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Research report

Opioid receptors modulate recovery from consummatory successive negative contrast

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Abstract

Three experiments explored the role of the opioid system in consummatory successive negative contrast. In Experiment 1, rats treated with the nonspecific opioid-receptor antagonist naloxone (2 mg/kg) exhibited increased suppression after a shift from 32% to 6% sucrose solution (32 → 6), relative to 6 → 6 unshifted controls. A similar but shorter effect was observed with the delta-opioid receptor antagonist naltrindole (1 mg/kg). In Experiment 2, naloxone increased suppression after a more conventional 32 → 4 sucrose shift. In Experiment 3, rats classified as expressing slow recovery from contrast (after a 32 → 4 sucrose downshift) were more sensitive to naloxone in an activity test than fast-recovery rats. Whereas it was previously known that contrast was reduced by the extrinsic administration of opioid agonists, the effects reported here with antagonists provide the first evidence that the opioid system is intrinsically engaged by situations involving surprising reward loss.

Keywords: Opioid receptors; Naloxone; Naltrindole; Consummatory successive negative contrast

1. Introduction

When rats exposed to daily trials of free access to a 32% sucrose solution are downshifted to a 4% solution, they exhibit a sharp suppression of consummatory behavior typically followed by a gradual recovery of normal drinking levels over the next 2–5 trials [30]. This phenomenon is known as consummatory successive negative contrast (cSNC). The present experiments are concerned with the role of the opioid system in cSNC, a role suggested by Gray's frustration = fear hypothesis [12,14,15].

Gray [14] reviewed behavioral, pharmacological, and brain lesion studies suggesting a fundamental mechanistic similarity among learning phenomena involving the conditioning of frustration (e.g., SNC and the partial reinforcement extinction effect) and fear (e.g., passive avoidance and one-way avoidance). For example, partial reinforcement and partial punishment, involving conditioned frustration and fear, respectively, increase persistence in extinction [5]. Moreover, signals paired with reward omission or electric shock can be used to potentiate the startle reflex [7,31]. On the basis of this type of evidence, Gray suggested that a similar brain network mediates the anticipatory responses of frustration and fear. Gray's hypothesis could be extended from the conditioned responses (frustration = fear), to the unconditioned events that provide support for conditioning in each case [21]. Thus, the unconditioned response to surprising reward loss is termed primary frustration, whereas the conditioned, anticipatory form of such response is termed secondary frustration [1], whereas the unconditioned response that supports fear conditioning is usually pain induced by electric shock [14]. If secondary frustration = fear, then primary frustration = pain. This analogy suggests a number of counterintuitive predictions, including the hypothesis of a connection between frustration and the opioid system, known to play a major role in pain [3].

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The primary frustration = pain hypothesis correctly predicts that opioid-receptor agonists, which have a powerful analgesic effect, should also reduce the size of cSNC when administered before postshift trials. Rowan and Flaherty [28] confirmed that administration of the nonselective opioid-receptor agonist morphine (4 and 8 mg/kg, i.p.) before the first or second postshift trial reduced cSNC in rats shifted from 32% to 4% sucrose, without affecting consummatory behavior in unshifted, 4% controls. Similarly, the delta-opioid receptor agonist DPDPE also reduced the size of cSNC, but it did so selectively on the first postshift trial [32]. DPDPE had no effect on consummatory behavior when injected on the second postshift trial or on unshifted controls. Such selectivity demonstrates that the opioid system is implicated in surprising reward loss, and not just in consummatory behavior.

