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Anisomycin Disrupts Consummatory Behavior after Incentive Downshift via Conditioned Taste Aversion

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Abstract

The protein synthesis inhibitor anisomycin was tested in the consummatory successive negative contrast paradigm (cSNC). The cSNC effect involves suppression of consummatory behavior induced by 4% sucrose in animals that previously received 32% sucrose (downshifted), relative to animals that always received 4% sucrose (unshifted). Systemic anisomycin (25 mg/kg, ip) induced suppression in both downshifted and unshifted groups when injected before the first or second downshift trial (Experiment 1) or after the first downshift trial (50 mg/kg, ip; Experiment 2). The effect of anisomycin (50 mg/kg, sc) administration after the first downshift trial was observed only in downshifted animals in Experiment 3, but the same dose and route of administration induced significant conditioned taste aversion in Experiment 4. It was concluded that a conditioned taste aversion to the 4% sucrose solution accounts most parsimoniously for all the results. Implications for other experiments involving posttrial anisomycin administration are discussed.

Key words: Anisomycin; consummatory successive negative contrast; conditioned taste aversion.

Novelty and Significance

What is already known about the topic?

Incentive downshift induces the consolidation of new memories.

Disruption of protein synthesis affects the formation of new memories.

However, protein-synthesis inhibitors may also have peripheral effects.

What this paper adds?

Disruption of protein synthesis affects consummatory behavior after a downshift.

However, these effects are not selective to the incentive downshift condition.

Anisomycin disrupts consummatory behavior via conditioned taste aversion.

A demonstration of this aversion effect includes controls for nonassociative effects.

Recent research suggests that the emotional memory of the incentive downshift can be modulated by the administration of the stress hormone corticosterone (Bentosela, Ruetti, Muzio, *et al*., 2006; Ruetti, Justel, Mustaca, & Papini, 2009) and by D-cycloserine, a partial agonist of the N-methyl-D-aspartate receptor (Norris, Ortega, & Papini, 2011). In both cases, the effects were observed when these drugs were administered immediately after the first downshift trial (Trial 11). However, it is not clear whether these effects on memory are mediated by protein synthesis, as it is the case with long-term memories in many other tasks (e.g., Routtenberg & Rekart, 2005). Thus, a potential role of protein synthesis on cSNC was assessed by administering the protein-synthesis

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inhibitor anisomycin before (Experiment 1) and after (Experiments 2 and 3) the first downshift trial. If new memories cannot be consolidated because of disruption of protein synthesis (memory-erasure hypothesis), then one would expect that whatever experience was acquired on the trial affected by anisomycin would be effectively erased. Thus, it was predicted that recovery from cSNC in anisomycin-treated animals would start on the trial after anisomycin administration as if the previous trial had not occurred.

Anisomycin is an antibiotic derived from the bacterium *Streptomyces griseolus* that inhibits protein synthesis in many types of cells, including neurons, by binding to the 60S translation step in ribosomes and blocking the formation of peptide bonds (Barbacid & Vázquez, 1974; Grollman, 1967). When administered systemically (intraperitoneal, ip, or subcutaneous, sc), as in the present experiments, anisomycin can also induce a state of sickness and cause a variety of behavioral effects (Davis & Squirre, 1984). These post-effects of anisomycin administration can support conditioning of antecedent cues (conditioned stimuli, CSs) by acting as unconditioned stimuli (USs). Thus, any demonstration of an anisomycin effect after systemic administration must control for the possibility that the behavioral deficit is not due to interference with long-term memory, but to the Pavlovian conditioning of an aversion to antecedent cues. Pairing of various types of food (including sucrose) with several protein synthesis inhibitors (including anisomycin), resulted in a dose-dependent reduction of consummatory behavior that was specific for the food paired with the drug (Hernández & Kelly, 2004; Squire, Emanuel, Davis, & Deutsch, 1975; Ungerer, Marchi, Ropartz, & Weil, 1975). However, none of these experiments included an unpaired control, that is, a condition in which food and drug were not contiguous in time (only saline controls were included). Thus, nonassociative factors cannot be discarded and given that protein synthesis inhibitors have a variety of effects, including changes in hormone release and locomotor activity (Davis & Squire, 1984; Martínez, Jensen, & McGaugh, 1981), such factors provide plausible alternatives to either a protein synthesis or a conditioned aversion account of the behavioral changes. A conditioned taste aversion account of the effects of systemic anisomycin on cSNC was tested in Experiment 4.

EXPERIMENT 1

Experiment 1 tested the hypothesis that anisomycin causes a retardation of the process of recovery from negative contrast. The memory erasure hypothesis also predicts that recovery from incentive downshift would start a session later for anisomycin-treated animals. To the best of our knowledge, no previous research has been reported using anisomycin or any other protein-synthesis inhibitor in an incentive downshift situation.

