). Recent research with memory enhancing drugs administered after Trial 11, such as corticosterone (Bentosela et al., 2006; Ruetti et al., 2009) and D-cycloserine (Norris et al., 2011), have shown that the cSNC can indeed be increased and extended, thus retarding the recovery of consummatory behavior. The present series of experiments follows a similar logic, but using the anxiolytic CDP, a drug that acts at the benzodiazepine site of the type-A gamma-amino butyric acid receptor. CDP was selected because it has been shown to affect cSNC, as described above, and it has been shown to affect memory in other situations. CDP and other benzodiazepines have been described as causing memory impairment in avoidance conditioning, spatial learning, and step-down inhibitory avoidance (Flood et al., 1998; Ghoneim, 1992; Herzog et al., 2000; Izquierdo et al., 1990; Olaman and McNaughton, 2001; Silva and Frussa-Filho, 2000). If CDP is a memory-interfering drug in the cSNC situation, then it should either (1) cause animals to recover faster from the downshift (interpreted as interference with egocentric memory), or (2) cause animals to recover more slowly (interpreted as interference with allocentric memory). This series of experiments starts by asking whether the trial-selectivity of pretrial CDP in the cSNC situation is reproduced under the current conditions. Subsequent experiments explore the effects of posttrial CDP administration on cSNC and the extent to which such effects depend on an experience of incentive downshift.

2. Experiment 1: Pretrial 11 vs. 12

The main outcome consistent with an anxiolytic effect of CDP on cSNC is the selective attenuation of this effect with pretrial drug administration before the second downshift trial (Trial 12), but not when CDP is administered before the first downshift trial (Trial 11). Although several studies reported such a trial selectivity of CDP administration on cSNC (see above), in one study (Genn et al., 2004), pretrial CDP (5 mg/kg, ip) administration reduced cSNC on both the first and second downshift trials. Experiment 1 had two aims: first, to demonstrate the anxiolytic effect under the conditions used in the rest of the experiments and, second, to determine whether the attenuating effect of CDP under these conditions is trial selective (i.e., present on Trial 12, but not on Trial 11).

The Genn et al. (2004) study differed from previous research (see above) in terms of the dependent measure (solution intake, rather than lick frequency), testing environment (home cage, rather than separate conditioning box), and the rat strain (hooded Lister rats, rather than Sprague–Dawley or other commercially available strains). The procedure used in the present and previous studies from our lab differed from other studies in several respects (e.g., Norris et al., 2008, 2011). First, we routinely use goal-tracking time (cumulative time in contact with the sipper tube) as the dependent measure. Goal-tracking time has produced orderly results in a variety of experiments (see Papini, 2009; Papini et al., 2006) and it has been shown to significantly and positively correlate with fluid intake (Mustaca et al., 2002). Similar results were obtained when both goal-tracking time and fluid intake were recorded in the same experiment (Papini et al., 1988; Riley and Dunlap, 1979). Second, training was carried out in a separate conditioning box. Finally, we used Long–Evans rats.

2.1. Method

2.1.1. Subjects

The subjects were 60 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. Animals were bred in the TCU colony, housed in wire-bottom cages with water continuously available during the course of the experiment. At 90 days of age, food was restricted until animals were 81–84% of the free food weight. Temperature (around 23 °C) and humidity (around 50%) were maintained relatively constant and the colony was on a 12 h of light–dark cycle (lights on at 07:00 h). Behavioral testing occurred during the light phase of the cycle. Housing and testing were carried out in an USDA-inspected research facility. All experimental procedures were approved by the Institutional Committee on Animal Care and Use. Animal health was evaluated daily by researchers and periodically by a consulting veterinarian.

2.1.2. Apparatus

Animals were tested in 4 conditioning boxes constructed of aluminum and Plexiglas, 29.3 cm long, 21.3 cm high, and 26.8 cm wide. The floor was made of steel rods 0.4 cm in diameter and 1.6 cm apart that ran parallel to the feeder wall. A tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine. A sipper tube (1 cm in diameter and protruding 1.5 cm from the feeder wall when fully inserted) was automatically inserted and retracted to deliver the sucrose solution. This sipper tube was inserted through an elliptical hole in the feeder wall, 1 cm wide, 2 cm high, and 4 cm from the floor. Contact with the sipper tube was recorded automatically by the closing of an electric circuit between the sipper tube and the steel floor. Each conditioning box was enclosed in a sound-attenuating chamber 57.5 cm long, 36.9 cm high, and 39.4 cm wide. This chamber also had a speaker and a fan, which together register 80.1 dB (SPL, scale C). The control of the sipper tube and recording of the response were performed by a computer located in an adjacent room.

2.1.3. Training procedure

When the weights reached the target deprivation criterion, animals were randomly assigned to one of six groups ($n = 10$) depending on the drug administered before Trial 11 or 12, either saline or CDP: 32/Sal/Sal, 32/CDP/Sal, 32/Sal/CDP, 4/Sal/Sal, 4/CDP/Sal, and 4/Sal/CDP. In this design, the same saline controls can be used for each of the two CDP conditions, Trial 11 or Trial 12. For two groups (one downshifted and one unshifted), the two injections were equal-volume saline injections (32/Sal/Sal and 4/Sal/Sal). Two other groups received CDP (5 mg/kg, ip) before Trial 11 and vehicle before Trial 12 (32/CDP/Sal and 4/CDP/Sal). The final two groups received the vehicle injection before Trial 11 and CDP before Trial 12 (32/Sal/CDP and 4/Sal/CDP).

All animals received training during 12 daily trials each lasting 5 min starting after the first contact with the sipper tube was detected. For 3 groups (downshifted groups), 32% sucrose was available during Trials 1–10, followed by 4% sucrose during Trials 11–12. For the other three groups, 4% sucrose was available during the 12 trials. Solutions were prepared w/w by mixing 32 g (or 4 g) of commercial sugar with 78 g (or 96 g) of distilled water and administered at room temperature. All animals received two injections (before Trials 11 and 12), 30 min before the start of each trial.

Rats received training in squads of four. Each animal was always in the same squad and trained in the same conditioning box, but the order of squads was randomized across days. Conditioning boxes were cleaned with a damp paper towel after each trial. 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. A recording period

started when a rat contacted the sipper tube and lasted 5 min. Retraction of the sipper tube was followed by a variable interval of 30 s (range: 15–45 s).

2.1.4. Drug preparation

CDP was prepared by mixing the appropriate amount of chlordiazepoxide hydrochloride (Sigma-Aldrich, Saint Louis, MO) with 1 ml of saline. The stock solution was then diluted to the 5 mg/kg dose. This dose has been shown effective in previous cSNC studies (see Flaherty, 1996). CDP was prepared 24 h prior to Trial 11. Isotonic saline solution was used as vehicle.

