Behavioural Brain Research 193 (2008) 28–36

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Trial-selective effects of U50,488H, a κ-opioid receptor agonist, on consummatory successive negative contrast

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ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 14 April 2008
Accepted 19 April 2008
Available online 26 April 2008

Keywords:
Incentive contrast
Opioid system
U50,488H
Consummatory behavior
Conditioned taste aversion
Allocentric memory
Egocentric memory

ABSTRACT

A series of experiments studied the effects of the κ-opioid receptor agonist U50,488H on consummatory successive negative contrast (cSNC) in rats. In cSNC, previous experience with a 32% sucrose solution leads to greater rejection of 4% sucrose than exclusive experience with 4% sucrose. Experiments 1 and 2 revealed that U50,488H failed to influence cSNC when administered before the first downshifted trial, but either attenuated (1 mg/kg) or enhanced (3 and 10 mg/kg) cSNC when administered before the second downshift trial. Experiment 3 showed that U50,488H administered immediately after the first downshift trial had no effect on cSNC at the 1 mg/kg dose, but tended to increase cSNC at the 3 mg/kg dose. However, Experiment 4 suggested that the apparent enhancement of cSNC after 3 mg/kg U50 administered posttrial 11 may have reflected the development of a conditioned taste aversion. The trial-selective attenuating effect of the low dose may reflect an anxiolytic-like property of U50,488H.

1. Introduction

The opioid system is one component of a set of mechanisms mediating the response to surprising reward loss, as studied in the consummatory successive negative contrast (cSNC) situation [41]. In the cSNC situation, the consummatory performance of rats given free access to 32% sucrose solution during short daily trials is compared after a downshift to 4% sucrose with that of an unshifted group always exposed to 4% sucrose. Downshifted rats exhibit a transient suppression of consummatory behavior relative to unshifted controls, followed by the recovery of normal levels of consummatory behavior after 2–4 daily trials of access to the downgraded solution [15]. Experiments show that the nonselective opioid receptor agonist morphine (4–8 mg/kg, i.p.) administered before either the first or second downshift trial (usually trials 11 and 12, respectively) attenuates cSNC on both trials, thus accelerating recovery [47]. Complementary to these results, the nonselective opioid receptor antagonist naloxone (2 mg/kg, i.p.) administered before trials 11 and 12 enhances cSNC on both trials, thus interfering with recovery from reward loss [44]. Both morphine and naloxone are considered nonselective opioids as far as their receptor-binding properties. However, greater affinity for the μ receptor in both drugs has been reported [29]. If correct, the μ-opioid receptor subsystem may not play a selective role on the first versus second postshift trials in cSNC.

Additional evidence suggests that the δ-opioid receptor subsystem selectively mediates the initial response to reward loss, but not the recovery that follows. For example, the selective δ-opioid receptor agonist D-Ala2-,N-MePhe4,Phe4,Gly-ol (DPDPE, 24 μg/kg, i.p.) attenuates the initial impact of reward downshift on trial 11, but fails to affect recovery from reward downshift on trial 12 [54]. Moreover, the selective δ-opioid receptor antagonist naltrindole (1 mg/kg, i.p.) administered before trials 11 and 12 enhances cSNC on trial 11, but has no detectable effect on trial 12 [44]. Whereas several drugs are known to attenuate cSNC selectively on trial 12 (e.g., benzodiazepine anxiolytics; [15]), the selective effects of δ opioids on trial 11 provide the first evidence identifying a neurochemical system involved in modulating the initial reaction to surprising reward downshifts. This discovery provided the impetus for exploring the action of other opioid receptor selective peptides.

This paper reports evidence on the effects of pretrial and posttrial administration of the selective κ-opioid receptor agonist U50,488H (heretofore U50) on cSNC. The general goal is to determine whether activating the κ-opioid receptor subsystem would affect cSNC and, in the affirmative case, whether the effect is selective for trial 11 vs. trial 12 performance. Virtually nothing is known about the role of the κ-opioid receptor subsystem in situations involving reward loss. Following a well-known parallel between the mechanisms subserving pain-fear and frustration [19,39,41], it may be profitable to look for potentially relevant evidence

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doi:10.1016/j.bbr.2008.04.016

in experiments involving fear conditioning. For example, freezing responses in Pavlovian fear conditioning increase following administration of the μ antagonist CTOP, but decrease following administration of the κ antagonist nor-binaltorphimine [14]. Administration of cyprenorphine and naltrindole (δ-opioid receptor antagonists) revealed no effects compared to saline controls. Furthermore, Osaki et al. [39] tested the effects of naloxonazine (μ antagonist) and nor-binaltorphimine (κ antagonist) administered into the periaqueductal gray and inferior colliculus on fear-like behaviors (running and jumping) after electrical stimulation of the central nucleus of the inferior colliculus. Naloxonazine increased and nor-binaltorphimine decreased the defensive threshold, suggesting that the κ-opioid receptor subsystem may regulate μ-mediated behaviors. These results suggest, first, that the μ- and κ-opioid receptor subsystems can oppose each other and, second, that these different opioid subsystems can have differential effects on fear conditioning. Together with the differential effects of DPDPE on cSNC reviewed previously, all three major opioid receptor subtypes are implicated in different roles in cSNC.

Four experiments are reported in this article. Experiment 1 addressed the effects of U50 when administered before postshift trials 11 and 12. Experiment 2 evaluated the effects of U50 administered only before postshift trial 12. Experiment 3 looked at the effects of U50 administered after postshift trial 11. Finally, Experiment 4 tested the hypothesis that the posttrial effects observed in the previous experiment were due to the rapid development of a U50-induced conditioned taste aversion. All together, these experiments demonstrate another selective action of an opioid agonist on cSNC, suggesting that under some conditions, the δ- and κ-opioid subsystems modulate cSNC selectively on trials 11 and 12, respectively.

2. Experiment 1

As mentioned previously, various opioid treatments can have opposite effects on behavior. For example, Ilyutchenok and Dubrovina [23] demonstrated that reacquisition of one-trial passive avoidance was enhanced by ICI 174,864 (δ antagonist) and dynorphin (κ agonist). Similarly, morphine self-administration [16] and morphine-induced locomotion [42] were suppressed by U50 in adult rats. In addition, preadolescent rats showed impaired morphine-induced place preference [8] and increased vocalizations [35] following U50 administration. Vocalizations were suppressed following administration of the κ-opioid receptor antagonist nor-binaltorphimine. Narita et al. [34] reported that the straub tail reaction induced by intracerebroventricular injections of morphine was significantly antagonized by beta-funaltrexamine (μ antagonist), and by U50 (κ agonist). Analogous opposing effects were obtained in some neurophysiological studies. For example, Walker et al. [53] found that activation of the κ-opioid receptor subsystem by U50 suppressed the firing of neurons activated by morphine in the substantia nigra. The fact that κ modulation leads in some cases to opposite effects from those found after μ and δ modulation is consistent with the differential distribution of these opioid receptors in the rat brain [30].

In addition to U50 showing opposite effects to those of μ- and δ-opioid receptor agonists, the dose level can affect behavior differentially. For example, Schnur and Walker [50] observed locomotor hyperactivity after administration of a low dose of U50 (1 mg/kg, i.p.), but locomotor hypoactivity after administration of a high dose of U50 (10 mg/kg, i.p.), relative to saline controls in hamsters. Apart from the potential species differences, this study suggests the possibility that the level of κ activation induced by the administered dose can cause opposite behavioral effects. Experiment 1 had two goals. First, to determine whether opposing effects similar to those described for locomotor activity are observed in the cSNC situation after administration of U50, and second, to determine whether the effects were behaviorally selective for the first vs. second postshift trials. Independent groups of rats received treatment with U50 (0, 1, 3, and 10 mg/kg, i.p.) before trials 11 and 12, while being either downshifted or unshifted in terms of incentive magnitude. Thus, two sets of controls were implemented: a drug control (saline treatment) and a contrast control (unshifted incentive treatment).