METHOD

Subjects and Apparatus

Sixty male, experimentally naive, Long-Evans rats served as subjects. They were around 90 days old at the start of the experiment. Rats were bred in the TCU

vivarium from rats originally purchased at Harlan (Indianapolis, IN), maintained under a 12:12 h light:dark cycle (lights on at 07:00 h), and housed in wire-bottom cages from around post-natal day 40 till the end of the experiment. The housing room had variable temperature (18-23 °C) and humidity (40-70%). Animals were deprived of food to 81-84% of their free-food weight. Free-food weights were defined as the average of each animal's weight during 2 successive days before the start of deprivation at 90 days of age. Water was continuously available throughout the experiment. Rats were trained during the light phase of the daily cycle.

Training was conducted in 4 conditioning boxes (MED Associates, St. Albans, VT) constructed of aluminum and Plexiglas, and measuring 29.4 x 28.9 x 24.7 cm (L x H x W). The floors were made of steel rods, 0.5 cm in diameter and 1.2 cm apart, and 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 is an elliptical opening 1 x 2 cm (W x H), 3.5 cm from the floor, through which a sipper tube, 1 cm in diameter, was inserted. When fully inserted, the sipper tube is flush against the wall of the box. A house light (GE 1820) located in the center of the box's ceiling provided diffuse light. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube. When rats contacted the sipper tube, a circuit involving the steel rods in the floor and the sipper tube was closed and the signal recorded by the computer. Each conditioning box was placed in a sound-attenuating chamber that contained a speaker to deliver white noise and a fan for ventilation. Together, the speaker and fan produced noise with an intensity of 78.0 dB (SPL scale C).

Procedure

Training took place in 15 daily trials. Trials 1-10 were preshift trials and trials 11-15 were postshift trials. Before each trial, animals were transported to a waiting room in squads of four. The transport rack fits up to 4 squads. The composition of each squad and the assignment to a training box were maintained constant, but the order in which squads are run was changed randomly across days. All trials lasted 5 min starting from the first contact with the sipper tube. During trials, the house light, white noise, and fan were on continuously. Immediately after a trial, animals were placed back in their cages and the conditioning boxes were wiped with a damp paper towel, feces removed, and bedding material replaced as needed. When all squads were run, animals were carried back to the colony room. This was repeated until all animals have been run for the day. Sufficient food to maintain target body weights was delivered in the home cage not less than 15 min after the squad ended its daily training trial.

During preshift trials, rats received either 32% or 4% sucrose solution (w/w), prepared by mixing 32 (or 4) g of commercial sugar for every 68 (or 96) g of distilled water. During postshift trials, all rats received 4% sucrose solution. This experiment was run in two replications; the 42 rats from one replication and the 18 rats from the other replication were randomly assigned to one of 6 groups (n = 10, with 7 and 3 animals from each replication per group). Rats trained with 32% sucrose during preshift trials were randomly assigned to either Group 32/Ani/Sal, 32/Sal/Ani, or 32/Sal/Sal, whereas

rats trained with 4% sucrose during preshift trials were assigned to either Group 4/Ani/Sal, 4/Sal/Ani, or 4/Sal/Sal. Groups with the same preshift sucrose concentrations were behaviorally matched before assignment to postshift condition. All rats in this experiment received two injections, one 30 min before Trial 11 and the other 30 min before Trial 12. Group labels refer to these two injections: anisomycin (Ani; 25 mg/kg, ip) or isotonic saline (Sal; equal volume). Thus, administering two injections to all animals allowed testing of the effects of anisomycin on Trials 11 *vs*. 12 using a single pair of saline controls -32/Sal/Sal and 4/Sal/Sal- while simultaneously matching injection-related variables across groups. Anisomycin or saline were administered in a separate room. Anisomycin (Sigma-Aldrich, Saint Louis, MO) was dissolved into isotonic physiological saline to a concentration such that each subject received a 1 ml/kg injection.

The dependent variable, labeled goal-tracking time and measured in 0.01-s units, was the cumulative amount of time (up to 5 min) in contact with the sipper tube. Goal-tracking times were subjected to analysis of variance with an alpha value set at the 0.05 level. Pairwise comparisons using the LSD test were derived from the main analysis whenever justified by appropriate significant interactions. SPSS was used to compute all the statistics. For brevity, only significant F and p values are reported in the text.