2.1.5. Dependent variable and statistics

The dependent variable was goal-tracking time: the cumulative time in contact with the sipper tube (recorded in 0.01-s units), up to a maximum of 5 min. Goal-tracking time typically yields nonsignificant preshift differences, but this is not uncommon with lick frequency data (for one example, see Flaherty, 1996, p. 56). Goal-tracking times were subject to nonparametric analyses using the Mann–Whitney *U* test, with an alpha value set at the 0.05 level, for a 2-tailed distribution. The analyses in the present and subsequent experiments are restricted to pairwise comparisons among the groups of interest in specific trials. The simplicity of nonparametric tests combined with their equivalent power (for the sample sizes used in the present experiments) to the more typical analysis of variance determined our choice. Moreover, given that the cSNC effect sometimes results in low levels of responding for downshifted animals, relative to unshifted controls, at least during the initial postshift trials, violating the equal-variance assumption is irrelevant for nonparametric tests. All statistics were computed using the IBM SPSS Statistics 21 package.

2.2. Results and discussion

Performance during the preshift trials was similar across conditions. The mean (±SEM) goal-tracking times during Trials 1–10 were 154.56 s (±5.7) and 157.19 s (±6.0) for animals exposed to 32% or 4% sucrose, respectively. The difference was not significant, $U(30, 30) = 414$, $p = 0.60$. Notice that the average across all preshift trials (Trials 1–10) implies that these scores will necessarily be lower than those obtained on any individual postshift trial; this is because goal-tracking times start at a low level and then increase gradually across preshift trials. This applies to all the experiments testing for cSNC in this series. The goal-tracking times for Trial 10, the last preshift trial, were 214.58 s (±7.8) and 221.50 s (±8.2) for 32% and 4% sucrose animals, respectively. The difference was not significant, $U(30, 30) = 427$, $p = 0.73$.

The main results are presented in Fig. 1 in terms of the first (Trial 11) or second (Trial 12) downshift trial. For the first downshift trial, pairwise comparisons between 32/Sal/Sal vs. 4/Sal/Sal and between 32/CDP/Sal vs. 4/CDP/Sal indicated that both differences were significant, $Us(10, 10) < 9$, $ps < 0.003$. Furthermore, comparisons between 4/Sal/Sal vs. 4/CDP/Sal and 32/Sal/Sal vs. 32/CDP/Sal yielded nonsignificant results, $Us(10, 10) > 29$, $ps > 0.13$. Thus, there was no evidence that pretrial CDP administration affected the cSNC effect during the first downshift trial (Trial 11).

Pretrial CDP administration before the second downshift trial (Trial 12) eliminated the cSNC effect. Thus, whereas 32/Sal/Sal was significantly suppressed relative to 4/Sal/Sal, $U(10, 10) = 20$, $p = 0.023$ (i.e., a cSNC effect in saline groups), 32/Sal/CDP and 4/Sal/CDP did not differ from each other, $U(10, 10) = 29$, $p = 0.112$ (i.e., no evidence of cSNC in CDP-treated groups). Additionally, goal-tracking times were not different between 4/Sal/Sal vs. 4/Sal/CDP, $U(10, 10) = 41$, $p = 0.496$, and, although there was a trend, 32/Sal/Sal and 32/Sal/CDP were also not different from each other, $U(10,10) = 27$, $p = 0.082$.

The trial-selective effects of CDP observed in the present experiment are consistent with the results obtained with lick rate (Flaherty et al., 1990), but differ from those reported for fluid intake (Genn et al., 2004). The latter dependent variable showed a reduction of cSNC on both Trials 11 and 12 with the 5 mg/kg dose used here. The reasons for this discrepancy are unclear. As noted earlier, in addition to the dependent variable, Genn et al. (2004) ran their experiment in the animal's home cage and used a different rat strain. For the present purpose, however, these results serve to validate the procedure used to induce and measure the cSNC effect.

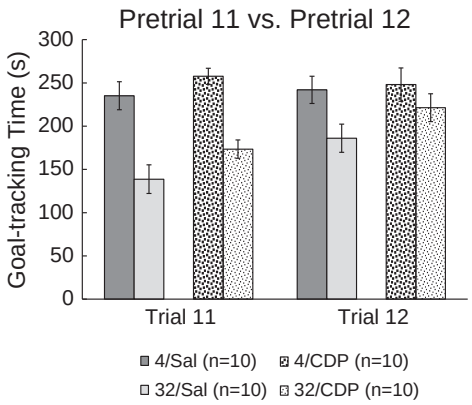

Fig. 1. Mean (±SEMs) goal-tracking time for groups downshifted from 32% to 4% sucrose (32) and unshifted controls receiving only access to 4% sucrose (4). These groups received pre-trial administration of either chlordiazepoxide (CDP, 5 mg/kg, ip) or saline (Sal, equal volume) during the first downshift trial (Trial 11) or second downshift trial (Trial 12). The design adopted in this experiment allowed us to use the same unshifted controls for comparison with groups given CDP on Trial 11 or 12. Thus, the bars for saline controls come from the same two groups, but the bars for CDP groups come from four different groups, for a total of six groups.

3. Experiment 2: Posttrial 11 CDP administration

Our starting hypothesis assumed that posttrial CDP administration would interfere with the egocentric memory of the downshift, thus facilitating recovery from cSNC. There appears to be no published research using the posttrial administration procedure with CDP. Other benzodiazepine ligands have been shown to have no measurable effect on passive avoidance when administered after training (Izquierdo et al., 1990).

3.1. Method

The subjects were 45 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. Other aspects of maintenance and apparatus were as described in Experiment 1.

Training started when animals reached the target deprivation criterion. Animals were matched by ad lib weight in pairs and then randomly assigned to one of two groups depending on the sucrose solution received during the 10 preshift trials, either 32% or 4%. For Trials 11–12, all animals had access to 4% sucrose. Prior to Trial 11, animals within each preshift condition were matched by preshift performance and randomly assigned to one of two groups receiving either saline (4/Sal and 32/Sal) or CDP (4/CDP and 32/CDP). CDP (8 mg/kg, ip) or saline (equal volume) was administered immediately following Trial 11. CDP was prepared as described in Experiment 1. A larger CDP dose was chosen (8 vs. 5 mg/kg in Experiment 1) to facilitate diffusion into the central nervous system, on the assumption that memory encoding of the events occurring on Trial 11 would be time dependent (McGaugh, 2000). This dose is also known to be effective when administered before Trial 12 (see Flaherty, 1996).

Because the aim of posttrial drug administration was to determine the effect of a specific compound on cSNC, an animal had to show a minimum amount of response suppression following the downshift to be included in this experiment. The criterion used was that an animal's

goal-tracking time on Trial 11 had to be 85% or less than the goal-tracking time exhibited by that animal on Trial 10. This criterion was applied in all the experiments that followed. Only failures to pass this minimum suppression criterion were noted hereafter. All other features were as described in Experiment 1.

3.2. Results and discussion

Preshift performance for 32% and 4% sucrose averaged over Trials 1–10 was not significantly different, $U(22, 23) = 242$, $p = 0.803$. The goal-tracking times for 32% and 4% groups, on Trial 10 (Fig. 2), were not different from each other, $U(24, 21) = 241$, $p = 0.80$.

Performance on Trial 11, the first postshift trial and the one before CDP administration, was significantly lower in 32-to-4% downshifted groups, whether assigned to the saline or CDP groups, $Us = 10$, $ps = 0.001$. Moreover, there were no differences between the two 4% groups, $U(12, 12) = 71$, $p = 0.954$, and the two 32% groups, $U(10, 11) = 53$, $p = 0.888$. Thus, both groups exhibited a similar cSNC effect before CDP was administered.

