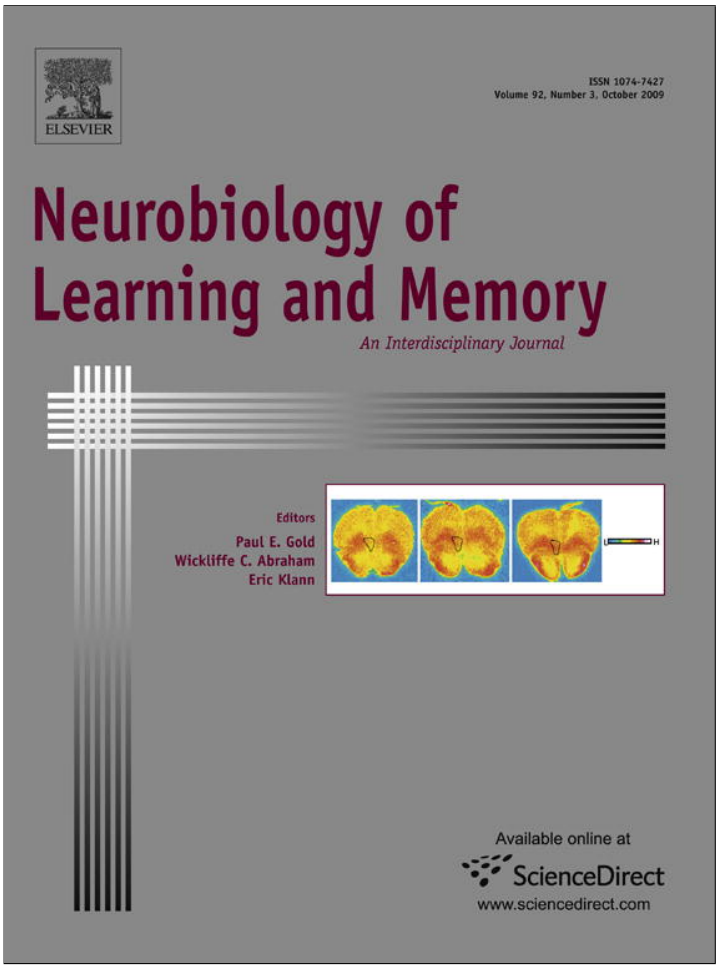

Neurobiology of Learning and Memory 92 (2009) 439–450

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Role of the opioid system in incentive downshift situations

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ARTICLE INFO

Article history:
Received 23 March 2009
Revised 14 May 2009
Accepted 10 June 2009
Available online 14 June 2009

Keywords:
Opioid system
Incentive contrast
Consummatory successive negative contrast
Appetitive extinction
Naloxone
Naltrindole
DPDPE
Rats

ABSTRACT

Previous research has shown that opioid blockage enhances consummatory successive negative contrast (cSNC)—a suppression of consummatory behavior following a downshift from 32% to 4% sucrose solution. In Experiment 1, administration of the nonselective opioid receptor antagonist naloxone (2 mg/kg, ip) distorted the comparison between expected and received incentives. The results of Experiment 2 discarded the alternative that naloxone enhances cSNC by inducing a conditioned taste aversion. The results of Experiments 3a–3c provided no evidence that opioid administration after the first downshift trial modulated subsequent consummatory performance. The opioids tested included naloxone (2 mg/kg, ip), the δ-opioid receptor selective antagonist naltrindole (1 mg/kg, ip), and the δ-opioid receptor selective agonist DPDPE (24 μg/kg, ip). The selected doses have proven in earlier experiments to be effective when administered before training. Experiments 4–5 failed to uncover any effects of posttraining opioid blockage with naloxone in an appetitive extinction task (autoshaping with lever-food pairings). These results add to our previous understanding of opioid function in situations involving incentive downshifts, suggesting a role in the comparison process that triggers cSNC, but no apparent function in memory consolidation related to the downshift event.

1. Introduction

Vogel, Mikulka, and Spear (1968) reported that rats given access to 32% sucrose solution for 5 min daily for 11 trials exhibited consummatory suppression after a downshift to 4% sucrose, relative to an unshifted control group only exposed to the 4% sucrose. Consummatory behavior recovered over the subsequent six postshift trials to the level of the unshifted controls. This phenomenon is called consummatory successive negative contrast (cSNC). Flaherty (1996) characterized cSNC in terms of a multistage hypothesis consisting of two distinct stages. The first stage involves the detection of the downshift, the rejection of the downshifted incentive, and the searching for the missing reward. Failure to locate the missing reward initiates a second stage, called recovery, during which conflict and stress are involved. Based primarily upon pharmacological data, Flaherty (1996) described the first stage in the multistage hypothesis as purely cognitive, while the second stage involved an emotional reaction of frustration.

The multistage hypothesis has since been adapted to Amsel's (1992) frustration theory, which has the advantage of making some of the components more explicit (Papini, Wood, Daniel, & Norris, 2006; Wood, Daniel, & Papini, 2005). Amsel's (1992) theory of frustration attributes the emotional reaction resulting from surprising reward loss to the violation of an incentive expectancy by the presentation of a smaller reward than expected. The main differences between Flaherty's sequential hypothesis and Amsel's frustration theory are the following. In the first stage, frustration theory interprets "rejection" as resulting from the elicitation of primary frustration, an internal aversive state induced by surprising reward loss. Thus, the initial stage is not purely cognitive, but it also contains an emotional unconditioned response to the downshift. In the second stage, frustration theory suggests that the avoidance component of the approach-avoidance conflict reflects secondary frustration, that is, a conditioned anticipatory version of primary frustration. Frustration theory also suggests two additional conditioning processes that contribute to recovery from cSNC. One involves the counterconditioning of secondary frustration by its pairing with the downshifted incentive and the other is the update of the incentive expectation to match the postshift incentive value. The counterconditioning of secondary frustration leads to a reduction in competing responses that interfere with drinking behavior, whereas the memory updating process reduces the discrepancy between expected and obtained incentives, thus weakening primary frustration and, consequently, promoting consummatory behavior.

The modified multistage model of cSNC suggests a sequence of stages that can be characterized as involving detection, rejection, search, approach-avoidance conflict, counterconditioning, and memory update. Although these stages occur in rapid succession, the pharmacological evidence alluded to below suggests that the

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doi:10.1016/j.nlm.2009.06.003

effects of conflict peak after at least 5 min after the initial downshift experience. Because each trial lasts 5 min, this implies that pharmacological manipulations acting on the conflict (e.g., administration of benzodiazepine anxiolytics such as chlordiazepoxide) tend to be more effective on the second postshift trial (usually trial 12) than on the first (e.g., Becker, 1986; Flaherty et al., 1990; Flaherty & Rowan, 1989). However, anxiolytics can be effective on the first postshift trial provided that the trial is lengthened beyond the typical 5 min (Flaherty, Grigson, & Rowan, 1986; Mustaca, Bentosela, & Papini, 2000) or when the animal is downshifted repeatedly (Flaherty, Clarke, & Coppotelli, 1996). Anxiolytics attenuate cSNC only after some experience with the downgraded solution.

Based on the characterization of cSNC provided by the modified multistage model, it could be argued that this phenomenon is based on three fundamental processes: detection (a perceptual–cognitive process), rejection (a motivational–emotional process), and learning (acquiring information about the new incentive conditions, called allocentric learning, and about the aversive experience of the downshift, called egocentric learning; Papini, 2003). Previous research shows that the opioid system is involved in both the rejection and the recovery process in a surprisingly selective manner. For example, the δ-opioid receptor subsystem is selectively involved in modulating the initial reaction to the downshift. Thus, the agonist DPDPE ([D-Pen2,D-Pen5]-Enkephalin) attenuates cSNC when administered before the first downshift trial, but has no effect when administered before the second downshift trial (Wood et al., 2005). Conversely, the antagonist naltrindole enhances cSNC when administered before the first downshift trial, but has no effect on the second downshift trial (Pellegrini, Wood, Daniel, & Papini, 2005). A second set of experiments suggest that the κ-opioid receptor agonist U-50,488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide) attenuates cSNC when administered before the second downshift trial, but has no effect when administered before the first downshift trial—just the opposite trial selectivity as that exhibited by δ-opioid receptor modulators (Wood, Norris, Daniel, & Papini, 2008). Interestingly, the nonselective opioid receptor antagonist naloxone administered before the first and second downshift trials enhances cSNC, suggesting that the downshift experience naturally induces the release of endogenous opioids (Pellegrini et al., 2005). Consistent with the effects of naloxone, the nonselective opioid receptor agonist morphine attenuates cSNC when administered before the first and second downshift trials (Rowan & Flaherty, 1987).