RESULTS

The results are presented in Figure 1. Notice that Groups 32/Sal/Sal and 4/Sal/Sal are the same in both panels of this figure. A Sucrose (32%, 4%) x Trial (1-10) analysis indicated a significant interaction, $F(9, 531) = 3.08$, $p < 0.002$, indicating that animals exposed to 32% sucrose developed consummatory behavior faster than animals exposed to 4% sucrose. The main effect for trial was also significant, $F(9, 531) = 170.15$, $p < 0.001$.

Postshift data were analyzed separately for groups that received anisomycin prior to Trial 11 (Figure 1, top) and prior to Trial 12 (Figure 1, bottom). Contrast (32%, 4%) x Anisomycin (Ani, Sal) x Trial (11-15) analyses were computed in each case and the results were identical. In none of the two analyses was the triple interaction or the contrast by anisomycin interaction significant. This implies that the effects of anisomycin were similar in both downshifted and unshifted groups, as shown in Figure 1. There were significant contrast by trial interactions, Fs > 3.17, ps < 0.02, indicating greater consummatory suppression in downshifted than unshifted groups, and significant anisomycin by trial interactions, Fs > 4.86, ps <0.002, indicating greater suppression in anisomycin than saline groups. The main effects of contrast and trial were also significant in both analyses, Fs >13.46, ps < 0.002. Other effects were nonsignificant.

To test whether anisomycin-treated rats started recovery from incentive downshift on the trial following anisomycin administration (Trial 12 or 13, depending on the group), as predicted if this treatment erased all memories of the downshift event, Groups 32/Ani/Sal and 32/Sal/Sal were compared on Trials 12-15 and 11-14, respectively, whereas Groups 32/Sal/Ani and 32/Sal/Sal were compared on Trials 13-15 and 12-14, respectively. Anisomycin x Trial analyses indicated nonsignificant differences

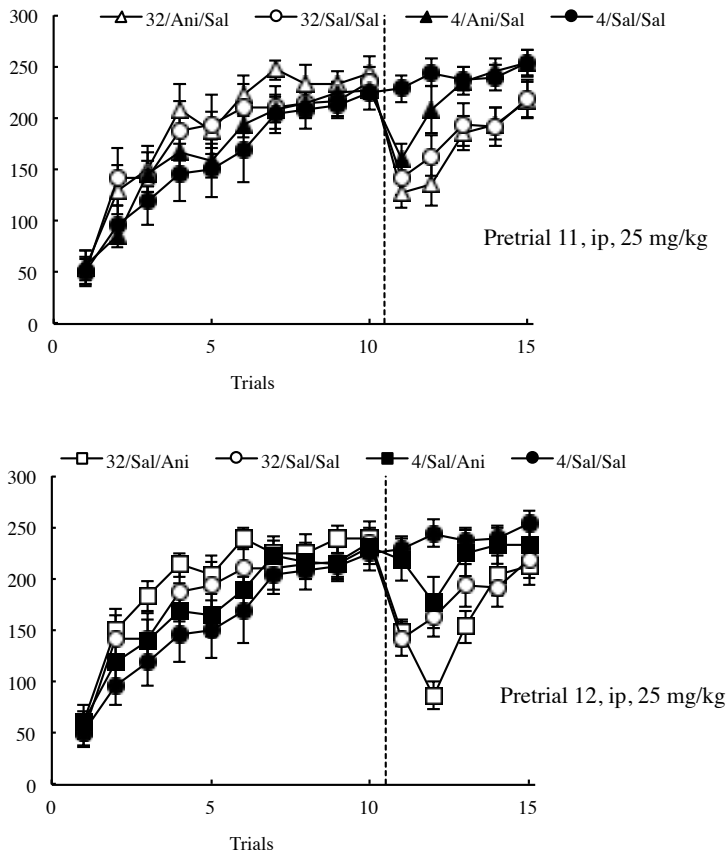

Figure 1. Goal-tracking time (means ±SEMs) of groups of rats given access to 32% sucrose (32) or 4% sucrose (4) and given pretrial anisomycin (Ani) or saline (Sal) either before Trial 11 (top panel) or before Trial 12 (bottom panel). Groups 32/Sal/Sal and 4/Sal/Sal are the same in both figures. Results from Experiment 1.

for the interactions and for the anisomycin effects, in both comparisons, *F*s <1. Only the trial effect was significant in both comparisons, *F*s > 16.09, *p*s < 0.001. These results are consistent with a complete memory erasure of downshift events by anisomycin.