Fig. 2 also shows the consequence of Posttrial 11 CDP administration on Trial 12, the following day. There was recovery of goal-tracking times in downshifted saline animals, such that the difference between 32/Sal and 4/Sal was no longer significant, $U(12, 10) = 34$, $p = 0.086$. However, Posttrial 11 CDP administration reduced the performance of downshifted animals such that the difference between 32/CDP and 4/CDP on Trial 12 was highly significant, $U(12, 11) = 10$, $p = 0.001$. Whereas CDP also seemed to affect the performance of unshifted controls, the difference between 4/Sal and 4/CDP on Trial 12 was not significant, $U(12, 12) = 60$, $p = 0.488$. However, 32/CDP suppressed consummatory behavior significantly more than 32/Sal on Trial 12, $U(10, 11) = 8$, $p = 0.001$.

Administration of CDP immediately after Trial 11 enhanced the cSNC effect on Trial 12, the day following drug administration. The remaining experiments were designed to characterize the time limits of this effect and the extent to which it was specific to the incentive downshift experience.

4. Experiment 3: Immediate vs. delayed CDP administration

If posttrial CDP affects cSNC by interfering with allocentric memory, then the effect should be time dependent (McGaugh, 2000). Thus, for example, Posttrial 11 corticosterone enhances cSNC when administered immediately after the trial, but not when administered 3 h after the trial (Bentosela et al., 2006). The same design was used in this experiment to test for time dependency.

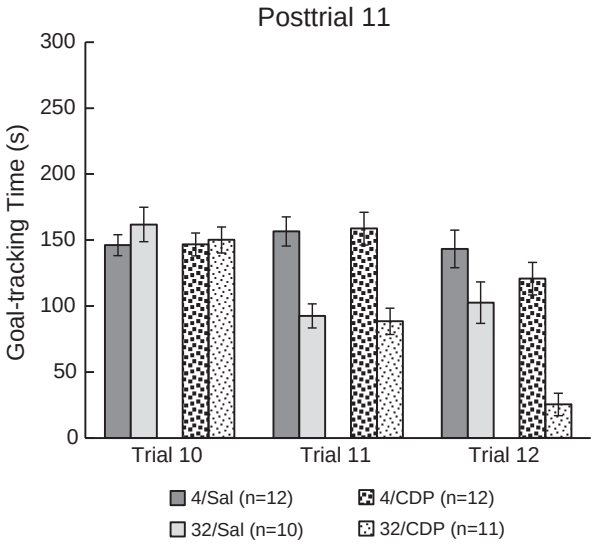

Fig. 2. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline were administered immediately after Trial 11. The performance on Trial 10, the last preshift trial, is also shown.

4.1. Method

The subjects were 60 Long-Evans experimentally naïve, male rats, approximately 90 days of age at the start of the experiment, and maintained as described in Experiment 1. Animals were tested in 8 conditioning boxes similar to those described in Experiment 1.

Animals were matched by ad lib weight and randomly assigned to the two sucrose conditions, 32% and 4%, administered during preshift Trials 1–10. Following Trial 10, animals within each sucrose concentration condition were matched in triplets by preshift performance and randomly assigned to one of three groups depending on the timing and type of drug administration, either immediately after Trial 11 or 3 h later, and either CDP (8 mg/kg, ip) or saline (equal volume). Six groups were thus established: 4/Sal/Sal, 32/Sal/Sal, 4/Sal/CDP, 32/Sal/CDP, 4/CDP/Sal, and 32/CDP/Sal. Therefore, animals were matched in terms of the number of injections (two) and timing of the injections (immediately after the trial or 3 h after the trial). All other features were as described in Experiment 2.

4.2. Results and discussion

There was a significant difference between groups during preshift trials (Trials 1–10), with 32% sucrose supporting higher levels of consummatory behavior than 4% sucrose, $U(29, 31) = 275$, $p = 0.01$. Fig. 3 shows the performance on Trial 10. By the time groups reached the last preshift trial, the differences between 32% and 4% sucrose groups were no longer significant, $U(29, 31) = 363$, $p = 0.20$.

On Trial 11, right before drug administration, all downshifted vs. unshifted groups exhibited a similar cSNC effect, $Us < 21$, $ps < 0.02$. Moreover, 4% and 32% sucrose groups to be treated with saline did not differ from any of the groups to be treated with CDP, $Us > 40$, $ps > 0.62$.

Fig. 3 also shows the results for Trial 12. There were significant cSNC effects in comparisons between Groups 32/Sal/Sal vs. 4/Sal/Sal and between Groups 32/CDP/Sal vs. 4/CDP/Sal, $Us < 22$, $ps < 0.03$. However, Groups 32/Sal/CDP and 4/Sal/CDP fell short of statistical significance on Trial 12, $U(10, 10) = 25$, $p = 0.059$. None of the CDP-treated 4% or 32% groups differed from saline controls, $Us > 39$, $ps > 0.29$; this includes the comparison between 32/CDP/Sal and 32/Sal/Sal. Although in the same direction as that observed in the previous experiment, this time the difference was not significant.

This experiment provided only a weak confirmation of the enhancing effect on cSNC by Posttrial 11 CDP administration observed in the previous experiment. The effect is weak in that cSNC was observed both in saline animals and in animals that received CDP immediately after Trial 11. However, given the confirmation of this result obtained in subsequent experiments, this partial failure to confirm the effect is taken with caution. What this experiment did provide is a lack of evidence that CDP administration 3 h after Trial 11 made a measurable impact on consummatory behavior the following day. This result is consistent with at least two hypotheses: (1) That CDP has no effect after a time window for the consolidation of the downshift memory has elapsed, or (2) that delayed CDP administration does not support measurable conditioned taste aversion in this preparation.

5. Experiment 4: Testing for conditioned taste aversion

Posttrial drug administration can have at least two effects. One is that it can modulate (enhance or interfere) memory consolidation. This is the effect being tested in this series of experiments. However, the effects of a drug administered after a given experience may induce

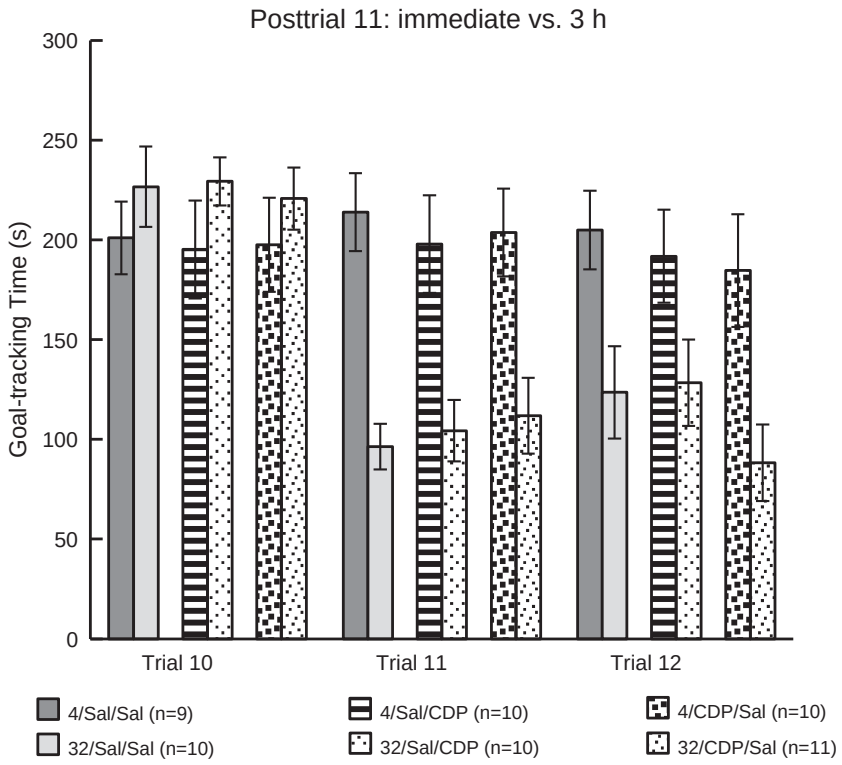

Fig. 3. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 11 or 3 h after the end of that trial. The performance on Trial 10, the last preshift trial, is also shown.