Opioid modulation of cSNC can be understood in terms of effects on detection, rejection, and/or learning. The available evidence does not distinguish between these potential effects, providing only evidence of trial and receptor selectivity. Notice that the effects of opioid peptides on cSNC cannot be accounted for in terms of altered sucrose palatability because these drugs had no effect on consummatory behavior in unshifted control groups. Therefore, although opioids can modulate sucrose palatability under some conditions (e.g., Kelley et al., 2002), such modulation does not appear to be a factor in the cSNC situation. Similarly, whereas opioids may be less effective in modulating feeding when animals are food deprived (Lowy, Maickel, & Yim, 1980), this was not a factor in cSNC experiments given the lack of opioid effects in unshifted controls and the trial-selective effects of some opioids such as DPDPE and U50,488H described above. The experiments reported here were designed to test the role of the opioid system on detection of the incentive downshift (Experiment 1), on rejection based not on frustration, but on conditioned taste aversion (Experiment 2), and on the modulation of egocentric memory consolidation (Experiment 3). In addition, Experiments 4–5 explored the role of the opioid system on appetitive extinction, a training situation that shares with cSNC the incentive downshift operation.

2. Experiment 1

Papini and Pellegrini (2006) reported that incentive downshifts of different magnitudes but with the same ratio of discrepancy between solutions resulted in similar amounts of consummatory suppression. Based on this evidence (see also Pellegrini, Lopez Seal, & Papini, 2008; Pellegrini & Papini, 2007), it was argued that the detection of an incentive downshift operates under constraints similar to those described by Weber's law for sensory systems. If opioids influence the comparison between the solutions, then administration of naloxone will distort this scaling property. For example, if opioid receptor blockage enhances the disparity between the preshift and postshift incentives, then naloxone administration should cause the groups with greater absolute disparity between solutions to show enhanced consummatory suppression compared to groups with the same ratio but smaller disparity.

2.1. Method

2.1.1. Subjects

The subjects were 66 male, experimentally naive Long–Evans rats, 90 days old at the start of the experiment. Rats were bred in the TCU vivarium from parents purchased at Harlan (Indianapolis, IN) and maintained under a 12:12 h light:dark cycle (lights on at 07:00 h). The vivarium temperature (18–23 °C) and humidity (40–70%) were monitored daily. 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 three successive days before deprivation started. Water was continuously available in each individual wire-mesh cage. Animals were trained during the light phase of the daily cycle.

2.1.2. Apparatus

Training was conducted in four conditioning boxes (MED Associates, Fairfax, VT) constructed of aluminum and Plexiglas (29.3 × 21.3 × 26.8 cm, $L \times H \times W$). The floors were made of steel rods, 0.4 cm in diameter and 1.6 cm apart, running parallel to the feeder wall. A bedding tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine. Against the feeder wall was an elliptical opening 1 cm wide, 2 cm high, and 4 cm from the floor, through which a sipper tube, 1 cm in diameter, was inserted. When fully inserted, the sipper tube was 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 was 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 80.1 dB (SPL scale C).

2.1.3. Procedure

Training lasted 15 daily trials. All trials lasted 5 min starting from the first contact with the sipper tube. The first 10 were the preshift trials and the last 5 were postshift trials. For all the groups, each preshift trial consisted of access to either 32% or 16% sucrose solution (w/w; e.g., 32% was prepared by mixing 32 g of commercial sugar for every 68 g of distilled water). At the end of the preshift, groups given either 32% or 16% sucrose were each divided into two subgroups matched for preshift responding. The 5 postshift trials were exactly like preshift trials, except for the concentration of the sucrose solution. The rats originally trained with 32% sucrose were assigned to either the 32-6 or 32-12 conditions,

whereas those originally trained with 16% sucrose were assigned to either the 16-3 or 16-6 condition. Each condition was divided into two subgroups, behaviorally matched for preshift performance, one assigned to the naloxone condition (Nlx) and the other to the vehicle condition (Veh). The final eight groups were the following: 32-6/Veh ($n = 8$), 32-12/Veh ($n = 8$), 16-6/Veh ($n = 8$), 16-3/Veh ($n = 8$), 32-6/Nlx ($n = 9$), 32-12/Nlx ($n = 8$), 16-6/Nlx ($n = 8$), and 16-3/Nlx ($n = 9$).

The selected concentrations were chosen so as to generate specific postshift/preshift ratios. Because naloxone enhances contrast (Pellegrini et al., 2005), smaller downshifts than the usual 32-4 downshift were used to reduce the possibility of floor effects (Papini & Pellegrini, 2006). For Groups 32-6 and 16-3, the ratio of disparity between solutions was 0.1875, whereas for Groups 32-12 and 16-6 the ratio was 0.375. Ratio scaling makes two predictions for the first downshift trial: (1) equal ratios will produce equal levels of consummatory behavior, and (2) the larger the ratio, the lesser the consummatory suppression.

All animals were weighed every day starting three days before food deprivation and ending on the day of the final training trial. Before each trial, animals were transported to a waiting room in squads of four. The transport rack fit up to four squads. The composition of each squad and the assignment to a training box was maintained constant, but the order in which squads were run was changed randomly across days. Naloxone or saline were administered in the waiting room. Naloxone (Sigma–Aldrich, Saint Louis, MO) was dissolved into isotonic physiological saline as a vehicle to a concentration such that each subject received a 1 ml/kg injection. The dose (2 mg/kg), administration route (ip), and timing (15 min before postshift trial 11) were previously shown to be effective in the cSNC situation (Pellegrini et al., 2005). Immediately after the trial, animals were placed back in their cages, the boxes were wiped with a wet paper towel, and the animals returned to the waiting room. When all squads had been run, animals were carried back to the colony room. This was repeated until all animals had 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 last squad had ended its daily training trial.

The dependent measure was the accumulated time in contact with the sipper tube (called goal-tracking time and measured in 0.05-s units) up to a maximum of 5 min (the duration of each trial from the first contact with the sipper tube). This measure has shown orderly results under the present conditions of training, had been shown to correlate significantly with fluid consumption (Mustaca, Freidin, & Papini, 2002), and has provided similar results to those of fluid consumption and lick rate when both measures were used (Papini, Mustaca, & Bitterman, 1988; Riley & Dunlap, 1979). Goal-tracking times were subjected to conventional analysis of variance, with the alpha error set at the 0.05 level. Due to a computer malfunction, data were lost for two rats in Group 16-6/Veh on trial 13 and were replaced with the group average (Kirk, 1968).

2.2. Results

The overall results of the experiment are presented in Fig. 1. A Sucrose (32%, 4%) × Ratio (0.375, 0.1875) × Drug (naloxone, saline) × Trial (preshift trials 1–10) analysis revealed a significant increase of goal-tracking times across trials, $F(9, 65) = 149.63$, $p < 0.01$, but no other significant effects or interactions, Fs < 3.31, ps > 0.07.

An overall analysis of postshift performance with a Sucrose × Ratio × Drug × Trial (postshift trials 11–15) analysis indicated the following results. There was a significant change in goal-tracking times across trials, $F(4, 65) = 50.89$, $p < 0.01$, and significantly higher scores for groups exposed to a higher postshift/preshift solution ratio, $F(1, 65) = 16.24$, $p < 0.01$. There were also significant

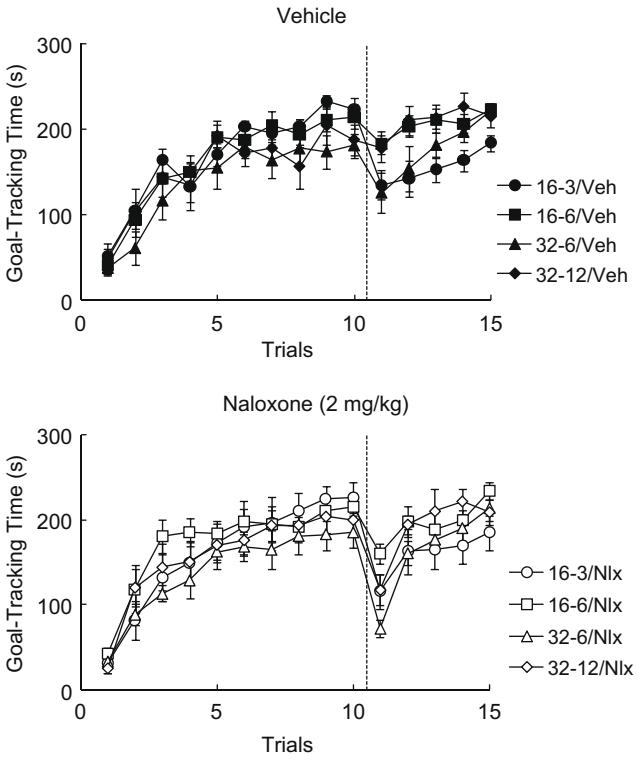

Fig. 1. Mean (±SEM) goal-tracking time for Experiment 1. The top panel shows the results for vehicle groups treated with saline solution. The bottom panel shows the results for groups treated with naloxone (2 mg/kg, ip). Injections were administered 15 min before trial 11. Groups differ in terms of the concentration of the sucrose solution received during preshift trials (either 32% or 16% sucrose) and postshift trials (12%, 6%, or 3% sucrose).

trial by preshift, $F(4, 65) = 5.65$, $p < 0.01$, and trial by drug interactions, $F(4, 65) = 4.52$, $p < 0.01$. All other effects and interactions were nonsignificant, Fs < 2.08, ps > 0.08.