Whereas the extrinsic administration of opioid agonists reduces cSNC, this does not imply that the opioid system is intrinsically engaged by reward downshift. In fact, Rowan and Flaherty [28] also reported that the general opioid receptor antagonist naloxone had no detectable effect on cSNC (0.25, 0.5, and 1.0 mg/kg). These negative results were clouded by two problems addressed in the present experiments. First, the results were presented in terms of a proportion measure (i.e., no absolute data were presented or analyzed). The response in each postshift trial, for each animal, was divided by the sum of responses in that trial plus responses on the last preshift trial. The authors pointed out that "analyses in terms of absolute lick frequencies led to essentially the same conclusions" (Rowan and Flaherty, p. 52 [28]), but this does not necessarily prove the occurrence of cSNC. To visualize this problem, imagine that a rat given access to 32% sucrose licks 1000 times in the last preshift trial, whereas one given access to 4% sucrose licks 430 times (arbitrary numbers). If both rats lick 430 times during the first downshift trial, both receiving 4% sucrose, then there would be no evidence of cSNC because the downshifted rat failed to suppress consummatory suppression below the level of the unshifted rat. However, the proportion index would be 0.3 for the shifted rat, but 0.5 for the unshifted rat, thus giving the appearance of a cSNC effect. Therefore, the absence of a naloxone effect could have been the consequence of a weak or absent cSNC effect. In the present Experiment 1, both absolute and proportional scores are provided.

Second, a potential floor effect in the licking measure may have obscured the suppressive effects of naloxone. A 32 → 4 downshift (typical of cSNC experiments [12]) could yield insufficient room for detecting further reduction of licking by naloxone. Fig. 4 in Rowan and Flaherty [28] indicates that naloxone (1 mg/kg) reduced the proportion of licks on trial 12 from 0.35 (saline) to 0.30, although this difference was not significant. The floor-effect problem was minimized in the present Experiment 1 by choosing a postshift concentration of 6%, known to weaken the cSNC effect [25].

2. Experiment 1

Rats received training with either 32% sucrose or 6% sucrose for 10 preshift trials. Subsequently, all rats received access to the 6% for an additional five trials. One pair of groups (32-6/Sal, 6-6/Sal) received saline injections before trials 11 and 12. Their performance was compared to that of two additional groups (32-6/Nlx, 6-6/Nlx) that received naloxone before the same trials. In Rowan and Flaherty's [28] failure to find naloxone effects on cSNC the highest dose was 1 mg/kg. Consequently, a larger dose was selected for this experiment: 2 mg/kg. Five postshift trials were included to determine whether naloxone affected the recovery process beyond the trial in which it was originally administered.

A third pair of groups (32-6/Nti, 6-6/Nti) was injected with the selective delta opioid receptor antagonist naltrindole (1 mg/kg). The role of the delta-opioid receptor was studied previously with the administration of the agonist DPDPE, which was shown to attenuate cSNC on trial 11, but not on trial 12 [32]. Consequently, we were interested in assessing the effect of a selective delta-receptor antagonist. The delta receptor has been implicated also in the modulation of pain-induced behavior [3]. For example, DPDPE attenuated the behavioral effects of a subcutaneous injection of formalin [10], and impaired the acquisition of one-way active avoidance based on electric shock [18].

2.1. Methods

2.1.1. Subjects

Forty-nine Long-Evans hooded rats, approximately 90 days old, were used in this experiment. Rats were bred and housed in the TCU vivarium under a 12-h light:12-h dark cycle (lights on at 07:00 h), and were deprived of food to 85% of the free-food weight. Nondeprived rats also exhibit cSNC, but the level of consummatory behavior tends to be quite low [27]. Because naloxone is predicted to suppress consummatory behavior, a higher level for the saline controls is desirable. In addition, most experiments on cSNC use levels of deprivation between 80% and 85% of ad libitum levels. 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, Vermont) constructed of aluminum and Plexiglas (29.3 cm × 21.3 cm × 26.8 cm, $L \times H \times W$). The floor was 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 hole 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 protruded 1 cm into 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 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 (scale C).

2.1.3. Procedure

Training lasted for a total of 15 daily trials. Rats were trained in squads of four; squads were constant, but the order of training of the squads was varied across days. The 15 daily trials were divided into a preshift phase (10 trials) and a postshift phase (5 trials). Rats were randomly assigned to one of six groups ($n = 8$, except $n = 9$ for Group 32/Nti). For three of the groups (Groups 32/Nlx, 32/Nti, and 32/Sal) the 10 preshift trials involved access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 78 g of distilled water), whereas the 5 postshift trials involved access to a 6% solution (w/w, 6 g of sugar for every 94 g of distilled water). For the remaining three groups (Groups 6/Nlx, 6/Nti, and 6/Sal), all 15 daily trials involved free access to a 6% sucrose solution.