2.1. Method

2.1.1. Subjects

The subjects were 64 adult Long-Evans rats, 90–110 days old at the start of the experiment. Thirty-two males and 32 females were used. Both male and female rats are used whenever possible, as done in most previous experiments on cSNC from our lab. Rats were bred in the TCU vivarium from parents purchased at Harlan. Animals were housed under a 12:12 h light:dark cycle (lights on at 07:00 h) and behaviorally tested during the light phase of the cycle. In preparation for the experiment, all rats were deprived of food to an 81–84% of their ad libitum body weight. Water was freely available in the home cage.

2.1.2. Apparatus

Animals were tested in four 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 flush against 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.

The 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 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. Procedure

Animals were exposed to the conditioning box for a total of 14 trials, 10 preshift trials and 4 postshift trials. Testing consisted of a 5-min access to the sucrose solution starting from the first contact of the rat with the tube. Each trial started and ended with a variable interval averaging 30 s during which the sipper tube was retracted. These intervals were introduced to remove the handling events that precede and follow a trial from consummatory behavior occurring during the trial. Downshifted animals received access to 32% sucrose on preshift trials 1–10 and then 4% on postshift trials 11–14. Unshifted animals received 4% in both pre- and postshift trials. One trial per day was administered. Sucrose solutions were prepared (w/w) by mixing 4 g (or 32 g) of sucrose per 96 g (or 68 g) of distilled water. Random assignment was used to determine which animals received access to 32% sucrose or to 4% sucrose during the preshift trials. Thereafter, the performance of rats on preshift trials was matched before randomly assigning individual animals to the various groups exposed to downshifted or unshifted conditions. Thirty-two animals received a 32-4 downshift, were matched, and then randomly assigned to four drug conditions ($n = 8$). Group 32/S, 32/1, 32/3, and 32/10 received, respectively, injections of saline, 1, 3, or 10 mg/kg. Thirty-two animals were matched and then randomly assigned to the unshifted 4-4 contrast control condition and randomly assigned to four groups ($n = 8$). Groups 4/S, 4/1, 4/3, and 4/10 received, respectively, injections of saline, 1, 3, or 10 mg/kg. All drugs were administered i.p. 20 min before the start of trials 11 and 12. Saline injections were of equal volume. These doses were based on Schnur and Walker's [50] experiment. *Trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide (U50,488H) was prepared by mixing the appropriate amount of desiccate with 1 ml of saline. The stock solution was then diluted to the appropriate doses. Doses were prepared 48 h prior to the first postshift trial (trial 11). Isotonic saline solution was used as vehicle. Drugs were purchased from Sigma–Aldrich Chemicals (Saint Louis, MO).

The dependent variable was the cumulative time in contact with the sipper tube (in 0.05-s units), up to a maximum of 5 min. Under the conditions used in the present experiments, this dependent variable (named goal-tracking time) produces less variable data than the more typical licking frequency measure used in other labs. Goal-tracking time has been reported in previous research [44,54], it has yielded significant positive correlations with amount of fluid intake [33], and it has recently replicated within a single experiment the selective effects of chlordiazepoxide on trial 12, but not on trial 11, previously reported in separate experiments [12]. Goal-tracking time yields nonsignificant preshift differences in some experiments, but this is not uncommon with lick frequency data (for one example, see [15], p. 56). Goal-tracking times were subject to conventional analysis of variance. Multiple comparisons were computed using Fisher's LSD post-hoc test. In all the statistical tests, the alpha value was set at the 0.05 level. Due to experimental error, two females in the 4% sucrose condition received fewer than 10 trials of testing; the data from these two animals were excluded.

2.2. Results

Fig. 1 displays the consummatory performance of the eight groups segregated by dose. Overall preshift performance shows a trend toward greater response level in groups receiving access to 32% sucrose than to 4% sucrose. A Contrast (32% vs. 4% sucrose) × Sex × Trial (1–10) analysis indicated the following results. There was a significant triple interaction, $F(9, 540) = 2.03$, $p < 0.04$, but neither the main effect of sex, the sex by trial interaction, or the sex by contrast interaction reached significance, $Fs < 2.26$, $ps > 0.13$. Goal-tracking times were significantly higher for rats drinking 32% sucrose than 4% sucrose, $F(1, 60) = 6.61$, $p < 0.02$, but tended to converged on later trials, as is usually the case, yielding a significant sucrose by trial interaction,

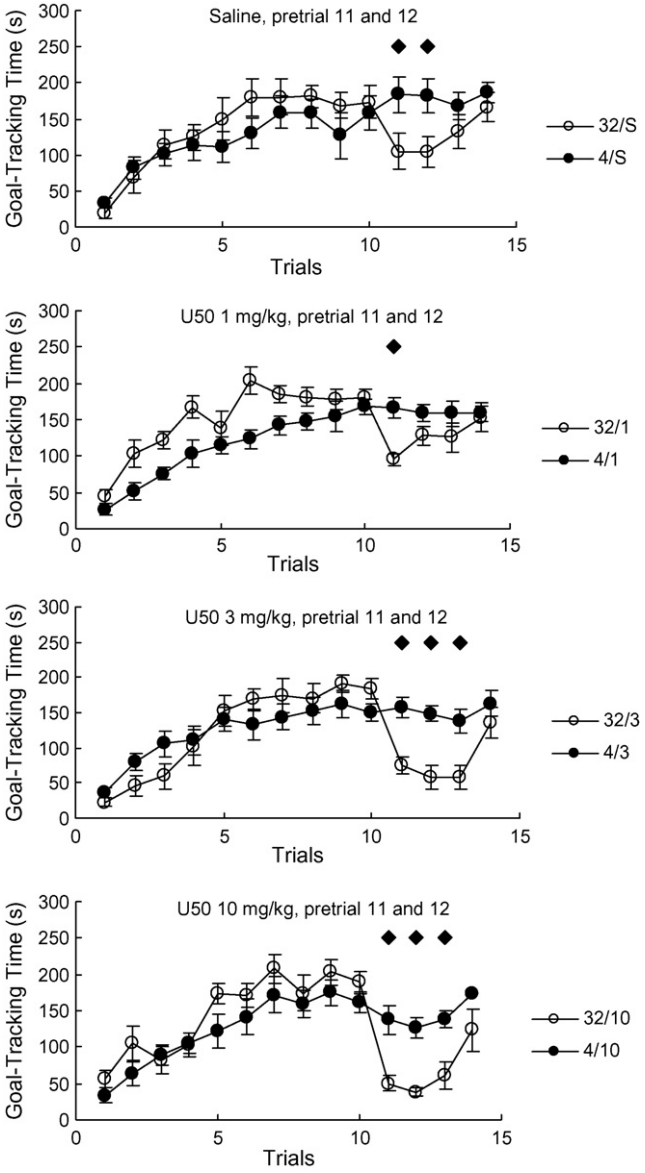

Fig. 1. Results of Experiment 1. Consummatory performance of the eight groups during preshift trials (1–10) and postshift trials (11–14). Each dose treatment for downshifted and unshifted groups is presented separately (saline, 1, 3, and 10 mg/kg of U50, i.p.). Drugs were administered before trials 11 and 12. In all the figures, means (±S.E.M.) are plotted and significant pairwise comparisons are marked with a black diamond ($p < 0.05$, LSD tests, only on postshift trials).

$F(9, 540) = 2.47$, $p < 0.01$. There was also a significant increase in goal-tracking times across preshift trials, $F(9, 540) = 103.84$, $p < 0.001$.