EXPERIMENT 2

The results of Experiment 1 provide no evidence that pretrial anisomycin selectively affects animals that are experiencing an incentive downshift event. Rather, the effects were evident in both downshifted and unshifted groups, although the degree of consummatory suppression was somewhat greater in downshifted than in unshifted groups. The effect of pretrial anisomycin on unshifted animals extensively exposed to 4% sucrose and displaying asymptotic behavior suggests that this is probably less connected to protein-synthesis inhibition than to other performance factors, such as the

development of an aversion to sucrose. Anisomycin seems to affect long-term memories that are being formed or updated, but not stable memories (Bracha, Irwin, Webster, Wunderlich, Stachowiak, & Bloedel, 1998; Rodríguez-Ortiz, De la Cruz, Gutiérrez, & Bermúdez-Rattoni, 2005). To target memory consolidation more directly, anisomycin was administered after, rather than before, Trial 11 in Experiment 2. Pretrial anisomycin administration can interfere with motor, perceptual, or motivational mechanisms underlying consummatory performance in the cSNC situation. Retardation of recovery following posttrial anisomycin administration would not be readily explained by direct performance disruption, given that potential unspecific effects could not be attributed to anisomycin circulating in the rat's system during Trials 11 or 12. As before, if anisomycin led to an erasure of Trial-11 memories, then the group thus treated should be delayed by one trial the onset of recovery from incentive downshift.

METHOD

Subjects and Apparatus

The subjects were 39 male, Long-Evans rats, experimentally naive and about 90 days old at the start of the experiment. The origin and maintenance of the animals, their food deprivation, and the training apparatus was as described in Experiment 1.

Procedure

Animals were randomly assigned to one of four groups: 32/Ani (n = 10), 32/Sal (n = 10), 4/Ani (n = 10), and 4/Sal (n = 9). The training procedure was as described in Experiment 1, with the exception that anisomycin was administered (ip) immediately after Trial 11 and the dose was increased to 50 mg/kg. An increased dose was used on the assumption that it should facilitate diffusion into the CNS, thus increasing the potential for affecting the memory consolidation process. Because posttrial administration aims at determining the effects of anisomycin on cSNC, only animals that meet a criterion for a minimum level of consummatory suppression were included in the analysis. An animal had to exhibit on Trial 11 a goal-tracking time that was 85% or less than that recorded for that animal on Trial 10.

RESULTS

Four rats in the downshifted groups, two in each group, failed to pass the suppression criterion and were thus excluded from the analysis, leaving Groups 32/Ani and 32/Sal with an n = 8. The results are presented in Figure 2. Although there was a trend toward faster change in animals with access to 32% sucrose than 4% sucrose, a Sucrose x Trial (1-10) analysis only revealed a significant change across trials, $F(9, 297) = 72.66$, $p < 0.001$.

The effects of Posttrial 11 anisomycin on consummatory behavior were drastic, as also shown in Figure 1. A Contrast x Anisomycin x Trial (11-15) analysis yielded

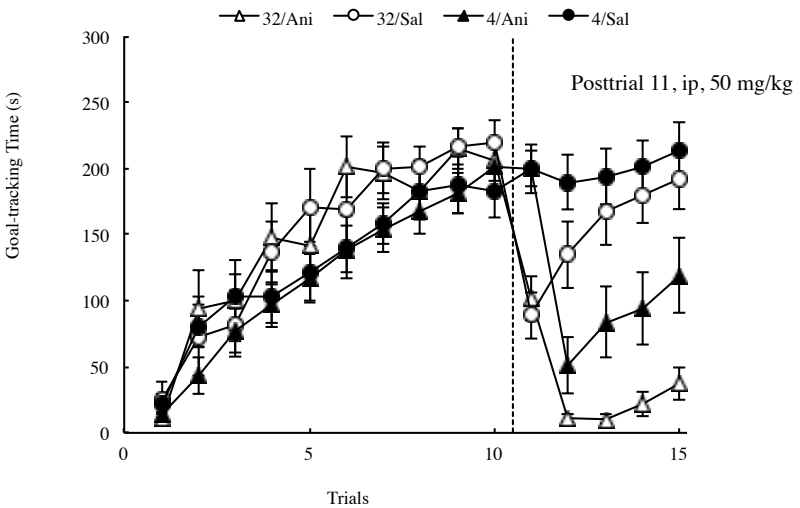

Figure 2. Goal-tracking time (means ±SEMs) of groups of rats given access to 32% sucrose (32) or 4% sucrose (4) and given Posttrial 11 anisomycin (Ani) or saline (Sal). Results from Experiment 2.