aversion to antecedent cues. In this case, the taste of the 4% sucrose on Trial 11 (the putative conditioned stimulus) paired with the effects of CDP administered after that trial (the putative unconditioned stimulus) may have generated sufficient conditioned taste aversion to suppress consummatory behavior on Trial 12. This effect may be stronger in downshifted animals because the 4% sucrose solution was relatively more novel than in unshifted controls, which had access to the solution during 10 previous trials without any negative consequence. Conditioned taste aversion is known to be attenuated by prior nonreinforced preexposure to the taste—a latent inhibition effect (Cannon et al., 1983; Lubow, 2009). The taste aversion hypothesis was tested under conditions that should maximize its occurrence: a single exposure to 4% sucrose followed by CDP administration immediately after the trial. It should be noted that, as far as the authors could determine, there appears to be no published evidence that CDP or any other benzodiazepine supports conditioned taste aversion. As is often the case with conditioned taste aversion studies, such demonstrations rely on a comparison between a taste-CDP condition and a taste-only control, that is, a group receiving access to the taste followed by an injection of saline vehicle (Cappell et al., 1973). Such CS-only group neglects to control for the potential nonassociative effects of CDP. The present experiments include both a saline control and also, most importantly, an unpaired control receiving both sucrose and CDP, but separated by a relatively long interval. In fact, benzodiazepines are known to enhance the palatability of sweet solution, in addition to their anxiolytic and memory impairment effects (e.g., Berridge, 1988).

5.1. Method

The subjects were 30 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. All other aspects of maintenance were as described in Experiment 1 and the apparatus were the same 8 boxes used in Experiment 3.

Triplets matched on ad lib weight were formed and randomly assigned to one of three groups: Sal/Sal, Sal/CDP, and CDP/Sal ($n = 10$). Animals received access to 4% sucrose during three trials. Immediately following Trial 1, all animals received two injections, one immediately after the trial and the other 3 h later (as done in Experiment 3). Group names refer to the immediate and delayed injections. Therefore, groups were matched in terms of the number and timing of the injections. Animals were administered either CDP (8 mg/kg, ip) or saline (equal volume).

This design involves a paired condition (Group CDP/Sal), in which the events in Trial 1 were paired with the effects of CDP, and two controls groups. First, an unpaired control (Group Sal/CDP), in which CDP was administered after 3 h. Paired and unpaired groups were matched in terms of the putative conditioned and unconditioned stimuli (sucrose and CDP effects, respectively). Second, an injection control (Group Sal/Sal).

The results were analyzed using the Kruskal–Wallis nonparametric test for multiple groups, taking each of the three trials separately. All other features were as described in Experiment 1.

5.2. Results and discussion

Fig. 4 shows the results of this experiment. The low goal-tracking times are typical of the initial trials in the cSNC situation. There was a tendency for Posttrial 1 CDP administration to reduce goal-tracking times relative to saline controls, whether administered immediately after the trial or three hours later. However, the comparison between the paired (CDP/Sal) and unpaired (Sal/CDP) groups, controlling for nonassociative factors, yielded no evidence of any conditioning trend. Consistent with the figure, an analysis of the three groups in each trial failed to reveal any significant difference, $\chi(10, 10, 10) < 5.82$, $ps > 0.05$. Thus, there was no support for the hypothesis that the effects of posttrial CDP administration reduce subsequent consummatory behavior due to the development of a conditioned taste aversion.

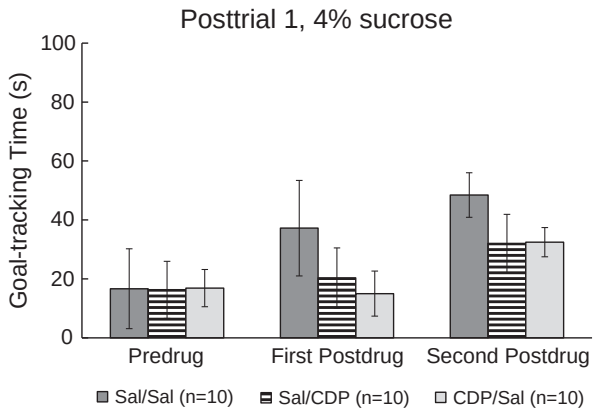

Fig. 4. Mean (±SEMs) goal-tracking time for three groups receiving only 4% sucrose in three trials. CDP (8 mg/kg, ip) or saline was administered immediately or 3 h after Trial 1 (Predrug).

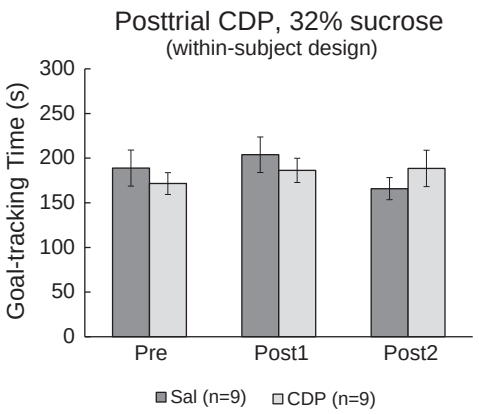

Fig. 5. Mean (±SEMs) goal-tracking time for a group of rats giving only access to 32% sucrose. CDP (8 mg/kg, ip) or saline was administered immediately after Trials 11 and 14, in a counterbalanced order. The average performance on the trial preceding the injections (Trials 11 and 14) is also shown (Pre).

6. Experiment 5: Posttrial CDP with only 32% sucrose experience

Posttrial CDP does not affect consummatory behavior when animals only had either one (Experiment 4) or 11 (unshifted controls, Experiment 2) trials of access to 4% sucrose (i.e., in the absence of an incentive downshift). Using a within-subject design, this experiment explored the possibility that posttrial CDP would affect performance when animals only had access to 32% sucrose, again in the absence of an incentive downshift.

6.1. Method

The subjects were 11 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. All other aspects of maintenance were as described in Experiment 1. The apparatus were the same 8 boxes used in Experiment 2.