The effects of naloxone on consummatory behavior were restricted to trial 11. A one-way analysis of variance comparing Groups 32-6/Nlx, 32-6/Veh, 16-6/Nlx, and 16-6/Veh yielded a significant difference between groups, $F(3, 29) = 10.77$, $p < 0.01$. LSD pairwise post hoc comparisons indicated that Group 32-6/Veh drank less than Group 16-6/Veh, $p < 0.02$, and Group 32-6/Nlx drank less than Group 16-6/Nlx. These comparisons provided evidence for a special case of cSNC (i.e., equal postshift incentives, but different preshift incentives; Papini & Pellegrini, 2006). However, while Groups 16-6/Nlx and 16-6/Veh did not differ significantly from each other, $p > 0.28$, Group 32-6/Veh drank more than 32-6/Nlx, $p < 0.02$, indicating that the suppressive effects of naloxone were directly related to the absolute disparity between solutions.

A Sucrose × Ratio analysis involving only the vehicle groups on trial 11 replicated the scaling property reported by Papini and Pellegrini (2006), with a significant main effect of ratio, $F(1, 31) = 8.15$, $p < 0.01$. The preshift sucrose and the sucrose by ratio interaction were nonsignificant, Fs < 1. However, a Sucrose × Ratio analysis on only the naloxone groups indicated that, like the vehicle groups, there was a significant effect of ratio, $F(1, 33) = 8.16$, $p < 0.01$, but, unlike in vehicle-treated groups, the preshift effect was also significant, $F(1, 33) = 7.41$, $p < 0.02$. The interaction remained nonsignificant, $F < 1$. This result illustrates that normally the ratio determines the level of behavioral decrement during cSNC, but when naloxone is administered, the absolute disparity between the preshift and postshift solutions also determines the level of responding. Fig. 2 shows goal-tracking time on trial 11 as a func-

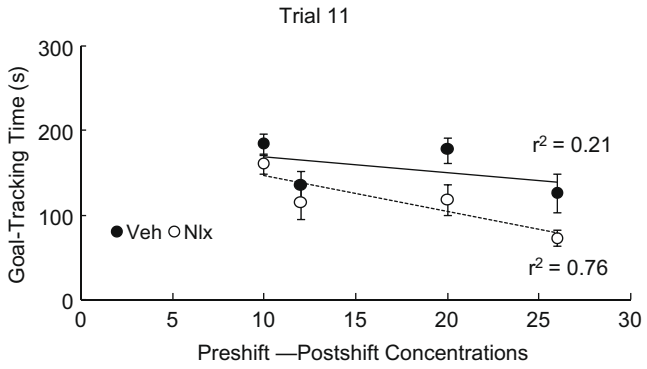

Fig. 2. Mean (±SEM) goal-tracking time for trial 11 in Experiment 1 as a function of the absolute difference between preshift and postshift sucrose concentrations. Ratio scaling in the vehicle groups distorts the linear relationship, lowering the coefficient of determination ($r^2 = 0.21$). By contrast, naloxone improves linearity ($r^2 = 0.76$).

tion of the absolute disparity between pre- and postshift concentrations. Vehicle groups exhibited similarity based upon the postshift/preshift ratio, but suppression in the naloxone groups was more linear with regard to the absolute discrepancy. A correlational analysis of each set separately indicated a higher linear fit for the naloxone-treated groups than for the vehicle-treated groups (see r^2 coefficients of determination in Fig. 2).

The present experiment replicated both the special case of contrast and the scaling property of cSNC reported by Papini and Pellegrini (2006). Downshifts with greater absolute disparity were more sensitive to the effects of naloxone, providing the first evidence supporting the hypothesis that opioid blockage affects the comparison between expected and received incentive magnitudes that is critical for cSNC. These results are consistent with the hypothesis that opioid blockage results in a shift from a ratio rule to a difference rule in assessing expected and current incentive values.

3. Experiment 2

Experiment 2 was designed to test the alternative hypothesis that the enhancing effect of naloxone on cSNC reported previously (Pellegrini et al., 2005) was caused not by rejection based on frustration, but on the development of a conditioned taste aversion. Whereas the results of Experiment 1 indicate that naloxone affected consummatory behavior only when it was given before trial 11, in the original report of this series (Pellegrini et al., 2005, Experiment 1), with naloxone administration before trials 11 and 12, naloxone treatment extended cSNC to trials 11–15, whereas the saline groups exhibited cSNC only during trials 11 and 12. The detection of an effect beyond drug administration trials suggests the hypothesis that opioid receptors may be involved in memory processes in the cSNC situation. This hypothesis is tested in the remaining experiments of this series.

If naloxone induces an aversive internal state, that state may act as an unconditioned stimulus (US) capable of supporting a conditioned taste aversion to the downshifted sucrose solution acting as a conditioned stimulus (CS). The lack of an effect in the unshifted controls relative to downshifted animals could be explained in terms of differential familiarity with the low-concentration sucrose. Unshifted controls received 10 trials with the low-concentration solution before their first CS–US pairing. By contrast, downshifted rats received 10 trials with the high-concentration solution, qualitatively similar but quantitatively more intense. Thus, for downshifted animals, the first trial with the low-concentration solution coincides with the first CS–US pairing. Consistent with this account, taste aversions induced by lithium chloride are known to be disrupted by preexposure to the CS (Best, 1975).

In addition, naloxone has been reported to affect consummatory behavior. For example, naloxone reduced water and food intake in deprived rats (Frenk & Rogers, 1979). Naloxone also seems to induce taste aversions, at least when compared to a saline-injection control in a one-bottle training procedure (Wu, Cruz-Morales, Quinan, Stapleton, & Reid, 1979). But apparently these two phenomena are not related, that is, consummatory suppression is not the result of taste aversion, as the two measures are not correlated (Wu et al., 1979). Furthermore, although the taste aversions induced by naloxone (2.5 or 20 mg/kg) were significantly weaker than those induced by lithium chloride (31.8 mg/kg), place aversions were stronger with naloxone than with lithium chloride (Lett, 1988). In this case, rats were exposed to solutions paired or not with naloxone, but in separate days (i.e., each test involved a single bottle). Other studies reported evidence of taste aversions induced by naloxone with comparison with other drugs. For example, naloxone dose-dependently reduced saccharin consumption more markedly than the delta-opioid receptor antagonist naltrindole, especially at 10 and 18 mg/kg, in one-bottle tests (Hutchinson et al., 2000). The use of one-bottle tests in previous experiments is especially relevant for the present purposes because rats are exposed to a single solution during cSNC experiments.

The design adopted here equated three groups in terms of exposure to the injection procedure, while simultaneously manipulating the temporal contiguity between the CS (5-min access to 4% sucrose) and the US (2 or 10 mg/kg naloxone). All animals received 3 injections, one 3 h before the CS, another immediately after the CS, and a third one 3 h after the CS. Naloxone was administered once in each of these three pairing conditions for three independent groups, yielding backward, paired, and unpaired conditions. The other two injections were vehicle control injections. In the absence of relevant information with naloxone, the choice of 3-h intervals for the pre- and post-CS injections was based on similar experiments involving corticosterone administration (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006; Ruetti, Justel, Mustaca, & Papini, 2009). Thus, rats were tested under conditions similar to those operating in a cSNC experiment, except that they did not experience an incentive downshift.

3.1. Method

3.1.1. Subjects and apparatus

Forty-eight male ($n = 23$) and female ($n = 25$), experimentally naive Long–Evans rats, 90 days old at the start of training, were used in this experiment. The origin and general maintenance of these animals was the same as described in Section 2.1.1. Females were included in this (and in some later experiments) only because of subject availability and not because of any specific aim at studying the effects of sex on consummatory behavior. Nonetheless, sex was included in statistical analyses whenever appropriate. The general effect is that males produce higher goal-tracking times than females, but these differences disappear when goal tracking is expressed as a ratio of ad libitum body weight (g). Thus, in general, sex effects appear to be reducible to sex differences in body size. The same conditioning chambers described in Section 2.1.2 were used in this experiment.