a significant triple interaction, $F(4, 124) = 4.07$, $p < 0.005$. All other effects were also significant, Fs > 7.62, ps < 0.004, except for the contrast by anisomycin interaction. Two LSD pairwise comparisons were computed with the error term derived from the main analysis. A comparison of 32% vs. 4% sucrose groups yielded the following results. For saline groups, 32/Sal vs. 4/Sal, the cSNC effect was observed only on Trial 11, $F(1, 31) = 21.90$, $p < 0.001$. However, for anisomycin groups, 32/Ani *vs*. 4/Ani, the cSNC effect was observed on Trials 11, 13, 14, and 15, Fs(1, 31) > 5.60, ps < 0.03. A comparison of anisomycin vs. saline groups provided the following results. For unshifted Groups 4/Ani *vs*. 4/Sal, they were not different on Trial 11, but differed on Trials 12-15, Fs > 9.71, ps < 0.005. For downshifted Groups 32/Ani *vs*. 32/Sal, again they did not differ on Trial 11, but they did on all subsequent trials, Fs > 17.66, ps < 0.001. Thus, neither unshifted nor downshifted groups differed before anisomycin administration on Trial 11, but anisomycin suppressed goal-tracking times in all subsequent trials.

To test the memory erasure hypothesis (i.e., that anisomycin administration erased the events of Trial 11), the performance of Group 32/Ani on Trials 12-15 was compared to that of Group 32/Sal on Trials 11-14. An Anisomycin x Trial analysis indicated a significant interaction effect, $F(3, 42) = 7.90$, $p < 0.001$, and significant main effects for anisomycin and trial, $Fs > 21.35$, $ps < 0.001$. Thus, unlike in Experiment 1, these results provided no support for the erasure hypothesis; anisomycin treatment caused a significant reduction in consummatory behavior beyond what was expected from memory erasure.

EXPERIMENT 3

Experiments 1-2 revealed a drastic reduction in consummatory behavior in animals treated with anisomycin. The effect was observed in downshifted and unshifted rats, although, in both experiments the suppressive effect was larger in the former than in

the latter. Systemic administration of anisomycin has been done via both ip and sc (e.g., Hernández & Kelley, 2004; Wanisch & Wotjak, 2008). Effective doses tend to be higher for sc than ip administration, so perhaps an effect more clearly attributable to interference with memory consolidation than to aversive learning can be observed with sc administration. Experiment 3 was designed to test whether sc administration of anisomycin after Trial 11 would selectively disrupt consummatory behavior in downshifted animals. This experiment also afforded a test of the memory erasure hypothesis, namely, that anisomycin deleted the memory of events occurring on Trial 11, thus retarding the onset of recovery from incentive downshift by one trial.

METHOD

Subjects and apparatus

The subjects were 40 male, Long-Evans rats, experimentally naïve. Animals were maintained as described in Experiment 1 and trained in the same apparatus.

Procedure

Animals were randomly assigned to one of four groups (n = 10). Group names and training procedure was as described in Experiment 2, except that all injections were sc, under the neck skin.

RESULTS

One animal in Group 32/Sal became sick and was dropped before the end of the experiment, thus leaving this group with 9 subjects. The results are shown in Figure 3. A Sucrose x Trial (1-10) analysis indicated significant preshift effects for their interaction, $F(9, 333) = 3.41$, $p < 0.001$, as well as for the main effects of sucrose, $F(1, 37) = 11.58$, $p < 0.003$, and trial, $F(9, 333) = 97.52$, $p < 0.001$.

Figure 3 shows that, in this case, the effect of Posttrial 11 anisomycin was restricted to the downshifted condition. The effect was also smaller in size compared to that observed with ip injections. A Contrast x Anisomycin x Trial (11-15) analysis only yielded a significant contrast by trial interaction, $F(4, 140) = 6.10$, $p < 0.001$. There were also significant contrast and trial effects, $Fs > 11.29$, $ps < 0.003$, but none of the effects involving anisomycin reached significance. Because the effect of anisomycin was observed rather clearly on Trial 12 (see Figure 3), a second analysis involving just Trials 11-12 was computed. This time, there was a significant contrast by anisomycin by trial triple interaction, $F(1, 35) = 8.00$, $p < 0.009$. The source of this triple interaction was determined with LSD pairwise comparisons derived from this main analysis. A comparison of Groups 32 *vs*. 4 indicated a significant difference for both Trials 11 and 12 for anisomycin groups, $Fs(1, 35) > 7.47$, $ps < 0.02$, but only for Trial 11 in saline groups, $F(1, 35) = 12.01$, $p < 0.002$. Thus, anisomycin extended the sSNC effect by at least one trial. Moreover, a comparison of Groups Ani *vs*. Sal revealed that whereas