Each triplet of groups differed in the drug treatment received before trials 11 and 12. Groups 32/Nlx and 6/Nlx received an i.p. injection of naloxone (2 mg/kg). Groups 32/Nti and 6/Nti received an i.p. injection of naltrindole (1 mg/kg). Groups 32/Sal and 6/Nti received an injection of saline solution, the vehicle in which the two opioid receptor antagonists were diluted (equal volume, 1 ml/kg). All injections were administered in a separate room, 15 min before the onset of the training trial. Naloxone and naltrindole (Sigma–Aldrich Chemicals, Missouri) were prepared by mixing 1 mg of the drug powder with 1 ml of isotonic saline solution. The stock solution was then diluted in saline solution to obtain the desired dosages for each drug. The drug was prepared before trial 11. All injections were administered 15 min before the start of trials 11 and 12.

Each trial started with a variable pretrial interval of 30 s (range: 15–45 s). At the end of this interval, the sipper tube was automatically presented. A trial started when a rat maintained contact with the sipper tube for a cumulative total of 5 s during any 30 s interval. This criterion was introduced to avoid initiating a trial after an accidental contact with the sipper tube that did not involve drinking. The trial lasted a minimum of 5 min; if a rat was drinking when the 5 min period ended, the solution remained available until the rat spontaneously interrupted drinking. This was done to avoid retracting the sipper tube while the rat was drinking from it, a "punishment" contingency that could potentially affect drinking behavior. After each trial, conditioning boxes were cleaned with a damp paper towel, feces removed when present, and bedding material replaced as needed.

The dependent variable was the cumulative amount of time in contact with the sipper tube, measured in 0.05 s units and labeled goal tracking time. For the occasional trials lasting longer than 5 min, goal tracking was set at 5 min (the scheduled trial length). Goal tracking time has been shown to yield results similar to more conventional dependent variables, such as licking rate measure [27] or amount of fluid consumed [22]. Furthermore, goal tracking time correlates positively and significantly with the amount of fluid intake during 5-min long trials [20]. Finally, in the procedure used in our experiments, goal tracking time yields data with less individual variability than the more typical licking frequency measure.

2.2. Results

Data were lost due to equipment malfunction in five preshift trials, from five different rats, assigned to all groups except 6/Nlx, and in one postshift trial, in a rat assigned to Group 6/Nti. Missing values were replaced by the group average for that particular trial, as suggested by Kirk [17]. A rat from Group 32/Nlx had a tooth malformation that prevented normal feeding and drinking, and it was therefore eliminated from the experiment.

2.2.1. Preshift performance

Fig. 1, top panel, shows the goal tracking times for each group averaged over each of the 5-min trials. Preshift performance shows a significant tendency for groups drinking 6% sucrose to spend more time at the tube than the groups drinking 32% sucrose, $F_{1,42} = 12.56$, $p < 0.002$. This result was not totally unexpected (for an analysis of this effect, see [25]); lower goal tracking times for the 32% are due to a decline of goal tracking times during the second half of the 5-min trial. Other measures, such as licking rate, occasionally exhibit a