3.1.2. Procedure

Rats were randomly assigned to one of six groups ($n = 8$) according to a 3×2 factorial design with pairing condition as one factor (Paired, Unpaired, Backward) and naloxone dose as the other (2 mg/kg, 10 mg/kg). Each of the six groups included four males and four females except for the group that received back-

ward pairings with 10 mg/kg naloxone, which had three males and five females. All rats received 5 min of daily access to 4% sucrose for three trials, as in a similar experiment involving the κ-opioid receptor agonist U50,488H (see Wood et al., 2008, Experiment 4). As in cSNC experiments, the 5-min count started on each trial after the first contact of the animal with the sipper tube was detected. The first trial served as an acquisition trial, in which the naloxone was administered as described below, and was equivalent to trial 11 in a cSNC experiment. The subsequent two trials served to test the effects of the CS–US pairing in extinction.

Naloxone (Sigma–Aldrich, MO) was prepared and administered as described in Section 2.1.3. On day 1, all groups received three injections, one 3 h before the start of the first training trial, a second one immediately after the first training trial, and the third one 3 h after the end of the first training trial. For each group, one of the injections contained naloxone (2 or 10 mg/kg, ip), whereas the other two were saline injections. This procedure equates handling and the injection procedure across groups. For two groups (Backward), naloxone was administered 3 h prior to the onset of the first trial (2 or 10 mg/kg); the other two injections were saline. For two groups (Paired), naloxone was administered immediately after the first training trial (2 or 10 mg/kg); the other two injections were saline. For two groups (Unpaired), naloxone was administered 3 h after the end of the first training trial (2 or 10 mg/kg); the other two injections were saline. On days 2–3, all animals had access to 4% sucrose but no injections were administered. Other aspects of the procedure were as described in Section 2.

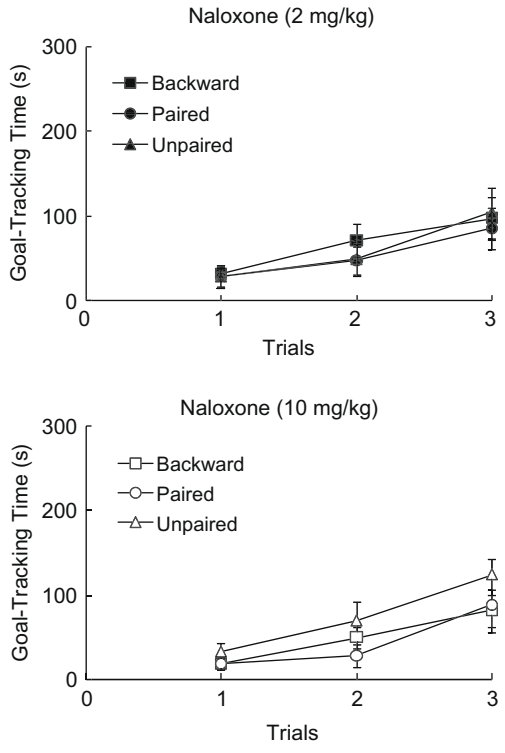

Fig. 3. Mean (±SEM) goal-tracking times for Experiment 2. A single naloxone injection was administered either immediately after the trial (paired), 3 h after the first trial (unpaired), or 3 h prior to the trial (backward), and either at 2 mg/kg (top panel) or 10 mg/kg (bottom panel). Animals were injected once with naloxone and twice with the vehicle, so that all animals received the same number of injections equally distributed in time (all injections were i.p. and of equal volume). Training (trial 1) was followed by two test trials (trials 2–3) in which only the CS (4% sucrose solution) was presented.

3.2. Results

The results are presented in Fig. 3. A Pairing (Paired, Unpaired, Backward) × Naloxone (2, 10 mg/kg) × Trial (1–3) analysis indicated nonsignificant effects for all factors, Fs < 4.07, ps > 0.05, except for a significant increase across trials, $F(2, 72) = 45.97$, $p < 0.001$, and significantly higher goal-tracking scores in males than in females, $F(1, 36) = 5.13$, $p < 0.04$. The sex effect was entirely due to sexual dimorphism in body size. When goal-tracking times (s) were expressed as a ratio of ad libitum weight (g), a similar analysis yielded no effect of sex, $F < 1$, or any other factor or interaction, Fs < 2.94, ps > 0.09, except for an increase across trials, $F(2, 72) = 37.58$, $p < 0.001$. A Pairing × Naloxone × Sex analysis of trials 2 and 3, using the goal-tracking scores of trial 1 as covariates was calculated in an attempt to control for the effect of response variability during the first (conditioning) trial on responding during trials 2 and 3 (tests). This analysis confirmed that none of the effects were significant, all Fs < 2.96, ps > 0.06, except for the increase across trials, $F(1, 35) = 15.87$, $p < 0.001$.

No evidence of conditioned taste aversion was found in this experiment, thus suggesting that the effects of opioid blockage on cSNC were probably unrelated to the development of a conditioned taste aversion to the relatively novel downshifted solution.

4. Experiment 3

The results of the two previous experiments suggest that opioid blockage may alter consummatory behavior in the presence of an incentive downshift event (Experiment 1), but it has no detectable effect on consummatory behavior in the absence of an incentive downshift event (Experiment 2). However, opioid blockage may also affect the consolidation of the emotional memory that encodes information about the downshift event. Such a memory is referred to as egocentric memory to distinguish it from the memory update that encodes information about the change in incentive value during postshift trials, or allocentric memory (Papini, 2003). However, the effects of pretrial drug administration are ambiguous with respect to a possible memory function of the opioid system in cSNC. Pretrial injection procedures may influence memory either indirectly, through performance factors such as perceptual, motor or motivational effects on acquisition, or directly by modulating the acquisition of new information. To dissociate these effects, the rest of the experiments reported here use a posttraining drug administration procedure. Posttrial drug administration can act either as a US or as a memory modulator. Experiment 2 provided no support for a role of naloxone as a US. The remaining experiments assess the potential role of opioid blockage on memory consolidation after the incentive downshift. This posttraining procedure has the advantage that memory acquisition is complete when the drug is administered, so changes resulting from the drug treatment are less likely to reflect the type of indirect (performance) effects described above (Gold, 2008).

There is substantial information suggesting that posttraining modulation of opioid receptors affects memory consolidation in a variety of tasks (Gold, 2008; McGaugh & Roozendaal, 2008). Using a step-down passive avoidance situation, Izquierdo and Dias (1983) reported that posttraining i.p. administration of β-endorphin (1 μg/kg) and naloxone (0.4 mg/kg) interfered and enhanced, respectively, retention performance a day later. The effects of posttraining opioid treatments on passive avoidance are mediated by noradrenergic activation in the pathway involving the amygdala and the bed nucleus of the stria terminalis (McGaugh, Introini-Collison, Juler, & Izquierdo, 1986; McGaugh, Introini-Collison, & Nagahara, 1988; Quirarte, Galvez, Roozendaal, & McGaugh, 1998). Similar effects were described by

posttraining administration of stress hormones, including corticosterone and epinephrine (Cottrell & Nakajima, 1977; Izquierdo & Dias, 1983).

In the cSNC situation, posttraining drug administration is providing some clues as to the role of memory in this situation. Consistent with the passive avoidance results cited previously, posttrial 11 (i.e., after the first downshift trial) administration of corticosterone enhanced cSNC when administered immediately after the trial, but not when administered 3 h after the end of the trial (Bentosela et al., 2006). This effect occurs when rats are downshifted from 32% sucrose to 4% sucrose, but not after an 8%-to-4% downshift, suggesting that the emotional significance of the downshift is an important determinant of this effect (Ruetti et al., 2009). Ruetti et al. also reported that the enhancing effect of corticosterone is not due to the development of conditioned taste aversion and is not present in a related contrast situation, anticipatory negative contrast, known to involve different neurochemical mechanisms (Flaherty, 1996). Posttraining administration of the κ-selective agonist U-50,488H also enhances cSNC, but the effect can be at least partially attributed to the development of a conditioned taste aversion to the downshifted solution (Wood et al., 2008). However, unlike in passive avoidance situations (McGaugh & Roozendaal, 2008), posttraining administration of cholinergic drugs (e.g., atropine, physostigmine) had no detectable effects on cSNC (Bentosela et al., 2005).

Experiment 3 explored the role of opioid receptors on memory for the downshift event in the cSNC situation by administering the nonselective opioid antagonist naloxone either after a 32-4 downshift (Experiment 3a) or a 32-6 downshift (Experiment 3b), and the δ-opioid receptor agonist DPDPE and antagonist naltrindole after a 32-4 downshift (Experiment 3c). In all cases, drugs were administered after the first postshift trial (i.e., trial 11).

4.1. Method

4.1.1. Subjects and apparatus

The subjects were Long–Evans rats, experimentally naïve and about 3 months old at the start of the experiment. Experiment 3a used 34 males and 37 females, Experiment 3b used 40 males, and Experiment 3c used 50 males. The origin and maintenance of the animals, their food deprivation, and the training apparatus were as described in Section 2.