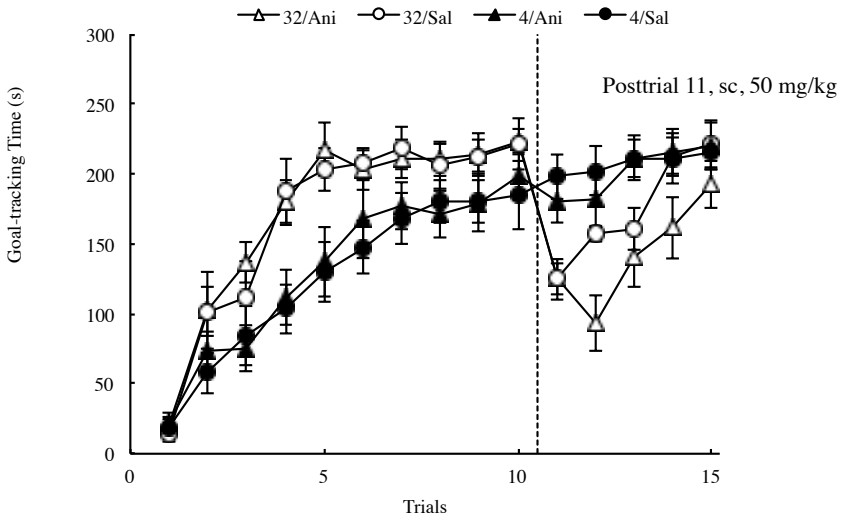

Figure 3. Goal-tracking time (means ±SEMs) of groups of rats given access to 32% sucrose (32) or 4% sucrose (4) and given Posttrial 11 anisomycin (Ani) or saline (Sal). Results from Experiment 3.

unshifted controls did not differ on Trials 11 or 12, and downshifted groups did not differ on Trial 11 (before administration), Group 32/Ani performed significantly below Group 32/Sal on Trial 12 (the trial after anisomycin administration), $F(1, 35) = 6.94$, $p < 0.02$. Posttrial 11 anisomycin administration had a more selective effect on downshifted animals, but the overall effect was weaker with sc administration (this experiment) than with ip administration (Experiment 2; see also, Hernández & Kelly, 2004).

As in the previous experiment, the memory erasure hypothesis (i.e., that anisomycin administration erased the events of Trial 11) was tested by comparing the performance of Group 32/Ani on Trials 12-15 with that of Group 32/Sal on Trials 11-14. In this case, the anisomycin by trial interaction and the main effect of anisomycin were both nonsignificant, $Fs < 1$. Only the trial effect was significant, $F(3, 51) = 21.02$, $p < 0.001$. This result is consistent with the memory-erasure hypothesis.

EXPERIMENT 4

The results of Experiments 2-3 based on Posttrial 11 anisomycin administration are partially consistent with an interference with protein synthesis affecting memory encoding. In the cSNC situation, fast recovery from incentive downshift depends, among other things, on a memory update that encodes the 4% sucrose in place of the 32% sucrose received during preshift trials. Recovery occurs in part because of a match between expected and obtained incentives -4% sucrose in both cases. Thus, interference with this memory update delays recovery because the actual incentive is compared to the preshift, 32% sucrose incentive. A similar interference with memory update occurs after Posttrial 11 administration of the benzodiazepine anxiolytic chlordiazepoxide (Ortega, Glueck, Daniel, *et al*., 2013). In Experiment 2, although anisomycin increased

suppression in both unshifted and downshifted groups, this effect was more extensive in downshifted animals. In Experiment 3, consummatory suppression was observed only in downshifted animals. Moreover, the results of Experiment 1 and 3 (but not the results of Experiment 2) were consistent with the memory-erasure hypothesis that anisomycin effectively deletes any memories being encoded as a result of the incentive downshift event.

However, the results of these experiments are also consistent with a conditioned taste aversion hypothesis. According to this hypothesis, the reason for a greater suppression in downshifted animals than in nonshifted controls relates to relative flavor novelty. By the end of Trial 11, downshifted rats were exposed to 4% sucrose only once, but unshifted animals were exposed on 11 previous trials. Although one would expect some generalization from 32% to 4% sucrose, the latter would be relatively more novel in the downshifted condition than in the unshifted condition. In most Pavlovian conditioning situations, CS-US pairings have less control over behavior after extensive preexposure to the CS, a phenomenon called latent inhibition (Lubow, 2009). A similar effect has been observed in experiments with anisomycin. For example, Hernández and Kelley (2004) reported that anisomycin (either ip or sc) did not affect consumption of a familiar incentive (sugar pellets), but it suppressed consumption of a novel flavor (chocolate pellets); this effect was attributed to the development of a conditioned taste aversion to the chocolate pellets. The goal of Experiment 4 is to assess the hypothesis that the anisomycin effects observed in Experiment 3 were due to the development of a conditioned taste aversion to the 4% sucrose.