The results of the postshift trials indicate some clear suppressive effects of U50 on goal-tracking times, especially at the 10 mg/kg dose. A Contrast × U50 × Sex × Trial (11–14) analysis provided the following results. The contrast by trial, contrast by sex by trial, and contrast by sex by U50 interactions were all significant, Fs > 2.88, ps < 0.05. Other interactions failed to reach significance, Fs < 1.76, ps > 0.08. There were also significant main effects of contrast, $F(1, 48) = 42.95$, $p < 0.001$, U50, $F(3, 48) = 6.17$, $p < 0.002$, and trial, $F(3, 144) = 17.63$, $p < 0.001$. The simple effect of sex was nonsignificant, $F < 1$. Further analyses were computed to clarify interactions involving U50 effects.

The main goal was to evaluate the presence or absence of cSNC on each downshifted–unshifted pair of groups exposed to the same U50 dose. This was achieved by computing a one-way ANOVA for each postshift trial followed by Fisher's LSD post-hoc pairwise tests. In all cases, one-way analyses included all the groups. In addition to being a standard practice (e.g., Wood et al., 2005), the downshifted-unshifted comparison assesses the presence of cSNC on each trial independently of U50 effects unrelated to the downshift manipulation. Fig. 1 indicates significant cSNC effects for each postshift trial as derived from these analyses.

On trial 11 there was a significant group effect, $F(7, 56) = 7.78$, $p < 0.001$. Pairwise comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for all the drug conditions (0, 1, 3, 10 mg/kg), ps < 0.005. LSD post-hoc analyses of the downshifted groups (32-4) revealed a significantly greater suppression of response in Group 32/10 than in Group 32/S, $p < 0.03$. Similar pairwise comparisons for the unshifted control groups (4-4) revealed nonsignificant differences, ps > 0.05. The downshifted vs. unshifted comparison indicated a significant cSNC effect for each drug dose level. Therefore, there was no evidence of an effect of U50 on cSNC on trial 11 at any of the doses used in this experiment. A nonspecific suppressive effect of U50 on goal-tracking time was detected at the 10 mg/kg dose in the downshifted groups, but not in the unshifted groups.

Trial 12 also revealed a significant group effect, $F(7, 56) = 10.03$, $p < 0.001$. Pairwise comparisons indicated significant differences between the two contrast groups (32% vs. 4%) for drug conditions 0, 3, and 10 mg/kg, ps < 0.002. The performance of Groups 32/1 and 4/1 was not significantly different, $p > 0.05$, indicating that the 1 mg/kg dose attenuated cSNC. LSD post-hoc analyses of downshifted groups showed a significant suppression of responding in Groups 32/3 and 32/10 relative to Group 32/S, ps < 0.05. Similarly, there was a significant suppression of responding in Group 4/10 when compared to Group 4/S, $p < 0.02$. These comparisons indicate a suppressive effect of U50 on consummatory behavior for the 10 mg/kg dose. Other pairwise comparisons among downshifted groups and among unshifted groups were nonsignificant, ps > 0.05. Unlike on trial 11, 1 mg/kg U50 eliminated the cSNC on trial 12. However, there was no evidence that the two larger doses had any effect on cSNC. There was also an indication that U50 at 3 and 10 mg/kg had a nonspecific suppressive effect on goal-tracking time for both downshifted and unshifted groups.

Trial 13 also revealed a significant group effect, $F(7, 56) = 5.04$, $p < 0.001$. Pairwise comparisons indicated significant differences between the two contrast groups (32% vs. 4%) for the 3 and 10 mg/kg doses, ps < 0.005, but nonsignificant differences for the 0 and 1 mg/kg doses, ps > 0.05. LSD post-hoc analyses of unshifted groups showed nonsignificant differences in suppression of responding in Groups 4/1, 4/3, and 4/10, when compared to 4/S, ps > 0.05. LSD post-hoc analyses of the downshifted groups showed a significant suppression of responding in Groups 32/3 and 32/10 when compared to Group 32/S, ps < 0.009. Because cSNC was not evident in the groups that received saline injections, but significant in groups that received either 3 or 10 mg/kg U50, it follows that these doses prolonged the cSNC effect.

Groups were not different on trial 14, $F(7, 56) = 1.11$, $p > 0.05$, indicating complete recovery from cSNC in all the drug conditions.

2.3. Discussion

Several conclusions follow from the results of Experiment 1. First, the administration of U50 before trials 11 and 12 dose-dependently suppressed consummatory behavior, as shown in the performance of unshifted control groups. Thus, this effect was unrelated to cSNC but it affected the dependent variable. A reduction in goal-tracking time suggests that either treated rats may have engaged nonconsummatory responses that interfered with drinking, or U50 may have induced an aversion to the sucrose solution. This last possibility is explored in Experiment 4.

Second, a comparison of downshifted vs. unshifted groups administered saline solution provides a picture of the cSNC effect under normal conditions for this experiment. There was significant consummatory suppression on trials 11 and 12, followed by recovery of normal levels of behavior on trials 13 and 14. The strength of this cSNC effect is similar to that reported in the literature [15]. Third, when the effects of U50 treatments were compared to the cSNC effect obtained under saline treatment conditions, it is clear that U50 had no detectable effects on the first postshift trial, but it significantly affected cSNC on the second postshift trial. In this respect, the effects of U50 are unlike those of morphine, which reduced cSNC in both the first and second postshift trials [47], and those of DPDPE, which reduced cSNC on the first, but not on the second postshift trial [54]. In fact, U50 exhibits the same trial selectivity described for benzodiazepine anxiolytics and ethanol [15], which attenuate cSNC only when administered before the second postshift trial. However, these conclusions must be carefully assessed.

The absence of a U50 effect on the first postshift trial must be considered with as much caution as any negative result. Some potential trivial explanations can be safely discarded. For example, because U50 had an effect on cSNC in subsequent trials, it cannot be argued that the doses were ineffective or the preparation was not sensitive enough to detect an effect. U50 also had an effect on consummatory behavior on trial 11, further showing that at least the 10 mg/kg dose was effective under the present conditions. Moreover, since the effects of U50 on cSNC were in opposite directions for different doses on trial 12, it cannot be argued that lack of an effect on trial 11 may reflect floor or ceiling effects in the dependent measure. Given that other pharmacological treatments have shown selectivity in terms of the first vs. second postshift trials, it is tentatively concluded that U50 does not affect cSNC on the first postshift trial.

The main problem interpreting the effects of U50 on the second postshift trial is that the rats had already received a similar dose a day earlier. Thus, it is not possible to determine whether the effects of U50 on trial 12 were selective, or just the consequence of repeated doses. Drug accumulation is not a strong possibility given that the analgesic effects of U50 are no longer evident 4 h after its administration [5]. Because there were about 23 h between successive administrations, it is likely that

the drug was completely metabolized by the time the animals received the second injection. Lynch and Burns [28] suggested that consecutive administration of U50 may enhance consummatory responsiveness to sucrose solutions. Thus, although U50 had no detectable behavioral effect on trial 11, it may have altered the rat's consummatory behavior on trial 12. While this possibility cannot be completely eliminated, it seems unlikely that all the results of trial 12 can be explained in such terms because of their bidirectionality. The enhancement of sucrose consumption by consecutive doses may explain the elimination of cSNC in the 1 mg/kg condition on trial 12, but it cannot explain the persistence of cSNC in the 3 and 10 mg/kg conditions to trial 13. None of these potential problems applies to the lack of effect of U50 on trial 11 because that was the first administration of the drug.