4.1.2. Experiment 3a: procedure

The training procedure was the same described in Section 2 with the following exceptions. Downshifted groups had access to 32% sucrose during preshift trials, followed by access to 4% sucrose during postshift trials. Unshifted groups had access to 4% sucrose throughout the 15 daily trials of the experiment. Because the goal of this experiment was to test the hypothesis that opioid blockage facilitates cSNC, a criterion for a minimum level of consummatory suppression was implemented (i.e., there is no basis to expect an effect of opioid blockage on cSNC in the absence of consummatory suppression following the downshift). Downshifted animals that exhibited a level of goal-tracking time on trial 11 greater than 90% of the goal-tracking time exhibited on trial 10 were excluded from the experiment. A greater proportion of rats were randomly assigned to the downshifted groups ($n = 41$) than to unshifted controls ($n = 30$) in anticipation of the possibility that some rats would not meet this suppression criterion. Ten rats were eliminated because of this criterion. After trial 10, the two original groups were further divided into a total of six groups matched by preshift responding: 32/Veh/Veh ($n = 10$; five males, five females), 32/Nlx/Veh ($n = 10$; four males, six females), 32/Veh/Nlx ($n = 11$; five males, six females), 4/Veh/Veh ($n = 10$; five males, five females), 4/Nlx/Veh ($n = 10$; five males, five females), and 4/Veh/Nlx ($n = 10$; five males, five females).

The preparation, dose, and administration route of naloxone were as described in Section 2.1.3. To minimize the number of control groups, each rat received two injections, one immediately after the end of trial 11 and another 3 h later. For the 32/Nlx/Veh and 4/Nlx/Veh groups, naloxone was administered immediately after the trial and an equal volume saline injection was administered 3 h after the trial. Groups 32/Veh/Nlx and 4/Veh/Nlx received a saline injection immediately after trial 11 and a naloxone injection after 3 h. For the vehicle groups, 32/Veh/Veh and 4/Veh/Veh, both the immediate and the 3-h injections were saline. The two-injection procedure allows the saline groups to serve as controls for both the immediate and the 3-h drug groups, reducing the number of necessary groups from eight to six.

4.1.3. Experiment 3b: procedure

The training procedure was in all respects equal to that described for Experiment 3a, except that the lower solution was 6% sucrose (prepared w/w by mixing 6 g of sucrose for every 94 g of distilled water). Animals received a single injection of either naloxone (2 mg/kg, ip) or equal-volume saline solution immediately after trial 11. Because naloxone enhances consummatory suppression following incentive downshift, it could be argued that its posttrial effects may be obscured by a floor effect. A 32%-to-6% sucrose downshift usually leads to a mild cSNC effect (Pellegrini, Muzio, Mustaca, & Papini, 2004), thus leaving room for detecting further suppression of consummatory behavior.

4.1.4. Experiment 3c: procedure

The training procedure was the same as that described in Section 4.1.2. Rats were randomly assigned to two groups ($n = 24$) balanced by weight and subjected to either the downshifted or unshifted procedures. After trial 10, each of these two groups was further subdivided into three groups balanced by preshift performance: 32/Veh ($n = 8$), 32/DPDPE ($n = 9$), 32/Nti ($n = 9$), 4/Veh ($n = 8$), 4/DPDPE ($n = 8$), and 4/Nti ($n = 8$). All injections were i.p. and administered immediately after trial 11. Groups 32/DPDPE and 4/DPDPE received an injection of DPDPE (24 μg/kg). Groups 32/Nti and 4/Nti received naltrindole (1 mg/kg). Groups 32/Veh and 4/Veh received an equal-volume saline injection. Doses were chosen based upon previous positive results in the cSNC situation (Pellegrini et al., 2005; Wood et al., 2005).

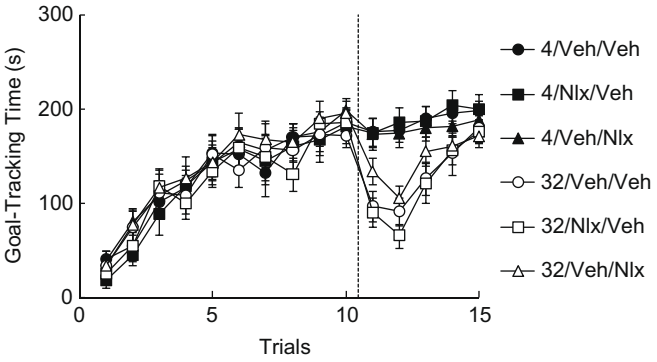

Fig. 4. Mean (±SEM) goal-tracking time for Experiment 3a. Group names reflect the preshift incentive magnitude (32% or 4% sucrose), the first injection administered immediately following trial 11 (Nlx or Veh), and the second injection administered 3 h after trial 11 (Nlx or Veh). Nlx: naloxone, 2 mg/kg, ip. Veh: equal-volume saline injection, the vehicle used to prepare naloxone.

4.2. Results

4.2.1. Experiment 3a

Matching groups in terms of responding on trial 11 was not a viable procedure because injections were administered immediately after that trial. As shown in Fig. 4, individual differences in consummatory suppression on trial 11 caused the groups to display unequal performance. Group 32/Veh/Nlx was higher than the other groups, obfuscating any interpretations made by analyses including the 3-h posttrial condition. A one-way analysis of variance with LSD pairwise post hoc comparisons on trial 11 [$F(5, 55) = 8.19$, $p < 0.01$] revealed that while Groups 32/Nlx/Veh and 32/Veh/Veh showed significant cSNC effects relative to their unshifted controls ($ps < 0.01$), Group 32/Veh/Nlx did not differ from Group 4/Veh/Nlx ($p > 0.06$). For this reason, the 3-h condition was excluded from further statistical analyses.

A Contrast (32-4, 4-4) × Drug (naloxone, saline) × Sex × Trial (preshift trials 1–10) analysis indicated a significant increase of goal-tracking times across preshift trials, $F(9, 39) = 8.44$, $p < 0.01$. As expected (see Section 2), males exhibited significantly higher scores than females, $F(1, 39) = 5.19$, $p < 0.04$. All other effects and interactions were nonsignificant, $Fs < 3.75$, $ps > 0.05$.

A Contrast × Drug × Sex × Trial (postshift trials 12–15) indicated significant changes in goal-tracking time across postshift trials, $F(3, 39) = 30.38$, $p < 0.01$, a significant contrast effect, $F(1, 36) = 22.03$, $p < 0.01$, and a trial by contrast interaction, $F(3, 39) = 14.26$, $p < 0.01$. All other main effects and interactions were nonsignificant, $Fs < 2.29$, $ps > 0.13$. Notably, all effects and interactions involving drug were nonsignificant, $Fs < 1$. Fig. 4 shows that Group 32/Nlx/Veh responded below Group 32/Veh/Veh on trial 12, suggesting a possible effect of naloxone. Because of the inability to balance for individual differences in responding on trial 11, Groups 32/Nlx/Veh and 32/Veh/Veh were subjected to an additional Drug × Sex × Trial analysis with goal-tracking time on trial 11 as a covariate. These results were consistent with previous conclusions, yielding a significant main effect of trial, $F(3, 19) = 8.36$, $p < 0.01$. The main effects of drug, sex, and all interactions were nonsignificant, $Fs < 2.89$, $ps > 0.10$.

Previous experiments showed that the dose of naloxone used in the present experiment (2 mg/kg, ip) was effective in enhancing cSNC (Pellegrini et al., 2005; Experiment 1). Thus, the effects of naloxone on cSNC appear to be limited to pretrial administration. This culls the consolidation of the aversive downshift memory from the list of possible mechanisms of cSNC modulation by the opioid system, thus narrowing the action of naloxone to two possibilities: acting on the intensity of primary frustration (consistent with previous results; Pellegrini et al., 2005) or acting on the comparison between preshift and postshift solutions (consistent with the results of Experiment 1).

4.2.2. Experiment 3b

Data were lost in seven trials (five in the preshift and two in the postshift), all in the unshifted control groups (no data loss was experienced in the downshifted groups). These scores were replaced by the group average for that trial (Kirk, 1968). The main results are presented in Fig. 5.