METHOD

Subjects and Apparatus

The subjects were 16 male, experimentally naive Long-Evans rats, 90-100 days old at the start of training. The origin and general maintenance of the animals were described in Experiment 1. Eight conditioning boxes similar to those described in Experiment 1 were used.

Procedure

Training lasted 5 daily trials identical to trials in the cSNC experiments reported above, except that all trials involved 4% sucrose. Rats were matched by *ad libitum* weight and then randomly assigned to one of two conditions: 4/Ani/Sal (n = 8) or 4/Sal/Ani (n = 8). Group names corresponded to the first and second injection administered after the first trial. The first injection (anisomycin for one group and saline for the other) was administered immediately after Trial 1, whereas the second injection (anisomycin for one group and saline for the other) was administered 3 h after the end of Trial 1. Thus, groups were matched in terms of the number and temporal distribution of injections. Group 4/Sal/Ani received exposure to both CS and US, but unpaired in time (i.e., unpaired control). Thus, this group controlled for nonassociative factors induced

by anisomycin administration as well as for injection-related effects. All other aspects of the procedure were as described in Experiment 1.

RESULTS

Figure 4 shows the results of this experiment. A Group x Trial analysis indicated significant effects for all three factors: the interaction, $F(4, 56) = 8.81$, $p < 0.001$, and the main effects for group and trials, $Fs > 9.43$, $ps < 0.004$. Pairwise LSD tests derived from this analysis indicated that whereas groups were not different on Trial 1, before the drug treatment, groups did differ on all subsequent trials, $Fs(1, 14) > 6.08$, $ps < 0.03$. Thus, the posteffects of anisomycin administration act as a US that supports conditioned taste aversion to the 4% sucrose solution under the same general conditions used during cSNC experiments, except for the absence of an incentive downshift.

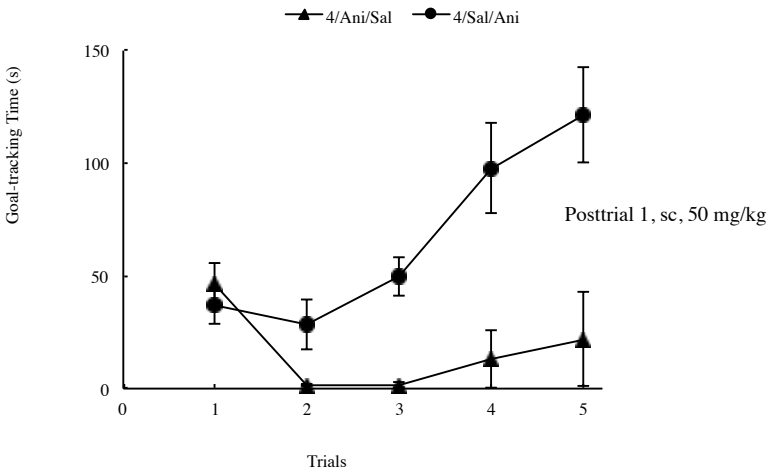

Figure 4. Goal-tracking time (means ±SEMs) of groups of rats given access to 4% sucrose (4) and Posttrial 1 anisomycin (Ani) or saline (Sal), either immediately after the trial or 3 h later. Results from Experiment 4.

GENERAL DISCUSSION

The generally accepted notion that the encoding of new memories requires de novo protein synthesis originally comes from the use of drugs that interfere with protein synthesis through a variety of molecular mechanisms (Alberini, 2008; Davis & Squire, 1984; Routtenberg & Rekart, 2005). The present experiments were designed to test the hypothesis that the incentive downshift experience induces the consolidation of new memories. Previous research with Posttrial 11 administration of memory enhancers provided initial evidence for memory consolidation in the cSNC situation (Bentosela *et al*., 2006; Norris *et al*., 2011; Ruetti *et al*., 2009). The key finding in these cases was the selective effect of memory enhancing drugs: They affected the behavior of downshifted animals, in which new learning is occurring, but did not affect the behavior

of unshifted animals, in which no new learning is occurring. In a similar way, because anisomycin is known to affect only new or updated learning (Bracha *et al*., 1998; Rodríguez-Ortiz *et al*., 2005), it was expected that it would display a profile similar to that of memory enhancers in the cSNC situation. This was the case in Experiment 3, with sc administration, but not in Experiments 1-2, with ip administration.