It is also worth considering the absence of an effect on trial 11 and the effects on trial 12 together. As in other cases of analogous behavioral selectivity [54], this combination of results suggests that the effects of U50 cannot be attributed to unspecific factors such as sensory-perceptual, motivational, or motor influences of the drug on consummatory behavior. Moreover, notice that the effects of the two largest doses were detectable on trial 13, a day after the last administration of U50 and in the absence of drug treatment. Such a carry-over effect suggests that U50 may be modifying the memory strength of the incentive downshift experience, a possibility that will be explored in Experiment 3.

The opioid system is known to exert an influence on feeding behavior, taste palatability, and gustatory responses. For example, Lynch and Burns [28] reported that a low dose of U50 increased sucrose consumption, an effect not observed in the unshifted control groups of Experiment 1. Li et al. [27] found that neurons in the nucleus of the solitary tract were suppressed following microinjections of met-enkephalin, whereas morphine administration in the parabrachial nucleus reduced taste palatability [48]. These nuclei are activated during sucrose drinking in rats [51] and at least one of them, the parabrachial nucleus, is critically involved in cSNC, as demonstrated by lesion experiments [20]. These potential influences of U50 on pontine nuclei may account for the suppression of consummatory behavior in unshifted controls, but could only explain the extension of cSNC to trial 13 on the assumption that these nuclei are involved in memory processes related to the incentive downshift experience. There is no evidence for such potential effects.

Motivational factors in consummatory behavior are also sensitive to opioid drugs. Drewnowski et al. [13] found that naloxone reduced overall consumption of bulimic patients and nonbulimic individuals, suggesting an opioid effect related to food palatability. Lynch and Burns [28] suggested that U50 dose levels may differentially affect consumption of sucrose solutions. Their findings revealed that after 10 days of U50 treatment, a 1 mg/kg dose had no effect on the volume of 20% sucrose consumed, but a 0.3 mg/kg dose increased sucrose consumption. Badiani et al. [4] found that 4 mg/kg U50 increased the consumption of high sucrose solutions (30%, 40%) while decreasing the consumption of low sucrose solutions (1%, 4%) over a 30-min period. Data collected on trial 12 of the present experiment show a similar pattern of results, revealing a dose-dependent suppression of U50 on consummatory responding in the unshifted groups. However, if sucrose palatability were the only mechanism affected by U50, one would expect to see this same pattern also in the downshifted groups, which showed either enhancement or suppression of consummatory behavior depending on the dose. Moreover, a palatability effect of U50 would require that downshifted groups exhibit the same effects on trials 11 and 12, which was not observed in Experiment 1.

Schnur and Walker [50] found a similar dose-dependent bidirectional response to U50 on locomotor activity. A high dose of U50 (10 mg/kg) significantly suppressed running wheel activity, whereas a low dose (1 mg/kg) significantly increased it. Changes in locomotor activity could influence consummatory behavior simply because of response competition. For example, an increase in activity levels would tend to drive the rat away from the sipper tube, thus yielding the appearance of consummatory suppression. However, the results reported by Schnur and Walker [50] are in the opposite direction to what might be predicted for the cSNC situation. For example, if the 10 mg/kg dose suppressed activity, then this should allow for a greater amount of consummatory behavior instead of the observed suppression in consummatory behavior. Similarly, if the 1 mg/kg dose increased activity, then one would expect suppression of consummatory behavior, when, again, the opposite was observed.

The present results suggest a dose-dependent bidirectional influence of U50 on cSNC. The medium and high doses of U50 enhanced cSNC, whereas the low dose attenuated it. With saline injections, cSNC was observed on trials 11 and 12; however, with 1 mg/kg U50, cSNC was observed only on trial 11, whereas with 3 and 10 mg/kg, cSNC was observed on trials 11, 12, and 13. All the drug groups showed recovery from cSNC on trial 14, thus demonstrating that these effects were transient. The possibility cannot be discarded that the effects of U50 on trials 12 and 13 may have been affected by drug administration on trial 11. The trial and dose selectivity effects of U50 were further analyzed in Experiment 2 by administering the drug only before trial 12.

3. Experiment 2

The selective effects of opioid receptors on cSNC were suggested by Wood et al.'s [54] results, who found that the δ-opioid receptor agonist DPDPE attenuated cSNC on postshift trial 11 without affecting cSNC on postshift trial 12. Moreover, Pellegrini et al. [44] reported that the δ-opioid receptor antagonist naltrindole enhanced cSNC on trial 11, but not on trial 12. In addition, the nonselective antagonist naloxone had a greater suppressive effect on consummatory behavior than naltrindole and saline, enhancing cSNC on postshift trials 11, 12, and 13. This suggests that μ, δ, and κ opioid receptors may differentially mediate the cSNC effect in terms of the initial impact of the downshift versus the subsequent recovery of normal levels of consummatory behavior.

Experiment 2 was designed to determine the effects of U50 on cSNC when administered only before trial 12. The low and medium doses used in Experiment 1 (1 and 3 mg/kg) were administered. If the opposite effects of these doses on cSNC during trial 12 observed in Experiment 1 were not caused by drug accumulation or some other effect of drug administration on trial 11, then the bidirectional effects of U50 on cSNC should be replicated in Experiment 2.

3.1. Methods

3.1.1. Subjects and apparatus

The subjects were 48 male Long-Evans rats, tested between 90 and 120 days of age. Experiment 2 was run in two replications, the first included 28 animals bred at the TCU vivarium and derived from Harlan parents (as in Experiment 1), while the second replication used 20 animals purchased from Harlan. Food deprivation started when all rats were 90 days old. Harlan rats were housed individually for 10 days prior to food deprivation. Other housing conditions and the conditioning boxes were as described in Experiment 1.

3.1.2. Procedure

A similar procedure to that of Experiment 1 was implemented. Groups 32/S, 32/1, and 32/3 ($n = 8$) received, respectively, an injection of saline, 1, or 3 mg/kg of U50. Twenty-four animals were assigned to the unshifted 4–4 control conditions and randomly distributed in three groups ($n = 8$) receiving saline, 1, or 3 mg/kg of U50 (Groups 4/S, 4/1, and 4/3, respectively). Drug treatments were identical to those described previously for the 32-4 groups except that only one injection was administered before trial 12. Other aspects of the procedure were also as described in Experiment 1.

3.2. Results

Due to computer malfunction, data were lost for five trials from Groups 32/3 (two trials in different animals), 4/1, 4/3, and 4/S, all in preshift trials. These data were replaced with the group average on that trial [24]. Two animals from Group 32/1 ($n = 6$) were eliminated, both from the first replication, because of a recording malfunction during postshift trials.

Fig. 2 displays the consummatory performance of the six groups segregated by dose. Overall preshift performance shows an early trend toward greater response level in groups receiving access to 32% sucrose than to 4% sucrose. However, a Contrast × Replication × Trial (1–10) analysis indicated no effect of sucrose concentration in any of the factors, Fs < 2.77, ps > 0.10. There was a significant main effect of replication, $F(1, 42) = 13.86$, $p < 0.01$, produced by higher scores in rats from the second replication (Harlan rats) than the first replication (TCU rats). There was also a significant increase across trials, $F(9, 378) = 97.47$, $p < 0.001$.

The goal-tracking time performance on trial 11, before the drug treatment, was very similar across groups. A Contrast × U50 × Replication analysis yielded a significant contrast effect, $F(1, 34) = 30.46$, $p < 0.001$. None of the other main effects and interactions reached significance, Fs < 1. Thus, drug groups were statistically indistinguishable before the treatment.

Fig. 2 also shows the performance on trial 12, after animals had received the drug treatment, and on the subsequent two trials after the treatment. U50 administered before trial 12 alleviated cSNC at the 1 mg/kg dose, but retarded recovery from cSNC at the 3 mg/kg. A Contrast × U50 × Replication × Trial (12–14) analysis revealed a trial by contrast interaction, $F(2, 68) = 4.47$, $p < 0.02$, and a contrast by U50 interaction, $F(2, 34) = 3.32$, $p < 0.05$. There were also main effects for contrast and trials, Fs > 12.58, ps < 0.002. None of the other effects or interactions, including those involving replication as a factor, reached significant levels, Fs < 2.22, ps > 0.11. The source of the contrast by drug interaction was sought by using the same procedure implemented in Experiment 1.