All animals received training during 17 daily trials with 32% sucrose. Immediately after Trial 11, one group ($n = 6$) received CDP (8 mg/kg, ip) and the other ($n = 5$) received saline (equal volume). After Trial 14, the animals that had received saline after Trial 11 were treated with CDP, whereas the animals that had received CDP after Trial 11 were treated with saline. Therefore, the order of drug administration was counterbalanced to create a within-subject design. These results were analyzed using a Wilcoxon signed-ranks test for dependent samples. Pairwise comparisons were done for each trial separately. All other features were as described in Experiment 1.

6.2. Results and discussion

Two animals were excluded from analysis because they were given CDP twice by mistake. Fig. 5 shows the results of this experiment. It was clear that posttrial CDP administration does not affect consummatory behavior when animals receive only exposure to 32% sucrose. Saline and CDP groups did not differ in any of the three trials plotted in Fig. 5, $Zs < -1.60$, $ps > 0.10$. Thus, Experiments 4 and 5 failed to detect any evidence that posttrial CDP administration affected goal-tracking times in the absence of a downshift experience, whether in animals that received only 4% sucrose or only 32% sucrose. This is consistent with the lack of differences among unshifted, 4% sucrose controls in previous experiments. Therefore, the effects of posttrial CDP appear to emerge when the animal has been exposed to an incentive downshift event.

7. Experiment 6: Posttrial 11 CDP tested with 32% sucrose on Trial 12

The present experiment tested whether Posttrial 11 CDP, after a typical downshift event, would affect performance even if the animal is tested with 32% sucrose on Trial 12, rather than 4% sucrose. If the posttrial effect of CDP were to suppress drinking behavior independently of the solution, then consummatory suppression should ensue even when the animal is tested with the preshift solution. If, however, CDP interferes with allocentric memory update, then changing the stimulus conditions from 4% sucrose (Trial 11) back to 32% sucrose (Trial 12) should reverse the effects of CDP on behavior, leading to little or no suppression in consummatory behavior.

7.1. Method

The subjects were 44 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. Other maintenance conditions were as described in Experiment 1. The apparatus were the 8 boxes used in Experiment 3.

Animals were matched in pairs by ad lib weight and randomly assigned to one of two groups depending on the preshift sucrose concentration: 32% or 4%. After Trial 10, animals within each sucrose condition were matched in pairs by preshift performance and randomly assigned to groups: 32/CDP, 32/Sal, 4/CDP, and 4/Sal. All animals had access to 4% sucrose on Trial 11, immediately followed by an injection of either CDP (8 mg/kg, ip) or saline (equal volume). On Trial 12, animals exposed to 32% sucrose on Trials 1–10 were again given 32% sucrose, whereas unshifted, 4% sucrose controls continued to receive 4% sucrose. All other features were as described in Experiment 3.

7.2. Results and discussion

Two animals were excluded due to equipment malfunction, one due to illness, and four due to a failure to pass the minimum suppression criterion (see Method, Experiment 2). There were no differences between the groups exposed to either 32% or 4% sucrose in terms of their average preshift performance over Trials 1–10, $U(18, 19) = 159$, $p = 0.715$. However, on Trial 10 (Fig. 6) the differences were significant, $U(18, 19) = 95$, $p = 0.02$.

Downshifted groups exhibited a significant decrease in goal-tracking times during Trial 11 that was virtually identical for groups that received saline or CDP immediately after the trial. A comparison of 32% vs. 4% groups yielded significant differences for both the saline and CDP conditions, $Us < 9$, $ps = 0.003$. Moreover, comparisons between the two 32% sucrose and the two 4% sucrose groups were not significant, $Us > 29$, $ps > 0.35$. Thus, these groups exhibited comparable cSNC effects before drug administration.

When animals in the saline and CDP conditions were tested the following trial with 32% sucrose (rather than the usual 4% downshifted

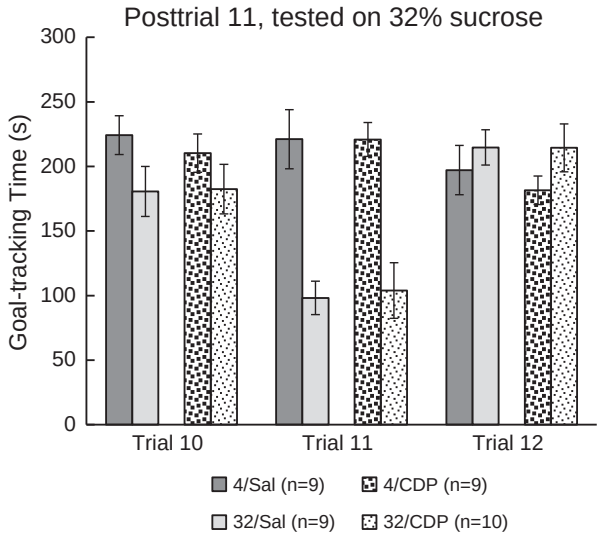

Fig. 6. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 11. However, downshifted groups were given access to 32% sucrose during Trial 12. The performance on Trial 10, the last preshift trial, is also shown.

concentration), they exhibited no evidence of suppression, *Us* >24, *ps* > 0.10. Moreover, neither the two 32% sucrose nor the 4% sucrose groups receiving different drug treatments showed differences, *Us* > 32, *ps* > 0.50. Thus, the Posttrial 11 effects of CDP administration were not observed when animals were tested with the highly palatable 32% sucrose solution on Trial 12.

8. Experiment 7: Posttrial 11 CDP tested after recovery from cSNC

Available evidence shows that once animals recover from cSNC, it is difficult to observe a relapse of the cSNC effect by manipulations known to cause spontaneous recovery in other situations (Norris et al., 2008). One implication is that the update of allocentric memory results in a complete replacement of the preshift memory of the 32% sucrose solution by the postshift memory of the 4% sucrose. A process of memory reconsolidation (Besnard et al., 2012) may underlie such memory update (Mustaca et al., 2009). This hypothesis suggests that posttrial CDP should not affect consummatory behavior in animals that experienced a typical incentive downshift, but have completely recovered from it.

8.1. Method

The subjects were 42 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. All other maintenance conditions were as described in Experiment 1 and the apparatus were the 8 boxes described in Experiment 3.

Animals were matched in pairs by ad lib weight and randomly assigned to a sucrose concentration for preshift trials: 32% or 4%. Animals received 10 preshift trials followed by a postshift phase (4% sucrose for all animals) that extended up to Trial 21 to ensure complete recovery from cSNC. All animals were injected with saline immediately after Trial 11, as in previous experiments. Prior to Trial 20, animals exposed to the downshift or unshifted controls were matched in pairs for prior performance and randomly assigned to one of two groups: 32/Sal ($n = 11$), 32/CDP ($n = 11$), 4/Sal ($n = 10$), and 4/CDP ($n = 10$). Immediately following Trial 20, all animals received an injection of either CDP (8 mg/kg, ip) or saline (equal volume) according to group assignment. All other procedural details were as described in Experiment 1.

8.2. Results and discussion

Rats exposed to 32% sucrose exhibited higher goal-tracking times than rats exposed to 4% sucrose during preshift Trials 1–10, $U(20, 22) = 139$, $p = 0.041$. However, the difference had dissipated by Trial 10, the last preshift trial (Fig. 7), $U(20, 22) = 179$, $p = 0.302$.