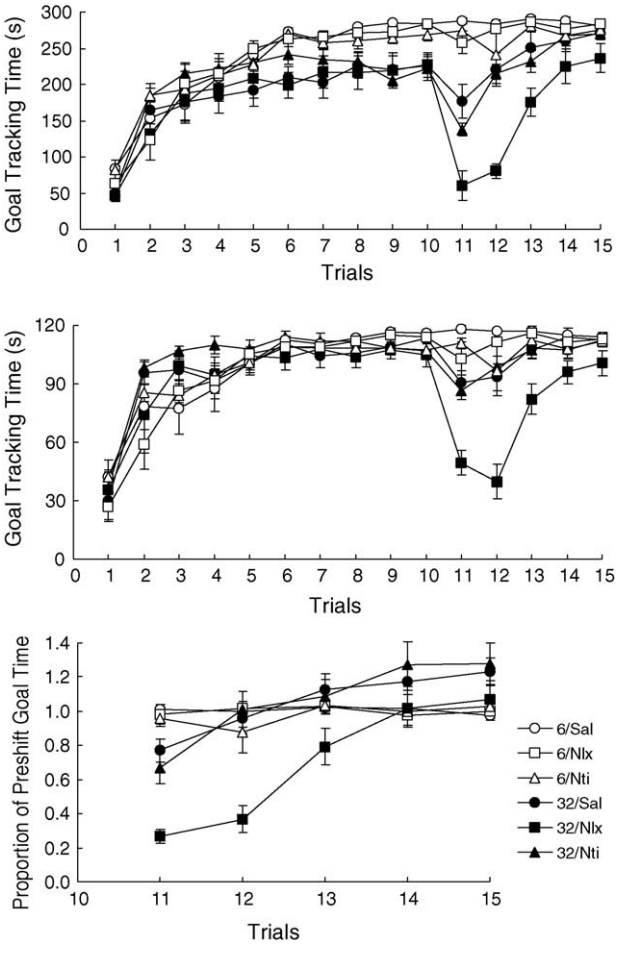

Fig. 1. Performance of groups given access to 32% sucrose solution during trials 1–10 and then 6% sucrose during trials 11–15, and groups given 6% sucrose during all 15 trials. Within each triplet, one group received treatment with saline solution (Sal), naloxone (Nlx), or naltrindole (Nti) before trials 11 and 12. The top panel shows goal tracking times for the entire 5-min trial. The middle panel shows goal tracking times for the initial 2 min of each trial. The bottom panel shows goal tracking time scores in each postshift trial relative to the score in the last preshift trial. Data from Experiment 1.

similar reversal (see p. 56 in Ref. [12]), particularly when rats are not deprived of food [13]. Rats given access to sucrose solution, water, and food in their home cages consume a constant amount of calories by regulating the amounts of each item. In particular, rats consume more 4% or 8% sucrose solution than 32% sucrose solution under such conditions [6]. These results suggest that the drop in consummatory performance during the ending part of the trial is probably due to satiation. There was also a significant increase in goal tracking across trials, $F_{9,378} = 121.06$, $p < 0.001$.

Fig. 1, middle panel, shows the preshift results when only the initial 2 min of each trial are plotted, when the effects of satiation would be minimized. In this case, the groups given access to 32% sucrose increased consumption somewhat faster than the groups drinking 6% sucrose during the initial trials, and then leveled their performance during later trials of the preshift phase. A Contrast (32%, 6%) by Drug (Sal, Nlx, Nti) by Trial (1–10) analysis indicated a significantly higher performance of the 32% sucrose groups than of the 6% group early in training in terms of a contrast by trial interaction, $F_{9,378} = 4.01$, $p < 0.001$. There were also significant changes across trials, $F_{9,378} = 120.57$, $p < 0.001$, and a significant drug by trial interaction, $F_{18,378} = 1.92$, $p < 0.02$. None of the other effects, including the main effect of contrast, were significant, Fs < 1.

Notice that all the groups received access to the same solution during the five postshift trials (i.e., all rats drank 6% sucrose). Thus, any difference in consummatory performance caused by access to solutions of different concentration during the preshift trials (as detected when data from the entire trial were analyzed), does not apply to the postshift data.