A memory consolidation effect of anisomycin is supported by three of the results reported here. First, Posttrial 11 anisomycin increased suppression on subsequent trials in Experiment 3, retarding recovery of normal levels of consummatory behavior, but had no effect on unshifted controls. This is consistent with an explanation in terms of interference with memory consolidation because only downshifted animals experience a change of conditions (i.e., the 32-to-4% sucrose downshift) that would induce the consolidation of new memories. The conditions of the unshifted controls remain unmodified throughout the experiment and, therefore, no induction of protein synthesis is expected to be present in these animals. As mentioned above, only new or updated memories require de novo protein synthesis (Bracha *et al*., 1998; Rodríguez-Ortiz *et al*., 2005). Second, although Posttrial 11 anisomycin also affected the performance of unshifted controls in Experiment 2, the suppressive effect on consummatory behavior was still more pronounced in downshifted animals. The strong suppressive effect of anisomycin on unshifted controls suggests that something other than inhibition of protein synthesis is affected. However, the fact that the suppressive effect on consummatory behavior was greater in downshifted animals suggests that inhibition of protein synthesis may have also been implicated. Third, Experiments 1 and 3 provided support for the memory erasure hypothesis, that is, that the anisomycin treatment would effectively delete from memory any events occurring on the trial affected by the drug. Such deletion would retard recovery from cSNC by one trial.

However, a memory interference account of these results has several problems. First, we chose the anisomycin doses used here (25 and 50 mg/kg) on the basis of previous behavioral experiments in which similar doses were effective (e.g., Bitrán & Kalant, 1993; Patterson, Rosenzweig, & Bennett, 1987). However, in the absence of a direct measurement, it is difficult to determine whether these doses effectively reduced protein synthesis in the brain (Flood, Bennett, Orme, & Rosenzweig, 1975).

The second and strongest argument against an account based on inhibition of protein synthesis comes from a conditioned taste aversion alternative. Such an account explains the effects of anisomycin on both downshifted and unshifted groups and it can also account for the relatively larger effect in downshifted than in unshifted animals. Conditioned taste aversion occurs when a relatively novel flavor (the CS) is paired with gastrointestinal disease (the US). Usually, disease is induced by lithium chloride, but many other drugs have been found to induce conditioned taste aversions (Welzl, D'Adamo, & Lipp, 2001). An aversion alternative becomes especially important in studies of memory consolidation based on a posttraining drug administration procedure. In the cSNC situation, for example, consummatory suppression induced by a drug administered after the trial may reflect a memory effect or the development of a conditioned taste aversion. It may be argued that a selective effect of the drug (present in downshifted, but not unshifted animals) controls for an aversion alternative, but this interpretation is clouded by the

phenomenon of latent inhibition. As mentioned above, nonreinforced preexposure to a CS, as would be the case in unshifted animals during preshift trials (i.e., exposure to 4% sucrose in the absence of aversive posteffects because no drug was administered), would tend to attenuate conditioned taste aversion (Cannon, Best, & Batson, 1983; Lubow, 2009). Because downshifted animals were exposed to an 8-times higher sucrose concentration during preshift trials (32% sucrose), the introduction of 4% sucrose during the postshift trials is relatively more novel than in unshifted animals, thus potentially enhancing the conditioned taste aversion effect. For this reason, studies involving postrial drug administration in the cSNC situation must eliminate the aversion alternative by demonstrating that the specific drug, administered under the same conditions, but in the absence of an incentive downshift, does not support a conditioned taste aversion to the 4% sucrose (Norris *et al*., 2011; Ruetti *et al*., 2009; Wood, Norris, Daniel, & Papini, 2008). Under the conditions of training used in these experiments, the taste aversion alternative cannot be adequately eliminated given the results of Experiment 4. Moreover, this alternative provides a parsimonious account for all the effects reported in these experiments. Although not directly tested, there is no reason to suppose that 10 trials of preexposure to 4% sucrose in the absence of anisomycin would not yield evidence of latent inhibition of taste aversion.

The present results have implications for other studies. Consider, for example, Milekic and Alberini's (2002) study. In their experiment, rats received training in passive avoidance and then received a Test 1 either 2, 7, 14, or 28 days after (memory reactivation in the absence of shock). In all conditions, anisomycin (210 mg/kg, sc) was administered immediately after Test 1. The effects were assessed 2 days later on Test 2. The goal was to determine whether the process of reconsolidation induced during Test 1 was sensitive to disruption by inhibition of protein synthesis only at certain Training-Test 1 retention intervals. Anisomycin affected Test-2 performance only in groups in which the Training-Test 1 interval was 2 or 7 days, but not when it was 14 or 28 days. The authors concluded that protein synthesis is necessary for reconsolidation only under relatively short intervals between original training and memory reactivation. However, notice that, in the absence of a control group that received the same treatment, except for passive avoidance training, it is difficult to discard the possibility that changes in latency are not related to inhibition of protein synthesis affecting reconsolidation, but to anisomycin's induction of a place aversion. The present results suggest the need for testing whether anisomycin can support place aversions.

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