On trial 12, the first trial after U50 administration, there was a significant group effect, $F(5, 40) = 5.42$, $p < 0.002$, and post-hoc pairwise comparisons showed significant differences between the two contrast groups (32% vs. 4%) of the 0 and 3 mg/kg drug conditions, ps < 0.005. However, Group 32/1 was not significantly different from Group 4/1, $p > 0.05$, indicating an attenuation of cSNC in the 1 mg/kg U50 condition. LSD post-hoc analyses of the downshifted conditions (32-4) revealed a significant difference between Groups 32/1 and 32/3, $p < 0.03$; other pairwise comparisons among the downshifted groups were not significant, ps > 0.05. A comparison of performance among unshifted conditions revealed nonsignificant differences between Groups 4/S, 4/1, and 4/3, ps > 0.05. This last result is consistent with a similar lack of a U50 effect on consummatory behavior on the 0–3 mg/kg range obtained in Experiment 1. In that experiment, only the 10 mg/kg U50 dose yielded evidence of a nonspecific suppressive effect on goal-tracking times.

An analysis of trial 13 showed a significant group effect, $F(5, 40) = 2.77$, $p = 0.04$. The comparisons between Groups 32/S vs. 4/S and between Groups 32/1 vs. 4/1 on

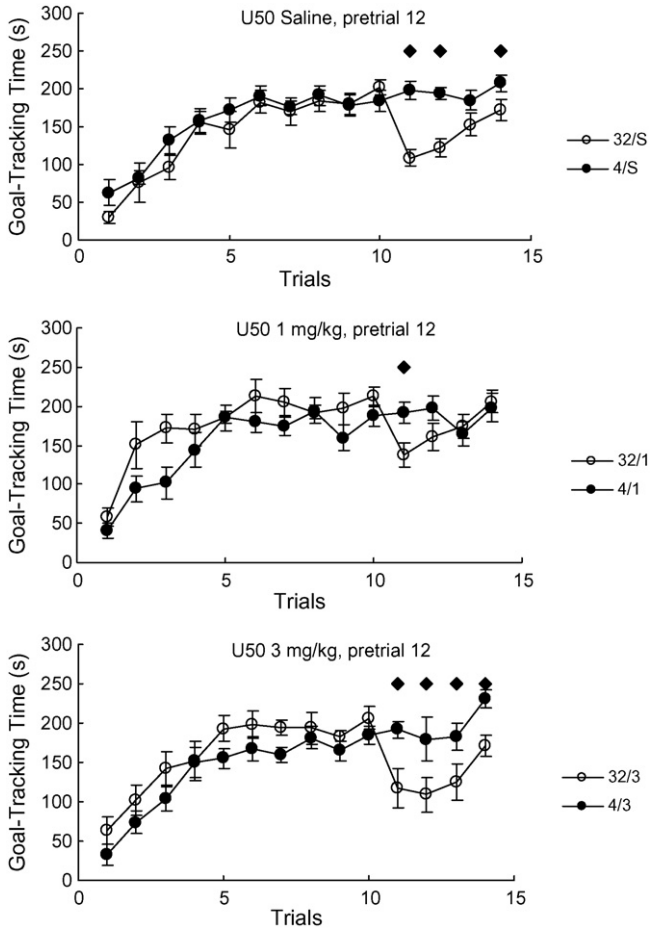

Fig. 2. Results of Experiment 2. Consummatory performance of the six groups during preshift trials (1–10) and postshift trials (11–14). Each dose treatment for downshifted and unshifted groups is presented separately (saline, 1, and 3 mg/kg of U50, i.p.). Drugs were administered before trial 12.

trial 13 were nonsignificant, $ps > 0.05$. However, post-hoc LSD analyses revealed that Group 32/3 performed significantly below Group 4/3, $p < 0.004$.

Trial 14 revealed a significant group effect, $F(5, 40) = 3.83$, $p < 0.007$, combined with a significant difference between Groups 32/S and 4/S, $p < 0.04$, and also between Groups 32/3 and 4/3, $p < 0.002$. The comparison between Groups 32/1 vs. 4/1 on trial 14 was again nonsignificant, $p > 0.05$.

3.3. Discussion

The evidence from Experiment 2 is consistent in all its details with the findings of Experiment 1. The low dose (1 mg/kg U50) selectively attenuated cSNC on trial 12, whereas the intermediate dose (3 mg/kg U50) selectively enhanced cSNC by extending it into trials 13 and 14. The results of Experiment 2, obtained after a single administration of U50 before trial 12, were very similar to those of Experiment 1, obtained after U50 administrations before trials 11 and 12. Relative to the saline groups, whose cSNC effect was detected in two trials in both experiments, the effect lasted one trial in the 1 mg/kg groups, and either 3 (Experiment 1) or four (Experiment 2) trials in the 3 mg/kg groups. Thus, U50 had a bidirectional effect on cSNC depending on the dose.

The bidirectionality of these effects of U50 on cSNC is difficult to explain in terms of nonspecific drug effects. For example, the 3 mg/kg dose may decrease goal-tracking time through an indirect activation of locomotor activity, a possibility suggested by other experiments [50]. However, the activity tests used on those experiments did not measure the effects of U50 within a period of time similar to consummatory trials implemented in these experiments. A more interesting possibility suggests that U50 influences the mechanisms involved in the posttrial processing of the downshift experience. Bentosela et al. [6] found that the level of consummatory suppression exhibited on trial 12 was enhanced by corticosterone administration immediately after trial 11. The effect of corticosterone administered after trial 11 implicates an effect on the consolidation and/or retrieval of the aversive memory of the downshift experience. This possibility will be addressed in Experiment 3.

There is some evidence suggesting that U50 can affect locomotor activity [21,50]. Schnur and Walker [50] reported that U50 had a dose-dependent bidirectional effect on running wheel activity in golden hamsters. Animals were tested 10 min after U50 administration and for a total of 120 min. Activity was then averaged into 10-min bins. Relative to saline controls, overall activity was higher in the low dose group (1 mg/kg U50) and lower in the high dose group (10 mg/kg U50). No evidence of an effect was found with a medium dose (3 mg/kg U50). However, the increase in activity in the low-dose condition emerged 50 min after drug administration. In the present experiments, U50 was administered 20 min before a trial that lasted 5 min. Thus, the present results are not easily explained in terms of the locomotor effects of U50.

4. Experiment 3

In Experiment 2, the low dose of U50 (1 mg/kg) reduced cSNC by one trial, whereas the medium dose of U50 (3 mg/kg) produced a more consistent cSNC effect across postshift trials, relative to saline controls. How were these effects accomplished? One possibility is that U50 influences the consolidation of the aversive memory of the downshift experience of trial 11. Experiment 3 was designed to assess this hypothesis.

The opioid system is involved in aversive learning. Gallagher and Kapp [17] reported that amygdala injections of the morphine analogue levorphanol and of the opioid antagonist naloxone following passive avoidance training affected retention in a time- and dose-dependent manner. Thus, levorphanol decreased retention when injected immediately following passive avoidance training, while naloxone injected immediately following passive avoidance training increased retention. Injections given 6 h posttrial were just as ineffective as saline controls. Hiramatsu et al. [22] found antiamnesia effects in the step-down passive avoidance task in mice subjected to amnesia induced by carbon monoxide. Their findings revealed that U50 administration before passive avoidance testing ameliorated carbon monoxide-induced deficits. The role of the κ-opioid system in memory is not well understood. Addressed above were examples of enhanced memory of aversive events. However, alternative evidence indicates that the κ-opioid system may induce memory deficits. For example, McDaniel et al. [31] identified spatial memory impairments on radial arm maze learning following administration of the κ-opioid agonist dynorphin into the dorsal (but not the ventral) hippocampus. This effect was reversed by naloxone.