2.2.2. Postshift: absolute scores

In terms of the absolute goal tracking scores (Fig. 1, top panel), a global analysis of postshift performance, with Contrast (32, 6), Drug (Sal, Nlx, Nti), and Trial (11–15) as factors, yielded the following effects. There was a significant triple interaction confirming that the rates of recovery were different across contrast and drug conditions, $F_{8,168} = 3.69$, $p < 0.002$. This was also confirmed in terms of a two-way contrast by drug interaction, $F_{2,42} = 8.52$, $p < 0.002$. In addition, downshifted rats showed a significantly lower consummatory level than unshifted controls, $F_{1,42} = 74.01$, $p < 0.001$, and their postshift performance changed significantly more across trials, as shown by a significant contrast by trial interaction, $F_{4,168} = 34.07$, $p < 0.001$. Furthermore, there was a significant effect of drug levels, $F_{2,42} = 9.91$, $p < 0.001$, as well as a significant drug by trial interaction, $F_{8,168} = 2.93$, $p < 0.005$. Finally, postshift performance changed significantly across trials, $F_{4,168} = 46.38$, $p < 0.001$. Separate analyses of groups at each postshift trial indicated significant group effects for each of the five postshift trials, Fs$_{5,42} > 2.97$, ps < 0.03. Pairwise LSD comparisons indicated the following patterns. A comparison among the saline groups (32/Sal versus 6/Sal) indicated a significant cSNC effect for trials 11 and 12, ps < 0.03. This short cSNC effect, lasting two trials, is consistent with previous results with these sucrose concentrations [25]. A comparison among the naloxone groups (32/Nlx versus 6/Nlx) yielded significant cSNC effects for all five postshift trials, ps < 0.01. For the two naltrindole groups (32/Nti versus 6/Nti), cSNC was present in trials 11 and 13, ps < 0.02. A comparison of the 32 → 6 shifted groups indicated that naloxone increased suppression relative to saline on trials 11–13, and 15, ps < 0.03, whereas naltrindole increased suppression relative to saline only on trial 11, $p < 0.04$. Moreover, naloxone was a more powerful suppressant of consummatory behavior than naltrindole on all postshift trials, ps < 0.03.

Fig. 1, middle panel, presents the results of the postshift trials when only the initial 2 min of each trial are taken into account. The pattern is virtually identical with the single exception that the effect of naltrindole is no longer visible. A Contrast by Drug by Trial analysis provided significant results for all the factors. The triple interaction, $F_{8,168} = 3.77$, $p < 0.001$, the contrast by trial interaction, $F_{4,168} = 15.14$, $p < 0.001$, the drug by trial interaction, $F_{8,168} = 3.22$, $p < 0.003$, the contrast by drug interaction, $F_{2,42} = 13.63$, $p < 0.001$, and all three main effects, Fs > 14.69, ps < 0.001. Group analyses for each trial separately indicated significant group effects for trials 11–14, Fs$_{5,42} > 2.89$, ps < 0.03. Pairwise LSD tests demonstrated a pattern very similar to that shown by the 5-min trial data. Among the saline groups, cSNC was observed on trials 11 and 12, ps < 0.03. For the groups treated with drugs, the two naloxone groups exhibited cSNC on trials 11–15, ps < 0.03, whereas the two naltrindole groups exhibited cSNC on trial 11, $p < 0.003$. Among the groups exposed to a sucrose downshift, naloxone increased suppression relative to saline on trials 11–13, and 15, ps < 0.03, and relative to naltrindole on trials 11–15, ps < 0.03.

None of the 6% unshifted groups differed in pairwise LSD tests, whether with the 5-min or with the 2-min trial data, demonstrating that neither naloxone nor naltrindole had any detectable unconditioned effect on consummatory behavior per se. This result is important for two reasons. First, it eliminates the possibility naloxone exerted its effects on cSNC by directly suppressing sucrose consumption. Naloxone is known to decrease sucrose intake under some conditions [4], but not under the present training protocol. Second, increased suppression in the 32 → 6 condition after naloxone treatment cannot be argued to reflect a naloxone-induced change in internal state (i.e., state dependency), because this would have also resulted in performance decrement in the 6% nonshifted controls.

2.2.3. Postshift: proportion scores

Because the goal tracking times for the 5-min trial data were higher for the 6% groups than for the 32% groups during the preshift trials, the results were analyzed also as a proportion of the final preshift trial (Fig. 1, bottom panel). This proportional transformation was applied by Flaherty et al. [13] when encountering a similar problem with the preshift