On Trial 11, the two pairs of groups to be given saline or CDP immediately after Trial 20 exhibited comparable cSNC effects. A comparison between 32/Sal vs. 4/Sal and between 32/CDP vs. 4/CDP indicated significant differences for both on Trial 11, *Us*(10, 11) > 16, *ps* < 0.04. Comparisons between the groups exposed to 4% sucrose and 32% sucrose revealed no differences, *Us* > 45, *ps* > 0.62. Thus, the cSNC effects were comparable.

The target trial for CDP administration was Trial 20. At this point, right before the treatment, downshifted and unshifted groups were responding very similarly. A 32% vs. 4% comparison, for each drug condition, yielded nonsignificant differences, *Us* > 37, *ps* > 0.23. Comparisons across both 4%-treated and 32%-treated groups were also not significant, *Us* > 43, *ps* > 0.27. Thus, downshifted groups had completely recovered from the cSNC effect.

Most importantly, Posttrial 20 CDP administration had no detectable effect on consummatory performance on Trial 21. This was shown by nonsignificant effects in comparisons between 32% vs. 4%, between 4%-treated groups, and between 32%-treated groups, *Us* > 42, *ps* > 0.39. Posttrial CDP administration in animals that had exhibited a normal cSNC effect was of no consequence when rats had completely recovered from the incentive downshift. This result is consistent with the reconsolidation hypothesis of allocentric memory as applied to the cSNC situation (Mustaca et al., 2009).

9. Experiment 8: Eight-day retention interval between Trials 11 and 12

Reconsolidation of the allocentric memory would require repeated reactivation of the original memory, which is normally achieved during postshift trials. Thus, it is not mere passage of time, but actual memory reactivation that would result in the replacement of the memory of the 32% sucrose by the memory of the 4% sucrose consumed during postshift trials. If this is correct, then interpolating a retention interval between Trials 11 and 12 should not interfere with the effects of

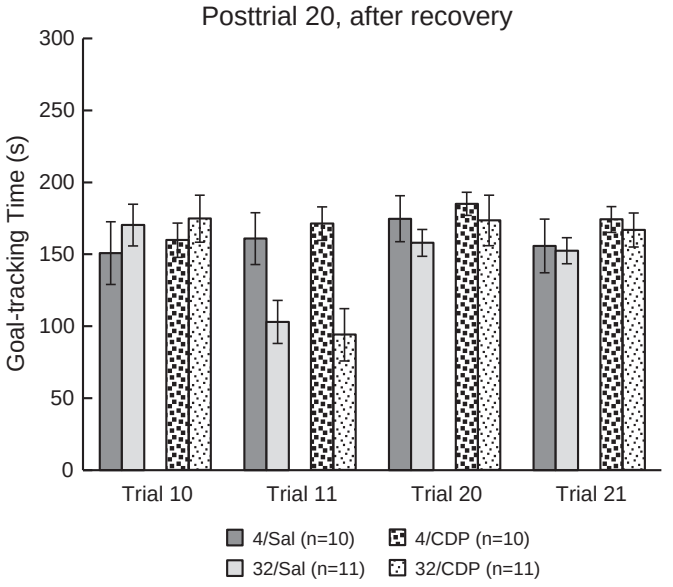

Fig. 7. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 20. The performance on Trial 10, the last preshift trial, is also shown.

Posttrial 11 CDP, although such an interval may weaken the cSNC effect in saline controls. Previous research in the cSNC situation involving retention intervals has focused on the time between the last preshift and first downshift trials (Flaherty, 1996). The goal of those experiments was to affect the memory of the preshift incentive. However, there seems to be no previous research interpolating a retention interval between the first and second downshift trials. In this case, the goal of a retention interval is to assess the memory of the downshift event. In the absence of prior information, an 8-day interval was chosen arbitrarily.

9.1. Method

The subjects were 45 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment. Maintenance conditions were as described in Experiment 1 and the apparatus were those used in Experiment 3.

Animals were matched in pairs according to ad lib weight and randomly assigned to the two sucrose concentrations used during preshift trials, 32% and 4%. Training proceeded as outlined in Experiment 1. Prior to Trial 11, animals were matched, within each sucrose condition, according to preshift performance and randomly assigned to the drug conditions, thus forming four groups: 32/Sal ($n = 10$), 32/CDP ($n = 11$), 4/Sal ($n = 10$), and 4/CDP ($n = 11$). On Trial 11, all animals received access to 4% sucrose. Immediately following Trial 11, animals were administered either CDP (8 mg/kg, ip) or saline (equal volume). Trials 11 and 12 were separated by an 8-day retention interval; during this interval, animals remained in the colony room and were kept on food deprivation. Following the retention interval, animals received an additional trial with access to 4% sucrose.

9.2. Results and discussion

Three animals were excluded from the experiment due to illness. Preshift performance (mean over Trials 1–10) was not significantly different among 32% and 4% sucrose groups, $U(21, 21) = 146$, $p = 0.061$. However, on Trial 10, the last preshift trial (Fig. 8), goal-tracking times were significantly higher for animals exposed to 32% sucrose than to 4% sucrose, $U(21, 21) = 129$, $p = 0.021$.

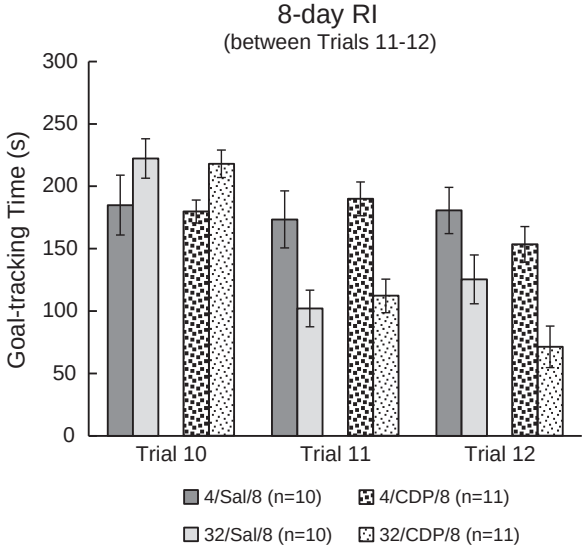

Fig. 8. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 11. Then, a retention interval (RI) of 8 days was interpolated before Trial 12. The performance on Trial 10, the last preshift trial, is also shown.

On Trial 11, both saline- and CDP-treated animals exhibited similar cSNC effects. The 32% vs. 4% comparison was significant for both drug conditions, $Us < 22$, $ps < 0.03$. Moreover, the two 4% groups and the two 32% groups that were to receive different drug treatments after the trial did not differ from each other, $Us = 48$, $ps = 0.622$. Thus, the size of the cSNC observed before implementing the drug treatment was similar for both saline and CDP conditions.