A Contrast (32%, 6% sucrose) × Drug (naloxone, saline) × Trial (preshift trials 1–10) analysis indicated that rats with access to 32% sucrose displayed higher goal-tracking times than rats exposed to 6% sucrose, $F(1, 36) = 5.57$, $p < 0.025$. There was also a significant increase in performance across trials, $F(9, 324) = 79.81$, $p < 0.001$. None of the other effects were significant, $Fs < 1.74$, $ps > 0.08$. A one-way analysis of goal-tracking times on trial 11, the first postshift trial immediately before the naloxone treatment, indicated a nonsignificant group effect, $F(3, 36) = 1.57$, $p > 0.21$. Post hoc pairwise comparisons with the LSD test showed that

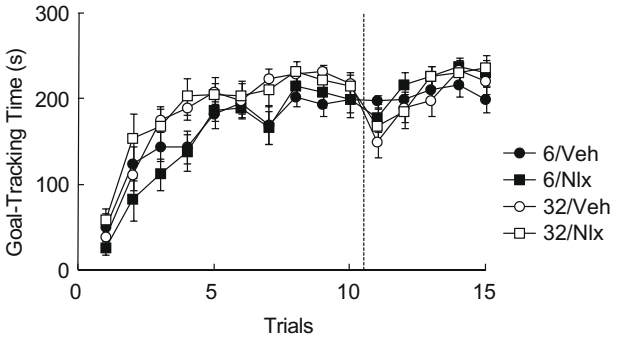

Fig. 5. Mean (±SEM) goal-tracking time for Experiment 3b. Group names reflect the preshift incentive magnitude (32% or 6% sucrose) and the injection administered immediately following trial 11 (Nlx or Veh). Nlx: naloxone, 2 mg/kg, ip. Veh: equal-volume saline injection, the vehicle used to prepare naloxone.

whereas the saline groups showed cSNC, $p < 0.05$, the naloxone groups did not show evidence of cSNC, $p > 0.67$. Subsequent performance also showed no indication of a naloxone effect on recovery from cSNC. A Contrast × Drug × Trial (postshift trials 12–15) analysis indicated a significant interaction between contrast and trial, $F(3, 108) = 3.10$, $p < 0.04$, and a significant change across trials, $F(3, 108) = 10.39$, $p < 0.001$, but no other significant effects, $Fs < 1.72$, $ps > 0.19$. When the goal-tracking times on trial 11 were used as a covariate, there was only a significant change across trials, $F(3, 105) = 3.44$, $p < 0.03$; other effects were nonsignificant, $Fs < 2.19$, $ps > 0.10$. Thus, posttrial 11 naloxone administration had no detectable effect on subsequent recovery from cSNC despite the use of parameters that reduce the possibility of a floor effect.

4.2.3. Experiment 3c

The data from six downshifted rats (one from Group 32/Nti, two from Group 32/DPDPE, and three from Group 32/Veh) failed the suppression criterion on trial 11 and were therefore discarded from statistical analyses. A data recording error affected trials 11 and 12 for one subject in Group 4/DPDPE; the missing values were replaced with group averages (Kirk, 1968). The results are shown in Fig. 6. A Contrast (32%, 4% sucrose) × Drug (naloxone, saline) × Trial (preshift trials 1–10) analysis revealed a main effect of trial, $F(9, 43) = 92.35$, $p < 0.01$, but no other significant main effects or interactions, $Fs < 1.45$, $ps > 0.18$. A Contrast × Drug analysis on trial 11 revealed a main effect of contrast, $F(1, 43) = 38.03$, $p < 0.01$. A

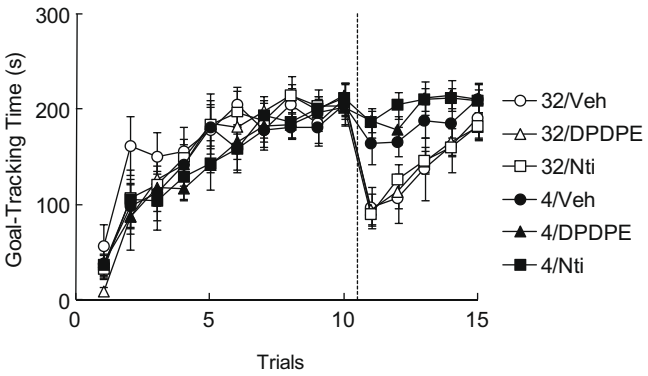

Fig. 6. Mean (±SEM) goal-tracking time for Experiment 3c. Group names reflect the preshift incentive magnitude (32% or 4% sucrose) and the injection administered immediately following trial 11 (DPDPE, Nti, or Veh). DPDPE: 24 μg/kg, ip, Nti: naltrindole, 1.0 mg/kg, ip. Veh: equal-volume saline injection, the vehicle used to prepare DPDPE and naltrindole.

Contrast × Drug × Trial (postshift trials 12–15) analysis revealed significant main effects of trial, $F(3, 43) = 30.60$, $p < 0.01$, contrast, $F(1, 43) = 16.74$, $p < 0.01$, and a trial by contrast interaction, $F(3, 43) = 6.73$, $p < 0.01$. There were no other significant effects, Fs < 1.65, ps > 0.14, indicating that neither of the drugs had a significant effect when given posttrial 11.

As in Experiment 3a, the inability to balance for individual differences in responding on trial 11 made it necessary to subject Groups 32/Nti, 32/DPDPE, and 32/Veh to an additional Drug × Trial (postshift trials 12–15) analysis with goal-tracking time on trial 11 as a covariate. This analysis confirmed previous results with a significant main effect of trial, $F(3, 18) = 11.29$, $p < 0.01$. The main effect of drug and all interactions were nonsignificant, Fs < 2.34, ps > 0.08.

Neither DPDPE nor naltrindole administered immediately after trial 11 measurably affected consolidation of the downshift memory. Combined with previous data showing the selectivity of these opioid compounds for trial 11 performance, Experiment 3c demonstrated that δ opioid receptors appears to play a role in modulating the direct impact of primary frustration, but not in the consolidation of the aversive downshift memory. It is important to note that the doses used in this experiment have been previously established as effective in modulating cSNC on trial 11. As with naloxone in Experiment 3, the action of naltrindole and DPDPE can be narrowed to two possibilities: acting on the intensity of primary frustration or on the comparison between preshift and postshift solutions. Together, the results of these experiments suggest that opioid receptors might not be involved in consolidation of the downshift memory in the cSNC situation. The role of opioid receptors in memory for other situations involving surprising reward loss is explored in the following two experiments.

5. Experiment 4

Experiments 3a–3c provided no evidence of an opioid function in the consolidation of the downshift memory. Some trivial possibilities can be safely discarded. For example, given the effectiveness of this dose and mode of administration of naloxone in previous studies, these results cannot be discounted on the basis of an inappropriate choice of drug parameters. At least three possibilities remain to be explored. First, posttrial naloxone could have effects in reward-loss situations other than the cSNC situation. Second, the process of memory consolidation might be essentially over when naloxone reaches its concentration peak in the critical brain areas. Third, opioid receptors might not be involved in memory consolidation in situations involving surprising incentive loss. The last two issues will be addressed in the Section 7, whereas the first one is examined in the next two experiments.

Experiments 4–5 explored the effects of naloxone on Pavlovian appetitive extinction. Appetitive extinction can be viewed as a special case of contrast in which the downshift is from a large incentive to no incentive, instead of small incentive. Autoshaping was chosen to study appetitive extinction. In autoshaping with rats, a lever (conditioned stimulus, CS) is presented for a fixed time period and its retraction is paired with the response-independent delivery of a food pellet (unconditioned stimulus, US). Although rats are not required to press the lever to obtain food, they nonetheless approach and contact the lever in anticipation of food delivery. In autoshaping, the dependent variable is an anticipatory response, rather than a consummatory behavior. Autoshaping also exhibits sensitivity to manipulations involving surprising reward loss. For example, a surprising reward omission increases response rate (i.e., frustration effect; Dudley & Papini, 1995; Dudley & Papini, 1997), preexposure to unsignaled 10% sucrose enhances subsequent autoshaping for pellets relative to preexposure to 30% sucrose (i.e., positive contrast; Papini, Ludvigson, Huneycutt, & Boughner, 2001), conventional incentive downshift also yields evidence of SNC (Papini et al., 2001), extinction is also faster after large, continuous reinforcement than after small reinforcement (i.e., magnitude of reinforcement extinction effect; Papini et al., 2001) or after partial reinforcement (Boughner & Papini, 2006). Appetitive extinction is also known to be accompanied by a response burst in the initial trials that is eliminated by adrenalectomy (Thomas & Papini, 2001). The partial reinforcement extinction effect is eliminated by pretrial administration of chlordiazepoxide (Boughner & Papini, 2008). Finally, whereas there are no data on the effects of presession naloxone administration on autoshaping extinction, such a treatment was shown to affect instrumental extinction of lever-pressing behavior previously reinforced with food or sucrose pellets, and escape induced by extinction of a consummatory response previously reinforced with 32% sucrose (Norris, Perez-Acosta, Ortega, & Papini, submitted for publication). The question posed in the present experiment was whether postsession opioid blockage modulates the aversive memory of appetitive extinction.

5.1. Method

5.1.1. Subjects

Twenty-five female, experimentally naive Long–Evans rats were used in this experiment. Housing and maintenance conditions were as described in Experiment 1.