data. Proportions were obtained by dividing the score each animal produced in each of the five postshift trials by the score of that animal on trial 10, the last preshift trial. The 5-min trial data were used for computing these proportions. As shown in Fig. 1, bottom panel, proportion scores tend to increase above 1 for the 32 → 6 downshifted groups, as they approach the relatively higher level of the 6% unshifted groups. To avoid misrepresenting the level of recovery, as noted by Flaherty et al. [13], the statistical analyses will concentrate on the initial three postshift trials. A global analysis of postshift performance, with Contrast (32, 6), Drug (Sal, Nlx, Nti), and Trial (11–13) as factors, yielded similar results to those found for absolute scores, with some exceptions. A significant triple interaction confirmed the presence of different rates of recovery in the various conditions, $F_{4,84} = 3.54$, $p < 0.02$. Consistent with this result, there was also a significant contrast by drug interaction, $F_{2,42} = 9.91$, $p < 0.001$. Downshifted rats also displayed a greater amount of change across postshift trials than unshifted controls, as captured by significant contrast, $F_{1,42} = 16.96$, $p < 0.001$, and contrast by trial interaction effects, $F_{2,84} = 24.30$, $p < 0.001$. Finally, postshift performance changed significantly across trials, $F_{4,168} = 53.48$, $p < 0.001$. The only nonsignificant effect was the drug by trial interaction, $F_{4,84} = 1.13$, $p < 0.35$. Separate analyses of groups at each postshift trial indicated significant group effects for trials 11 and 12, $Fs_{5,42} > 8.08$, $ps < 0.001$. Pairwise post hoc LSD comparisons indicated the following group differences. Group 32/Sal performed significantly below Group 6/Sal only on trial 11, $p < 0.008$; thus, as expected [25], the 32 → 6 downshift yielded a short-lived cSNC effect that was expected to help detect the suppressing effects of opioid antagonists. A comparison among the naloxone groups (32/Nlx versus 6/Nlx) yielded significant cSNC effects for trials 11–13, $ps < 0.04$. For the two naltrindole groups (32/Nti versus 6/Nti), cSNC was present only on trial 11, $p < 0.002$. A comparison of the 32 → 6 shifted groups indicated that naloxone increased suppression relative to saline on trials 11–13, $ps < 0.005$; however, unlike it was the case with the absolute scores, Group 32/Nti failed to increased consummatory suppression relative to Group 32/Sal. Naloxone was a more powerful suppressant of consummatory behavior than naltrindole on trials 11–13, $ps < 0.009$.

3. Experiment 2

Naloxone proved to be a potent modulator of cSNC, extending the effect in the 32 → 6 downshift from two to five trials. A second experiment was run to determine whether naloxone would have the same effect when administered under the more conventional 32 → 4 downshift. Only shifted groups were included because, under the present conditions, naloxone had no detectable effects on sucrose consumption in the unshifted controls. There was also no indication of state dependency affecting goal tracking scores in the unshifted controls. This design also cuts on the number of animals used in the experiment without any substantial loss of information.

3.1. Methods

3.1.1. Subjects and apparatus

The subjects were 12 rats similar to those used in the previous experiment, maintained under the same conditions, and trained in the same conditioning boxes.

3.1.2. Procedure

Rats were randomly assigned to two groups, $n = 6$, equivalent to Groups 32/Sal and 32/Nlx from Experiment 1. The only difference between experiments was in the administration of naloxone or saline, which was given before each of the five postshift trials (rather than only before trials 11 and 12). A more extensive naloxone treatment was selected in view of the negative results reported previously with 32 → 4 downshifts. Other details of procedure were the same as described in Experiment 1.

3.2. Results

Data were lost due to equipment failure in two preshift trials from different animals, both in Group Nlx, and were replaced by the group means [17]. Fig. 2, top panel, shows the results of Experiment 2 in terms of absolute scores. No differences were apparent during the preshift trials. An analysis