The effects of a medium dose of U50 (3 mg/kg) on the recovery phase of cSNC described in Experiments 1 and 2 may be the result of the activation of alternative systems, rather than direct mediation. For example, Taylor et al. [52] found that U50 stimulates pituitary-adrenal function via hypothalamic arginine-vasopressin and corticosterone releasing factor, which increased the levels of corticotropic releasing hormone and corticosterone in blood plasma as compared to saline controls. As mentioned previously, Bentosela et al. [6] reported a retardation of recovery from cSNC on postshift trials 12–15 after the administration of corticosterone immediately after trial 11. The effect was not present when corticosterone was administered 3 h after trial 11.

Experiments 1 and 2 suggest that U50 has a dose-dependent bidirectional influence on cSNC. To the extent that both effects are mediated by U50's influence on the consolidation and/or retrieval of the aversive memory of the downshift, posttrial 11 administration of U50 was predicted to have the following effects: (1) A low dose of U50 (1 mg/kg) should shorten the cSNC effect, whereas (2) a medium dose of U50 (3 mg/kg) should prolong the cSNC effect.

4.1. Method

4.1.1. Subjects and apparatus

The subjects were 36 male Long-Evans rats bred at the TCU vivarium and derived from Harlan parents. Housing, maintenance conditions, and conditioning boxes were as described in Experiment 1.

4.1.2. Procedure

The downshift procedure used in Experiment 3 was similar to that of Experiment 1. Groups 32/S, 32/1, and 32/3 ($n = 6$) were exposed to the downshifted treatment as described in Experiment 1, whereas Groups 4/S, 4/1, and 4/3 ($n = 6$) received unshifted control treatment. These animals received the i.p. administration of saline, 1, or 3 mg/kg U50 immediately following trial 11. Other aspects of the procedure were as described in Experiment 1.

4.2. Results and discussion

The goal-tracking score for one rat on trial 8 in Group 32/3 was lost and replaced with the group average [24]. Fig. 3 shows the results segregating groups according to drug dose. As in the previous experiments, consummatory performance increased steadily across the 10 preshift trials showing no evidence of a concentration effect. A Sucrose (32%, 4%) × U50 (0, 1, 3 mg/kg) × Trial (1–10) analysis yielded only a significant acquisition effect, $F(9, 270) = 80.27$, $p < 0.001$. All other factors were nonsignificant, $Fs < 1.86$, $ps > 0.18$.

The goal-tracking time performance on trial 11, before the drug treatment, was very similar across groups receiving immediate posttrial administration of U50

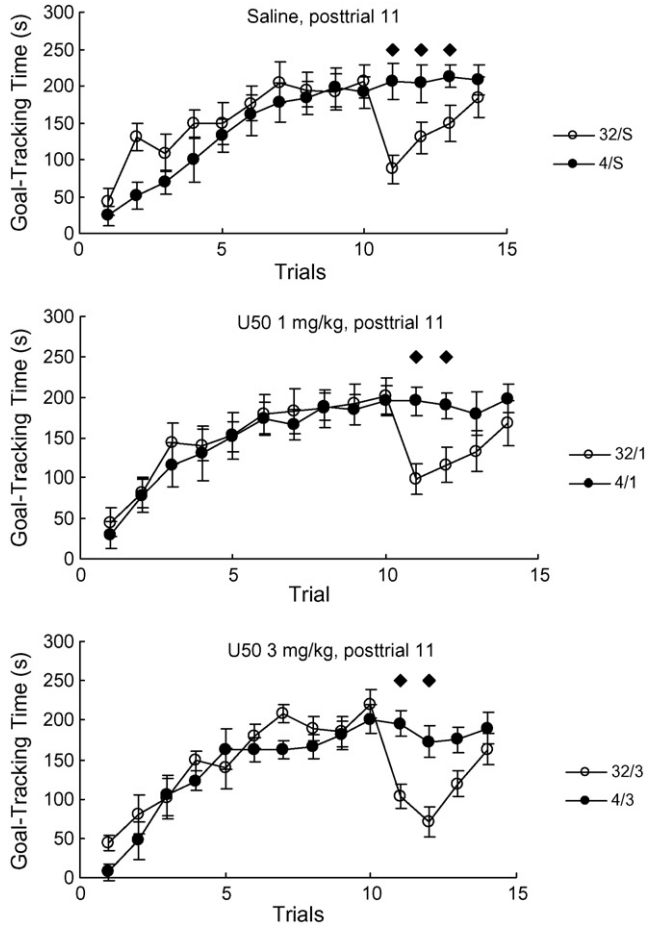

Fig. 3. Results of Experiment 3. Consummatory performance of the six groups during preshift trials (1–10) and postshift trials (11–14). Each dose treatment for downshifted and unshifted groups is presented separately (saline, 1, and 3 mg/kg of U50, i.p.). Drugs were administered immediately after trial 11.

(Fig. 3). All groups exhibited a cSNC effect of approximately the same size. A Contrast × U50 analysis yielded a significant contrast effect, $F(1, 30) = 31.18$, $p < 0.001$. None of the other main effects and interactions reached significance, $Fs < 1$. Thus, drug groups were matched before the posttrial treatment. A Contrast × U50 × Trial (12–14) analysis indicated a contrast by trial interaction, $F(2, 60) = 9.53$, $p < 0.001$, and main effects for contrast and trial, $Fs > 12.36$, $ps < 0.01$. Other $Fs < 1.48$, $ps > 0.24$. As in previous experiments, a trial-by-trial one-way analysis followed by LSD post-hoc pairwise comparisons followed.

Trial 11 revealed a significant group effect, $F(5, 30) = 6.32$, $p < 0.001$. Pairwise post-hoc comparisons revealed significant cSNC effects for each pair of contrast groups (32% vs. 4%) in each drug conditions (0, 1, and 3 mg/kg), $ps < 0.01$. LSD post-hoc analyses of the downshifted groups (32-4) revealed nonsignificant pairwise differences, $ps > 0.62$. Likewise, post-hoc analyses of the unshifted groups (4-4) revealed nonsignificant pairwise differences, $ps > 0.73$. These results confirm that before the posttrial administration of U50, the size of the cSNC effects and the consummatory behavior of the key groups were statistically similar.

Trial 12 yielded a significant group effect, $F(5, 30) = 4.93$, $p < 0.003$. Pairwise comparisons revealed significant differences between the two contrast conditions (32% vs. 4%) for all three drug conditions, $ps > 0.04$. Comparisons of downshifted groups indicated nonsignificant differences, $ps > 0.07$. Similarly, unshifted groups did not differ from each other, $ps > 0.34$, suggesting that the posttrial treatment did not affect the consummatory behavior of the unshifted controls.

Trial 13 produced a significant group effect, $F(5, 30) = 2.87$, $p < 0.04$. Pairwise comparisons revealed a significant cSNC effect for the saline condition, $p < 0.04$, but nonsignificant effects for the 1 and 3 mg/kg comparisons, $ps > 0.06$. No differences were also detected among the downshifted or the unshifted groups, $ps > 0.19$. Trial 14 revealed a nonsignificant group effect, $F < 1$. None of the post-hoc pairwise comparisons detected a significant cSNC effect, $ps > 0.31$.