The main results are those obtained on Trial 12, 8 days after the posttrial drug treatment. In this case, a comparison between 32/Sal vs. 4/Sal fell short of significance, showing recovery from cSNC, $U(10, 10) = 26$, $p = 0.07$. However, 32/CDP was significantly more suppressed on Trial 12 than 4/CDP, $U(11, 11) = 14$, $p = 0.003$. Although there was a reduction in goal-tracking times in 4/CDP relative to 4/Sal, that difference was not significant, $U(10, 11) = 39$, $p = 0.26$. Also nonsignificant was the reduction in goal-tracking times in 32/CDP relative to 32/Sal, $U(10, 11) = 30$, $p = 0.078$. Thus, whereas after the 8-day retention interval between Trials 11 and 12 there was no evidence of cSNC in saline-treated animals, the cSNC effect was detected after the retention interval in CDP-treated rats. These results are also consistent with the reconsolidation hypothesis of allocentric memory in the cSNC situation.

10. Experiment 9: Posttrial 12 CDP administration

The cSNC effect is relatively consistent across experiments, but its length varies substantially. It usually lasts between 1 and 3 postshift trials, which implies that the updating of allocentric memory requires at least that amount of training with the downshifted incentive. This observation implies that CDP should also cause consummatory suppression when administered immediately after Trial 12.

10.1. Method

The subjects were 41 experimentally naïve, male Long–Evans rats, approximately 90 days of age at the start of the experiment, maintained as described in Experiment 1, and trained in the same boxes used in Experiment 3.

Animals were matched in pairs by ad lib weight and randomly assigned to either 32% or 4% sucrose for the preshift trials. Training proceeded as described in Experiment 1 except for the following. Prior to Trial 12, animals were matched by prior performance and randomly assigned to one of two groups within each concentration group: 4/Sal ($n = 9$), 32/Sal ($n = 10$), 4/CDP ($n = 9$), and 32/CDP ($n = 10$). Immediately following Trial 12, animals received an injection of either CDP (8 mg/kg, ip) or saline (equal volume), according to group assignment. All animals received 13 trials in this experiment.

10.2. Results and discussion

The mean goal-tracking times averaged across Trials 1–10 were significantly higher for animals with access to 32% sucrose than to 4% sucrose, $U(18, 20) = 64$, $p = 0.001$. Fig. 9 shows the performance on the last preshift trial, Trial 10, where the same difference was also detected, $U(18, 20) = 110$, $p = 0.041$.

Additionally, there were significant cSNC effects in the comparison between 32% vs. 4% on Trial 11, $Us < 15$, $ps < 0.02$, but no detectable differences between the two 4% groups and between the two 32% groups, $Us > 34$, $ps > 0.62$. Thus, the cSNC effect was equivalent on Trial 11 in groups that were scheduled to receive either saline or CDP treatment immediately after Trial 12.

By Trial 12, however, the cSNC effect was statistically dissipated in both saline and CDP 32-vs.-4% comparisons, $Us > 23$, $ps > 0.08$, although there was a trend consistent with the effect in both groups. Trial 12 comparisons between both 4% and both 32% groups soon to receive different drug treatments yielded nonsignificant differences, $Us > 35$, $ps > 0.28$.

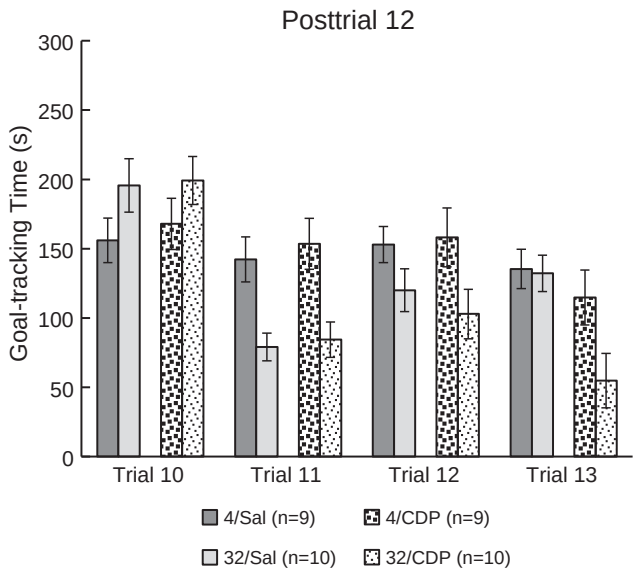

Fig. 9. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 12. The performance on Trial 10, the last preshift trial, is also shown.

The critical results are the performance of these groups on Trial 13, a day after the drug treatment. As can be seen in Fig. 9, 32/Sal and 4/Sal were virtually identical on Trial 13, $U(9, 10) = 44$, $p = 0.935$. As for 32/CDP and 4/CDP, whereas there was a trend toward more suppression of consummatory behavior in 32/CDP than 4/CDP, the difference failed to reach significance, $U(9, 10) = 22$, $p = 0.060$. However, although the two 4% groups did not differ from each other, $U(9, 9) = 27$, $p = 0.233$, goal-tracking times were significantly lower for 32/CDP than for 32/Sal, $U(10, 10) = 18$, $p = 0.016$. Therefore, these results suggest that although the cSNC was clearly dissipating by Trial 12, posttrial CDP administration led to greater consummatory suppression in downshifted animals relative to downshifted saline controls. However, the 32-vs.-4% comparison among CDP-treated rats fell short of significance.

11. Experiment 10: Effect of Posttrial 11 CDP in Wistar Rats

Experiments with immediate administration of CDP after either Trial 11 or 12 have yielded significant cSNC effects in a comparison between 32/CDP vs. 4/CDP in Experiments 2, 3, and 8, but not in Experiment 9. However, a comparison between 32/CDP and 32/Sal has yielded significantly more suppression after CDP treatment in Experiments 2 and 9, but not in Experiments 3 and 8. In this experiment, the basic design of Experiment 2 was applied to Wistar rats, rather than Long–Evans rats. In addition to helping determine the reliability of the posttrial effect of CDP on cSNC, this experiment sought to determine whether this effect was also present in a different strain of rats. Studies with several established rat strains have shown that cSNC is generally a reliable effect (Flaherty, 1996). However, cSNC was shown to be enhanced in Roman low-avoidance rats selected for poor active avoidance learning and known to be prone to show behaviors related to anxiety (Gómez et al., 2009). These findings suggest that the size of the cSNC effect may depend on the rat strain.

11.1. Method

The subjects were 38 experimentally naïve, male Wistar rats, approximately 90 days of age at the start of the experiment. Maintenance conditions were as described in Experiment 1 and the eight boxes used in Experiment 3 served again as apparatus.

Animals were matched in pairs by ad lib weight and randomly assigned to one of two sucrose concentrations, 32% or 4%. All other aspects of the procedure were as described in Experiment 2.

11.2. Results and discussion

Preshift data showed nonsignificant differences in goal-tracking time between rats exposed to 32% sucrose and 4% sucrose, $U(16, 17) = 112$, $p = 0.387$. Similarly, the performance on Trial 10, the last preshift trial (Fig. 10), was not significantly different across sucrose conditions, $U(16, 17) = 118$, $p = 0.533$.

On Trial 11 there were similar cSNC effects in groups to be treated with saline and CDP immediately after the trial, $Us < 5$, $ps < 0.003$. Moreover, there were no detectable differences between the two 4% groups and between the two 32% groups, $Us > 25$, $ps > 0.52$.