5.1.2. Apparatus

Four standard conditioning chambers were used, each enclosed in a sound-attenuating cubicle. The internal dimensions of each chamber were 20.1 cm wide, 28 cm long, and 20.5 cm high. The floor of each chamber was made of stainless steel bars 0.4 cm in diameter and spaced 1.6 cm apart, center to center. Located in the center of the front wall was a recessed magazine, 2 cm from the floor, into which the pellets (45-mg Noyes rat formula A/I) were delivered automatically. An aluminum retractable lever (4.8 cm wide, 1.9 cm deep, and 7 cm above the floor) was located 2 cm to the left of the magazine. Insertion (or retraction) of the lever took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber provided diffuse illumination and was positioned opposite the magazine. A speaker and fan provided background noise (75 dB, SPL scale C, measured in front of the magazine) and ventilation, respectively.

5.1.3. Procedure

Acquisition training involved 10 sessions. There were 10 trials per session separated by a variable intertrial interval (ITI) with a mean of 50.1 s (range: 33–64 s). Before the first trial in each session, there was an interval of variable duration and range equal to that of the ITI. Each trial started with the insertion of the retractable lever for 10 s (the CS). A computer recorded lever-press responses while the lever was inserted in the chamber. At the end of the 10 s, the lever was retracted and five pellets were delivered on the magazine cup at a rate of one pellet per 0.2 s (the US). Each rat consumed fifty 45-mg pellets per session. After the final acquisition session, triplets of rats matched for responding in acquisition were randomly assigned to one of the following groups: 0/Nlx ($n = 8$), 3/Nlx ($n = 7$), and Veh ($n = 7$). The data from three rats that failed to respond during at least 4 of the 10 acquisition sessions were removed from all statistical analyses.

Extinction training involved five sessions. The training conditions during these extinction sessions were the same as during acquisition sessions, except that all food delivery was withheld. Immediately after each extinction session, rats in Group 0/Nlx re-

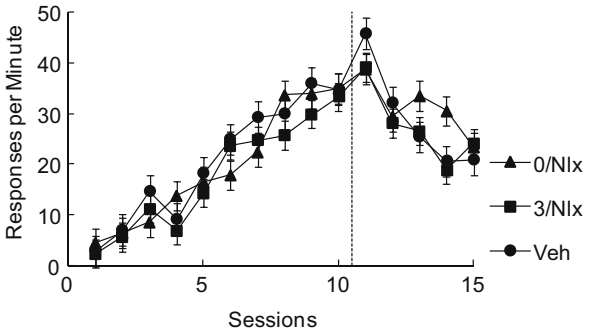

Fig. 7. Mean (±SEM) lever presses per minute for Experiment 4. Group 0/Nlx received naloxone (2 mg/kg, ip) immediately after each extinction session followed by an equal-volume saline injection 3 h later. Group 3/NAL received a saline injection immediately after trial 11 and a naloxone (2 mg/kg, ip) injection 3 h later. Group Veh received equal volume vehicle injections both immediately after and 3 h after trial 11.

ceived an injection of naloxone (2 mg/kg, ip), whereas rats in Groups 3/Nlx and Veh received an equal-volume saline injection. Conversely, 3 h after each extinction trial, the rats in Group 3/Nlx received an injection of naloxone (2 mg/kg, ip), whereas rats in Groups 0/Nlx and Veh received an equal-volume saline injection. Thus, animals were matched in terms of the number and timing of the two injections.

5.2. Results

The results are presented in Fig. 7. A Drug (immediate naloxone, delayed naloxone, saline) × Session (acquisition sessions 1–10) analysis revealed a significant increase across sessions, $F(9, 21) = 23.01$, $p < 0.01$, but nonsignificant effects for the drug groups or the drug by session interaction, $Fs < 1$, thus confirming that the matching procedure was effective. A similar Drug × Session (extinction sessions 11–15) analysis uncovered a significant extinction effect, $F(4, 21) = 11.78$, $p < 0.01$, but nonsignificant effects across drug treatments or in terms of the drug by session interaction, $Fs < 1.17$, $p > 0.34$. A Drug × Session analysis restricted to trial 10 (last acquisition trial) and 11 (first extinction trial) revealed a significant extinction spike, $F(1, 21) = 5.36$, $p < 0.04$, but, again, the drug and drug by session interaction were not significant, $Fs < 1$. The lack of a posttrial naloxone effect is consistent with the results of previous experiments in the cSNC situation. Thus, no evidence was found so far for the hypothesis that opioid receptors modulate the consolidation of an egocentric memory of the downshift experience.

6. Experiment 5

Experiment 4 was designed to match the training parameters used in the cSNC preparation as closely as possible in terms of session length and number of sessions. There are two aspects of the procedure that merit further examination. First, it is possible that five extinction sessions were not sufficient to reflect the effects of posttrial naloxone on extinction performance. Thus, Experiment 5 doubled the number of extinction sessions (sessions 10–20). Second, any effects of posttrial naloxone early in extinction would presumably interact with the increase in response rate (i.e., the extinction spike; Thomas & Papini, 2001), potentially leading to an increase in responding, rather than to a decrease typical of extinction. Thus, posttrial naloxone was administered either after the early (sessions 11–12) or after the late (sessions 14–20) extinction sessions in Experiment 5.

6.1. Method

6.1.1. Subjects and apparatus

Sixteen male, experimentally naive Long–Evans rats were used in this experiment. Housing and maintenance conditions were as described in Experiment 1. The same conditioning boxes described in Section 5.1.3 were used in this experiment.

6.1.2. Procedure

The training procedure was that described in the previous experiment with the following exceptions. After the final acquisition session, triplets matched in terms of acquisition performance were randomly assigned to one of three groups: Nlx/Veh ($n = 6$), Veh/Nlx ($n = 5$), and Veh/Veh ($n = 5$). Immediately after sessions 11 and 12, rats in Group Nlx/Veh received an injection of naloxone (2 mg/kg, ip), whereas rats in Groups Veh/Nlx and Veh/Veh received an equal-volume vehicle injection. This condition replicated the early extinction injections given in Experiment 4. After trial 13, all subjects received vehicle injections to maintain the same daily training routine. Immediately after trials 14–19, Groups Nlx/Veh and Veh/Veh received vehicle injections, whereas Group Veh/Nlx received naloxone injections. This condition was to assess the effects of naloxone on late extinction without the potential interference of the extinction spike. Thus, the number of injections was matched across groups and all injections were given immediately at the end of the extinction sessions.

6.2. Results

The results are presented in Fig. 8. A Drug (early naloxone, late naloxone, saline) × Session (acquisition sessions 1–10) analysis revealed a significant increase of response rates across sessions, $F(9, 117) = 26.44$, $p < 0.001$, but nonsignificant drug effect or drug by session interaction, $Fs < 1$. A similar analysis for extinction sessions 11–20 yielded the same general results. There was a significant extinction effect, $F(9, 117) = 19.73$, $p < 0.001$, but nonsignificant effects for drug or drug by session interactions, $Fs < 1$. An analysis of sessions 10 and 11 failed to reveal an extinction spike in this experiment. The trial effect was short of significance, $F(1, 13) = 3.72$, $p > 0.07$, whereas the drug and drug by session interactions were nonsignificant, $Fs < 1$. These results suggest that the lack of a posttrial naloxone effect in Experiment 4 was not due

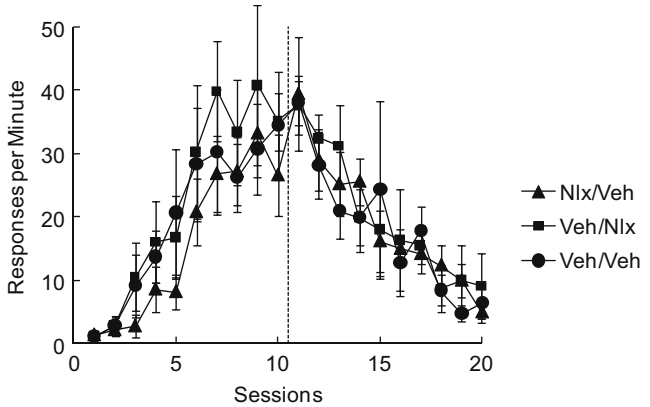

Fig. 8. Mean (±SEM) lever presses per minute for Experiment 5. Group Nlx/Veh received a naloxone (2.0 mg/kg, ip) injection immediately after extinction sessions 11 and 12, and vehicle injections after all subsequent sessions. Group Veh/Nlx received vehicle immediately after sessions 11–13, and naloxone after all subsequent sessions (sessions 14–20). Group Veh/Veh received saline injections after each extinction session. Thus, all groups received a saline injection after trial 13.

to a limited number of extinction sessions or an interaction of the presumably suppressive effects of naloxone with the extinction spike typical of early extinction trials. This result is consistent with the tentative conclusion that opioid receptors are not involved in the consolidation of the egocentric memory of the downshift experience.