Although the administration of U50 seemed to have shortened the cSNC effect relative to the saline controls, the borderline significance of the effect of U50 on trial 13 performance suggests caution in interpreting these results (see Fig. 3). Thus, it is tentatively concluded here that administration of the small (1 mg/kg) or medium (3 mg/kg) dose of U50 immediately after trial 11 had no effect on the recovery from cSNC during subsequent trials. As in previous experiments, posttrial 11 administration of U50 had no detectable effects on the consummatory performance of unshifted control groups. None of the effects reported in Experiments 1 and 2 with pretrial administration of U50 appears to be attributable to a role of the κ-opioid receptor subsystem on the consolidation of memory from the downshift experience.

5. Experiment 4

Despite the absence of a significant effect of posttrial U50 administration in the previous experiment, it is noteworthy that the performance of Group 32/3 on trial 12 was considerably lower than the performance of the same group on trial 11. Because the pretrial administration of that dose retards recovery of consummatory behavior (Experiments 1–2), it may be argued that this effect is attributable to the development of conditioned taste aversion (CTA). CTAs are usually supported by drug-induced sickness. For example, lithium chloride induces gastrointestinal sickness that acts as an effective unconditioned stimulus. However, drugs that do not induce sickness may nonetheless support the development of aversive conditioning. For example, the nonselective opioid antagonist naloxone supports relative strong place aversions [26], as well as increasing the cSNC effect [44]. U50 infused into several brain areas (including the nucleus accumbens, medial prefrontal cortex, and ventral tegmental area) has also been reported to induce similar place aversions [7]. These effects may be based on the induction of opioid-withdrawal symptoms or the anxiogenic properties of these opioids [32].

The CTA hypothesis is based on the procedural similarities between cSNC and CTA experiments involving drug administration. In both cases, rats are exposed to a taste while under the influence of a drug, either because of pre- or posttrial administration. In a typical posttrial administration procedure, rats in the unshifted control condition would be relatively less prone to exhibit CTA because of extensive exposure to 4% sucrose during preshift trials. However, 4% sucrose would be relatively novel for downshifted rats on trials 11 and 12. It is known that CTA proceeds faster with novel rather than familiar tastes [10]. Thus, the present experiment sought to determine whether CTA can be induced under the present conditions of training in the absence of a downshift experience. A positive answer to this question would suggest that the enhancing effects of U50 on cSNC at 3 and 10 mg/kg observed in previous experiments are attributable to the development of a transient aversion to the relatively novel 4% sucrose solution.

5.1. Method

5.1.1. Subjects and apparatus

The subjects were 22 female Long-Evans rats of the same characteristics and maintained under the same conditions as described in Experiment 1. The conditioning boxes were also those described in Experiment 1.

5.1.2. Procedure

Food-deprived rats were randomly assigned to two groups ($n = 11$). In a CTA experiment, the 4% sucrose solution operates as the conditioned stimulus and the administration of U50 as the unconditioned stimulus. All animals received three daily trials of access to 4% sucrose under the same conditions described in previous experiments. On trial 1, all rats received two injections, one immediately at the end of the trial and the other 3 h after the end of the trial. Group Paired was administered U50 immediately and saline solution 3 h after the trial, whereas Group Unpaired was administered saline immediately and U50 3 h after the trial. This design was chosen over the more typical use of a saline injection as a control procedure because it matches both groups in terms of the unconditioned stimulus (U50, 3 mg/kg, i.p.), as well as in terms of the administration procedures, while allowing the pairing of sucrose and U50 to vary across groups [12]. Other aspects of the procedure were as described in Experiment 1.

5.2. Results and discussion

Fig. 4 presents the main results of this experiment. The administration of U50 immediately at the end of the first trial caused a transient reduction in goal-tracking times during the subsequent two trials. A Group (Paired, Unpaired) × Trial (1–3) analysis indicated a significant interaction effect, $F(2, 40) = 3.41$, $p < 0.05$, and a significant change across trials, $F(2, 40) = 15.76$, $p < 0.001$. The group effect fell short of significance, $F(1, 20) = 3.67$, $p > 0.07$. Pairwise comparisons at each trial indicated that Group Paired scored significantly below Group Unpaired on trial 2, $F(1, 20) = 7.89$, $p < 0.02$, but not on trial 1, $F < 1$, or trial 3, $F(1, 20) = 2.37$, $p > 0.13$. Thus, U50 at 3 mg/kg administered immediately after the first access to 4% sucrose has the potential to at least attenuate the development of consummatory behavior and at most generate a transient CTA.

6. General discussion

The most surprising effect reported in this series of experiments involves the bidirectional effects of U50 on cSNC. The cSNC effect

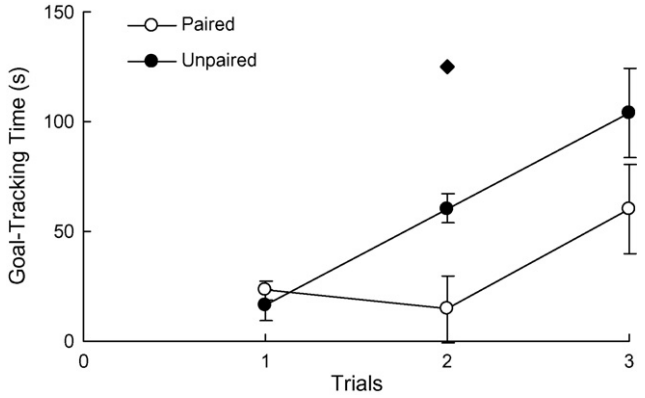

Fig. 4. Results of Experiment 4. Consummatory performance of the two groups on each of the three trials of this experiment. Each group received two injections, one immediately after trial 11 and the other after 3 h. In Group Paired, the immediate injection was U50 (3 mg/kg, i.p.) and the delayed was saline; for Group Unpaired the immediate injection was saline and the delayed was U50.

was either attenuated (1 mg/kg U50) or enhanced (3 mg/kg U50) depending on the dose administered before trial 12. Interestingly, both of these effects were behaviorally selective given that U50 failed to influence the cSNC effect when administered before trial 11. This behavioral selectivity and the lack of effects on consummatory behavior per se (except at the 10 mg/kg dose; see Experiment 1) suggest that the effects of U50 were not related to nonspecific actions on perceptual, motivational, or motor functions. Instead, these effects were dependent on the downshift experience. Another possibility explored in Experiment 3 was based on the hypothesis that U50 modulates the consolidation and/or retrieval of the downshift memory. No evidence was found that the effects of pretrial U50 administration were mediated by memory processes. Whereas the administration of the 3 mg/kg dose, whether pre- or posttrial (and also the 10 mg/kg dose in Experiment 1), interfered with recovery from cSNC on subsequent trials, Experiment 4 suggested that these effects were mediated by a transient conditioned aversion to the 4% sucrose. The rest of this section considers the theoretical implications of these findings.

Two hypotheses of cSNC consistent with Amsel's [3] frustration theory have been proposed [41,54]. First, consider the strong frustration hypothesis ("strong" because it includes all the major assumptions of frustration theory). Frustration theory suggests that the surprising reduction in sucrose concentration from 32% to 4% causes an unconditioned emotional response termed primary frustration that leads to consummatory suppression and to the invigoration of alternative behaviors (e.g., increased in activity and rearing during downshift trials; see [43]). Primary frustration is induced whenever the magnitude of an expected incentive exceeds the magnitude actually obtained and is the basis for the Pavlovian conditioning of an anticipatory form called secondary frustration. Secondary frustration provides an explanation for instrumental SNC (iSNC; [10]) and a basis to explain the ensuing approach-avoidance conflict [3]. Secondary frustration refers to the memory of the emotional reaction experienced by the animal during the downshift experience, also called egocentric memory [40]. According to this account, the suppression of consummatory behavior during cSNC occurs because of two mechanisms: first because of primary frustration (i.e., rejection of 4% sucrose on the first downshift trial) and subsequently because of both primary and secondary frustration (i.e., reactivation of the egocentric memory after the first downshift trial). Recovery from cSNC occurs because the continuous update of the memory record based on the new incentive conditions (called allocentric memory; [40]) makes it more likely for the organism to anticipate the incentive magnitude that it is actually receiving. As expectations and reality become more similar, the trigger for primary frustration is reduced. Furthermore, the association between secondary frustration and 4% sucrose leads to a counterconditioning process that reduces the avoidance component of the conflict, thus promoting consummatory behavior. Therefore, the strong frustration hypothesis explains cSNC in terms of four processes: (1) an allocentric memory of the preshift incentive, (2) primary frustration, (3) secondary frustration (egocentric memory of the downshift), and (4) counterconditioning. Factors selectively affecting cSNC on the second postshift trial, after some experience with the downshifted solution, have the potential to influence the consolidation or retrieval of the egocentric memory of the downshift experience.