The cSNC effect was still evident in both saline- and CDP-treated groups in a 32-vs.-4% comparison, $Us = 3$, $ps < 0.003$. However, whereas the two 4% groups did not differ from each other, $U(8, 8) = 22.5$, $p = 0.318$, Group 32/CDP performed significantly below Group 32/Sal, $U(8, 9) = 7$, $p = 0.005$. Thus, this experiment confirms the suppressive effects on consummatory behavior of CDP administered immediately after Trial 11 and extends this result to the Wistar strain.

12. General discussion

The effects of CDP on cSNC have traditionally been interpreted in emotional terms. The drug is labeled as an "anxiolytic" and it has precisely this role, that is, one of reducing the cSNC effect. The fact that its anxiolytic effects are restricted to the second downshift trial (see Experiment 1; Flaherty et al., 1990) led to the hypothesis that CDP acts to reduce the conflict generated by the tendency to approach the source of the 4% sucrose because of its absolute value and the tendency to withdraw and search for the remembered 32% sucrose (Flaherty, 1996). None of this applies to the basic effect reported here (Experiments 2 to 10), that is, that Posttrial 11 CDP enhanced consummatory suppression the following day in downshifted animals, but not in unshifted controls. Because CDP was administered after the trial, there are only two ways in which it could suppress consummatory behavior on the following trial. One is to induce a conditioned taste aversion; this possibility was tested and rejected in Experiment 4. The other possibility is that

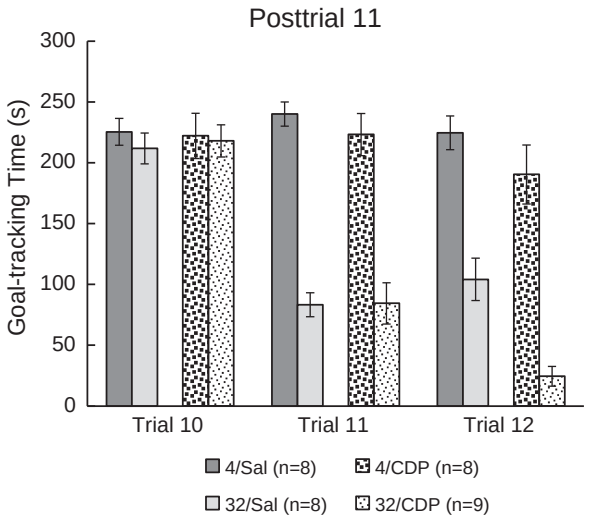

Fig. 10. Mean (±SEMs) goal-tracking time for downshifted (32) and unshifted groups (4). CDP (8 mg/kg, ip) or saline was administered immediately after Trial 11. The performance on Trial 10, the last preshift trial, is also shown. Wistar rats were used in this experiment (Long–Evans rats were used in all previous experiments).

it affected memory consolidation (McGaugh, 2000). This raises three questions.

First, why did Pretrial 12 CDP administration *reduced* cSNC, whereas Posttrial 11 or 12 CDP administration *enhanced* cSNC? Pretrial CDP administration seems to have an effect that does not extend to subsequent trials (Flaherty et al., 1986, 1990). Therefore, the timing of the administration relative to the training trial determines whether the effect would be restricted to that trial (when administered 30 min before the trial, as in Experiment 1) or extended to the next trial, 24 h later (when administered immediately after the trial, as in the other experiments). A timing effect suggests that as CDP administration relative to the trial moves closer to the posttrial administration protocol, the effect should become detectable the following day. If this is correct, CDP administration immediately *before* Trial 12 should lead to effects similar to those observed with Posttrial 12 CDP administration (see Experiment 9).

Second, is this memory effect related to the downshift experience? The present results show that a downshift experience is necessary, but not sufficient, for the posttrial suppressive effect of CDP. Without such an experience, posttrial CDP administered after either 4% sucrose (Experiment 4 and unshifted controls in Experiments 2 and 10) or 32% sucrose (Experiment 5) does not affect consummatory behavior. However, after having a downshift experience, CDP fails to affect consummatory behavior if animals are tested with 32% sucrose (rather than the downshifted 4% sucrose; Experiment 6) or if animals are allowed full recovery from the contrast effect (Experiment 7). The latter result suggests that the posttrial CDP effects require some downshifted experience (one or two postshift trials, Experiments 2 and 9), but not a substantial amount of experience with the downshifted solution (Experiment 7). CDP appears to affect memory formation, but not the retrieval of well-established memories (e.g., Maioli et al., 2012).

Third, what type of memory is being affected by posttrial CDP administration? If the cSNC effect is triggered by a mismatch between expected and obtained incentives–the comparator process (Papini and Pellegrini, 2006)–then reducing this mismatch must attenuate the contrast effect. Enhancing the consolidation/retrieval of the memory encoding the downshift incentive (4% sucrose) should adjust the expectation, thus reducing the mismatch and dissipating contrast. This memory-update process has been called allocentric learning (Papini, 2003). By opposition, interfering with this allocentric update would make it more difficult to retrieve the newly formed encoding of the downshift solution, thus preserving the mismatch longer than what is usually the case. We hypothesize that posttrial CDP administration increases consummatory suppression after the downshift because it interferes with the update of allocentric memory. This hypothesis is consistent with all the results reported in the present experiments and with CDP and benzodiazepines in general being characterized as memory interfering drugs (Flood et al., 1998; Ghoneim, 1992; Herzog et al., 2000; Izquierdo et al., 1990; Olaman and McNaughton, 2001; Silva and Frussa-Filho, 2000). There appears to be no reports describing CDP or any benzodiazepine drug as a memory enhancer. As argued above, interference with the encoding of the egocentric memory of the downshift would have produced the opposite result, namely, a reduction in the size of the cSNC effect.

These experiments also provide support for a memory reconsolidation account of recovery from cSNC. Memory reconsolidation was suggested by studies showing that the reactivation of a consolidated memory opens a transient period of instability resulting in a new memory trace that may not be equal to the original trace. Besnard et al. (2012, p. 69) suggest that "reconsolidation, as opposed to consolidation, may offer a unique opportunity to update memories," an idea that fits the notion of allocentric update driven by exposure to new incentive conditions (Papini, 2003). Reconsolidation was hypothesized to be responsible for the difficulty finding evidence of the spontaneous recovery of cSNC (Mustaca et al., 2009), and the present experiments provided at least two results consistent with this hypothesis. One was the absence of a posttrial CDP effect after complete recovery from cSNC (Experiment 7). Extensive exposure to the downshifted 4% sucrose incentive should have erased the memory of the preshift 32% sucrose incentive, or at least made it relatively inaccessible, such that posttrial CDP would have nothing to strengthen. According to this account, full recovery from cSNC is somewhat equivalent to having no exposure to 32% sucrose—as in unshifted 4% sucrose controls. The second result is the presence of a posttrial CDP effect even when the test trial is delayed 8 days in time (Experiment 8). Having had no exposure to the 4% sucrose solution during the retention interval prevents the allocentric update of the original, preshift memory. In the absence of reconsolidation, the original memory of the 32% sucrose incentive is still active to enhance the cSNC effect. Although these two results are consistent with a reconsolidation account of recovery from cSNC, they do not provide direct tests of this hypothesis. Because reconsolidation requires de novo protein synthesis (Nader et al., 2000), an ideal demonstration would involve microinfusing a protein-synthesis inhibitor in brain sites responsible for encoding allocentric memory.

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