7. General discussion

The present experiments explored the role of several potential mechanisms that could explain the effects of opioids in situations involving incentive loss. Previous research had shown that opioid blockage by naloxone administration before the first and second postshift trials enhanced the development of cSNC (Pellegrini et al., 2005). Based on such results it was argued in the introduction that the role of the opioid system on cSNC could be characterized in terms of three fundamental processes: detection (a perceptual–cognitive process), rejection (a motivational–emotional process), and learning (acquiring information about the new incentive conditions and about the aversive experience of the downshift). Experiment 1 demonstrated that pretrial naloxone administration disrupts the detection of the incentive downshift that triggers cSNC. Opioid blockage shifts the comparison between current and remembered incentives from a ratio comparison to one based on the absolute discrepancy of the sucrose concentrations. Experiments 3a–3b found no evidence that posttrial opioid blockage enhances subsequent cSNC, whereas Experiment 3c found no evidence that the more selective modulation of δ receptors, known to be regulate the initial stages of cSNC (Pellegrini et al., 2005; Wood et al., 2005), had any detectable effect on cSNC. Modulation of cSNC by posttrial administration of opioid peptides would implicate an opioid role in the consolidation of memories related to the incentive downshift manipulation. Experiments 4–5 generalized this lack of effect to appetitive extinction.

Null hypothesis significance tests as reported in the present experiments do not provide evidence in favor of the null hypothesis, whereas a Bayesian analysis provides such evidence (Gallistel, 2009; Kass & Raftery, 1995; Rouder, Speckman, Sun, Morey, & Iverson, 2009). Bayesian analysis tests the likelihood that two or more sets of data come from the same distribution (null hypothesis) or from different distributions. The outcome of a Bayesian analysis is a Bayes factor (BF) which gives the odds by which the null hypothesis is favored. A BF of 3.00 means that the null hypothesis is favored 3:1 over the alternative, and a BF of 0.33 means that the alternative is favored by 3:1 over the null. Odds less than 3 are generally considered "weak", odds greater than 10 "strong", odds greater than 30 "very strong" and odds greater than 100 "decisive" (Jeffreys, 1961; Rouder et al., 2009). We calculated the BF for instances in which we reported null effects after the downshift in the present experiments. A total of 30 BFs were calculated: Drug main effects for the five postshift trials in Experiment 3a, 5 Drug × Contrast interactions in Experiment 3a, Drug main effects for the five postshift trials in Experiment 3b, Drug main effects for five extinction sessions for Experiment 4, and for 10 extinction sessions for Experiment 5. In all cases the BFs favored the null hypothesis. The minimum BF was 6.92 (first quartile was 15.03), the median was 23.58 (third quartile was 37.20), and the maximum BF was 55.55. Thus, Bayesian analyses indicated that the null hypotheses reported here were favored by at least a 7:1 margin.

It is proposed here that the opioid system regulates two of the three fundamental processes involved in the cSNC phenomenon. Thus, normal opioid activation following incentive downshift regulates the detection of the downshift and the rejection of the downshifted incentive, but does not intervene in the learning processes triggered by the incentive change (i.e., learning of the new incentive conditions and learning of the emotional reaction to the incentive downshift; Papini, 2003). Such nonassociative hypothesis of opioid function in situations involving incentive downshift is consistent with all known effects.

Opioid agonists have been shown to reduce the size of the cSNC effect either selectively when administered before trial 11 (DPDPE; Wood et al., 2005) or before trial 12 (U50,488H; Wood et al., 2008), or nonselectively when administered before either trial 11 or 12 (morphine; Rowan & Flaherty, 1987). Although the selective effects on trial 12, after some experience with the downshift incentive, are suggestive of a role on learning about the incentive shift, this interpretation is not required. Whether the reaction is unconditioned (i.e., the initial reaction to the downshift) or conditioned (i.e., the retrieved memory of a previous downshift experience), agonizing the opioid system may be viewed as attenuating the emotional–motivational intensity of the reaction, without affecting memory encoding. Similarly, the enhancing effects of opioid antagonists on cSNC, whether selectively on trial 11 (naltrindole; Pellegrini et al., 2005) or nonselectively on trials 11 and 12 (naloxone; Pellegrini et al., 2005), may be viewed as reflecting the facilitating effect of opioid blockage on the emotional–motivational intensity of the reaction to incentive downshift.

Also consistent with this nonassociative hypothesis of opioid function are some results of opioid blockage on extinction. For example, pretrial naloxone administration hastens consummatory extinction in later portions of the session, without affecting behavior in the initial portions of the session (Norris et al., 2008). This can be viewed as the up-modulation of the aversive reaction to the empty tube by naloxone. A similar effect of pretrial naloxone administration has been found in instrumental lever-pressing extinction after acquisition with either sucrose or food pellets (Norris, Pérez-Acosta, Ortega, & Papini, submitted for publication). It may be posited that these extinction effects could be explained in terms of motor impairment; naloxone is known to impair motor activity (DeRossett & Holtzman, 1982; Sisti & Lewis, 2001). However, this alternative hypothesis does not apply to cSNC given the lack of naloxone effects on unshifted control groups. Furthermore, motor impairment should suppress consummatory extinction performance throughout the session, rather than selectively at the end of the session, as described above. In turn, these effects of naloxone on extinction performance exclude an explanation of its effects in terms of reinforcer palatability. Naloxone is known to reduce the palatability of sucrose solutions (Hayward, Schaich-Borg, Pintar, & Low, 2006), which could be a factor in cSNC situations given the incomplete reduction in reinforcer availability. By contrast, there is no incentive during extinction sessions, thus eliminating the possibility that naloxone influences behavior via changes in palatability. cSNC effects are also open to the possibility that extensive exposure to sucrose solutions (as in the 10 preshift trials typically administered in such experiments) increases sensitivity to opioid drugs (Jewett, Grace, & Levine, 2005). The instrumental extinction results previously described also argue against this possibility because the effect of naloxone on extinction occurs whether the instrumental response is reinforced with sucrose pellets or with regular food pellets.

The present failure to modulate recovery from incentive loss with the posttrial drug-administration procedure cannot be attributed simply to a presumptive impenetrability of cSNC to such manipulation. Posttrial 11 administration of corticosterone has proven an effective way to enhance the subsequent cSNC effect (Bentosela et al., 2006; Ruetti et al., 2009). Experiments show that corticosterone must be administered immediately after trial 11 (rather than 3 h later), immediately after a 32-4 downshift (but not after an 8-4 one), and in a successive contrast paradigm (but not in an anticipatory contrast paradigm). Furthermore, this posttrial corticosterone effect cannot be attributed to a conditioned

taste aversion and it occurs also in consummatory extinction. These results with corticosterone have been interpreted as providing evidence consistent with an associative interpretation, namely, that posttrial 11 corticosterone enhanced memory consolidation of the emotional experience of the downshift (Bentosela et al., 2006; Ruetti et al., 2009).

Before concluding that the opioid system plays no role in the consolidation of the egocentric memory of the downshift, a potential problem must be addressed. The naloxone dose used here was chosen because it proved effective in previous research, including the present Experiment 1. Yet one may argue that an effective pretrial dose may have no detectable effect when administered after the trial. Posttrial naloxone administration has in some experiments shown an inverted U-shaped function, with small and large doses having no effect (e.g., Messing et al., 1979). Furthermore, naloxone has a relatively short half-life in human patients (Handal, Schauben, & Salamone, 1983); although no reliable information seems to be available on the pharmacokinetics of naloxone in rats given ip injections, it would be safe to assume that it is probably less than 30 min (Tallarida, Harakal, Maslow, Geller, & Adler, 1978). The results reported in these experiments involving posttrial drug administration could simply reflect an asynchrony between the peak of naloxone's absorption in relevant brain areas and the temporal dynamics of memory consolidation after the downshift event. One procedure used by Messing et al. (1979) to compensate for the short half-life of naloxone involved administering naloxone twice after training, immediately after the trial and then again 30 min later. Furthermore, the effects of posttrial opioid blockage on memory consolidation in passive avoidance situations are mediated by β-adrenergic receptors in the amygdala (McGaugh et al., 1988). Flaherty (1996) summarized some published and unpublished data suggesting that blockage of neither α- nor β-adrenergic receptors seems to affect the course of cSNC. Thus, it seems plausible that memory processes are based on a different set of receptors in the case of the cSNC effect. One firm conclusion to be drawn from these experiments is that the same dose that readily produces pretrial effects in both cSNC (Pellegrini et al., 2005) and appetitive extinction (Norris et al., submitted for publication) has no detectable effects when administered after training.

Acknowledgements

The authors thank Jacob N. Norris for helpful comments on an earlier version of this manuscript. Correspondence may be addressed to M.R. Papini, Department of Psychology, Texas Christian University, Box 298920, Fort Worth, TX 76129 (m.papini@tcu.edu).

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