Recent data on the spontaneous recovery of consummatory behavior during postshift trials suggest an alternative explanation that can be dubbed the weak frustration hypothesis ("weak" because it does not require egocentric memory encoding). Norris et al. [37] reported that consummatory performance during postshift trials was high at the start of each trial and only subsequently dropped drastically during the trial. This observation suggests that cSNC occurs because at the onset of each postshift trial there is differential retrieval of the allocentric memory of the 32% sucrose vs. the allocentric memory of the new 4% sucrose or the egocentric memory of frustration. The retrieval of the 32% sucrose memory would then trigger primary frustration on each postshift trial. cSNC is reduced because of the update of the allocentric memory to the new incentive magnitude of 4% sucrose. Thus, whereas the strong frustration hypothesis requires an allocentric memory of 32% sucrose, a primary frustration process inducing consummatory suppression, the egocentric memory of the downshift, and the allocentric update to the new 4% sucrose incentive, the weak frustration hypothesis can explain cSNC without assuming the egocentric memory of the downshift. The ensuing conflict in the case of the weak hypothesis could be characterized as an approach-rejection conflict. "Avoidance" implies responding in anticipation to an object, whereas "rejection" implies responding to an actual contact with the object. Thus, a solution is avoided when a negative outcome is anticipated (cued recall), whereas a solution is rejected if it is found to be different than expected after some minimum consumption (recognition failure). Notice that egocentric memory is required to account for iSNC because it is measured in terms of a purely anticipatory response (i.e., running toward the goal box of a runway; e.g., [11]).

These hypotheses provide different interpretations for the effects of U50 on cSNC described in these experiments. Consider first the attenuating effect of 1 mg/kg U50 on trial 12, reported in Experiments 1 and 2. Nonspecific drug effects are discarded by the behavioral selectivity of the effect and memory encoding effects are discarded by Experiment 3. One hypothesis suggests that U50 acts like an anxiolytic. This anxiolytic hypothesis is consistent with the attenuating effects of low U50 doses on anxiety-related behaviors. For example, Privette [46] found that 1 mg/kg U50 increased exploratory behavior in the elevated plus maze. The κ-opioid receptor agonist U-69,593 showed a similar effect in the same test, indicating that a mildly active κ-opioid system may have anxiolytic properties. The elevated plus maze provides an assessment of fear of open spaces in rodents (agoraphobia), a type of behavior reduced by treatment with benzodiazepine anxiolytics [45].

Benzodiazepine anxiolytics also reduce cSNC on trial 12 [16]. Several studies have identified a direct connection between the effects of benzodiazepines and opioid drugs. For example, naloxone blocks the anxiolytic effects of benzodiazepines in several models of anxiety [2], whereas naloxone potentiates anxiety-related behavior [9,25,49]. Ågmo and Belzung [1] suggested that this effect

may be in part mediated by the κ-opioid receptor system. They found that the κ-opioid receptor antagonist nor-binaltorphimine dose-dependently blocked the effects of the benzodiazepine chlordiazepoxide. By contrast, Nemmani and Ramarao [36] found that diazepam dose-dependently attenuated analgesia induced by a large dose of U50 (40 mg/kg). Thus, κ-opioid agonists and benzodiazepine anxiolytics may interact depending upon their level of activation.

The weak frustration hypothesis suggests that an anxiolytic-like effect in the cSNC situation may be achieved by at least three mechanisms. One involves a reduction in the emotional intensity of primary frustration induced by the incentive downshift. This possibility applies well in the case of morphine [47] and DPDPE [54], but it is not viable for U50 because this drug has no detectable effect on cSNC during trial 11 (Experiment 1). The second possibility is that U50 attenuates the rejection component of the approach-rejection conflict presumably peaking during trial 12. This is superficially analogous to U50's attenuation of the usual rejection of open spaces in the elevated plus maze situation mentioned above [46]. A third possibility is that U50 enhances the approach component of the conflict, an effect consistent with the stimulation of sucrose consumption reported for small doses [28], but inconsistent with the lack of an effect on consummatory behavior in the present unshifted control groups. The anxiolytic-like properties of low U50 doses in the cSNC situation merit further analysis.

Consider next the enhancing effect of the 3 mg/kg U50 dose on cSNC. Again we may safely discard nonspecific explanations and, again, the possibilities point to two alternatives: either an effect on the strength of primary frustration (suggested by the weak frustration hypothesis) or on the conditioning of secondary frustration (suggested by the strong frustration hypothesis). The possibility that 3 mg/kg U50 increases the strength of primary frustration is discarded by the results of Experiment 1. In that experiment, U50 failed to enhance cSNC as one would expect if its role were to influence the emotional intensity induced by the incentive downshift experience. The selectivity of U50 for trial 12 suggests that its role in cSNC is related to secondary frustration, a conditioned internal response that builds up after some experience with the downshifted solution. U50 may enhance either the conditioning of secondary frustration, or its intensity once elicited, either contributing to prolonging the cSNC effect. However, the combined results of Experiments 3 and 4 suggest that an effect on the consolidation of the egocentric memory of the downshift experience is unlikely. Thus, it seems possible that the cSNC-enhancing effects of U50 are entirely due to the induction of a conditioned taste aversion, as shown in Experiment 4. Such conditioned aversions can sometimes develop even when the drug acting as the unconditioned stimulus is administered before access to the taste solution acting as the conditioned stimulus (e.g., with D-cycloserine as the unconditioned stimulus, see [38]). This could have been the case in the present Experiments 1 and 2, when U50 was administered before trials 11 and/or 12.

In conclusion, the function of the κ-opioid system in the cSNC situation is complex. On the one hand, U50 is behaviorally selective for the second postshift trial, a selectivity analogous to that displayed by benzodiazepine anxiolytics and different from the effects of at least two other opioid agonists, morphine (not trial selective; [47]) and DPDPE (selective for trial 11; [54]). This trial selectivity of the δ- and κ-opioid receptor subsystems confirms the dissociability of the psychological processes postulated to characterize the initial reaction to the downshift and the recovery that follows [41]. On the other hand, the strength of activation of the κ-opioid receptor subsystem determines whether cSNC is attenuated (1 mg/kg) or enhanced (3 and 10 mg/kg), probably due to the development of a conditioned taste aversion. Once the systemic effects of selective opioid-receptor compounds on cSNC are characterized, the next step would be to determine the locus of action of selective opioid-receptor drugs in the brain. Neurons that expressed opioid receptor genes or have membrane receptors are widely distributed in the rat brain [29]. It is expected that only some of these brain areas will participate in the control of behavior during cSNC.

Acknowledgments

The research reported in this paper was supported by research funds from the Department of Psychology, TCU. The authors appreciate comments made by Santiago Pellegrini and Leonardo Ortega Murillo to an earlier version of this paper.

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