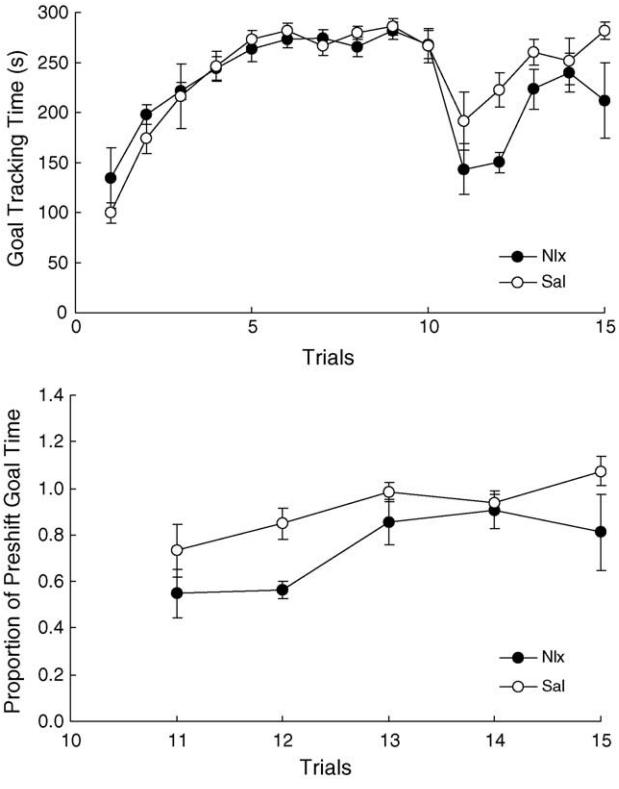

Fig. 2. Performance of groups exposed to a 32 → 4 sucrose downshift and treated with saline solution (Sal) or naloxone (Nlx) before each postshift trial (trials 11–15). Data from Experiment 2.

indicated only a significant acquisition effect, $F_{9,90} = 40.60$, $p < 0.001$, but no group or group by trial interaction, Fs < 1.

Postshift goal tracking time performance was generally lower in Group Nlx than in Group Sal. Naloxone administered before each postshift trial increased suppression consistently throughout this phase, $F_{1,10} = 15.37$, $p < 0.004$. There was also a significant recovery, $F_{4,40} = 5.61$, $p < 0.002$, but a nonsignificant interaction effect, $F < 1$. Fig. 2, bottom panel, shows the same results in terms of proportion scores; this was included for consistency with the analysis presented in Experiment 1. A Group by Trial analysis, including all postshift trials, yielded the same results as for the absolute measure, namely, Group Nlx performed significantly below Group Sal, $F_{1,12} = 9.12$, $p < 0.02$, and the recovery across trials was significant, $F_{4,48} = 8.13$, $p < 0.001$; the group by trial interaction failed to reach a significant level, $F_{4,48} = 1.09$, NS. The suppressive effects of naloxone on consummatory behavior after reward downshift can be extended to the more conventional 32 → 4 downshift.

4. Experiment 3

The opioid system is intrinsically activated by an episode of surprising reward loss. Further activation by administration of opioid agonists (morphine, DPDPE) reduces cSNC, whereas interference by administration of opioid antagonists (naloxone, naltrindole) enhances cSNC. These results are consistent with postsynaptic effects of opiates in neuronal populations involved in cSNC. Particularly interesting is the possibility that genetic variation in opioid receptor structure [19,34] could have relevance for an understanding of individual variation in recovery from cSNC. In such a case, animals that exhibit different patterns of recovery from reward downshift should show differential sensitivity to opioid treatment.

Experiment 3 was designed to expose the connection between individual variation in recovery from reward downshift and sensitivity to naloxone treatment. Published and unpublished data from our lab indicates that there is substantial individual variability in the speed of recovery from reward downshift. Fig. 3 shows individual consummatory responses from several experiments ($N = 25$) involving the same training conditions used in Experiment 3 (see Section 4.1), but recording the amount of fluid intake after each 5-min trial (similar results are obtained with other dependent measures). Some rats show higher performance on trial 12 than on trial 11 (fast recovery, shown in the top panel), whereas others show about the same or lower performance on trial 12 than on trial 11 (slow recovery, bottom panel).

In Experiment 3, rats were first exposed to the incentive downshift procedure and subsequently segregated according to their speed of recovery (see criteria for classification in Section 4.1.3). Fast- and slow-recovery groups were then independently tested for their sensitivity to naloxone. For such independent testing, conditions were selected so that they differed as far as possible for those prevailing in the

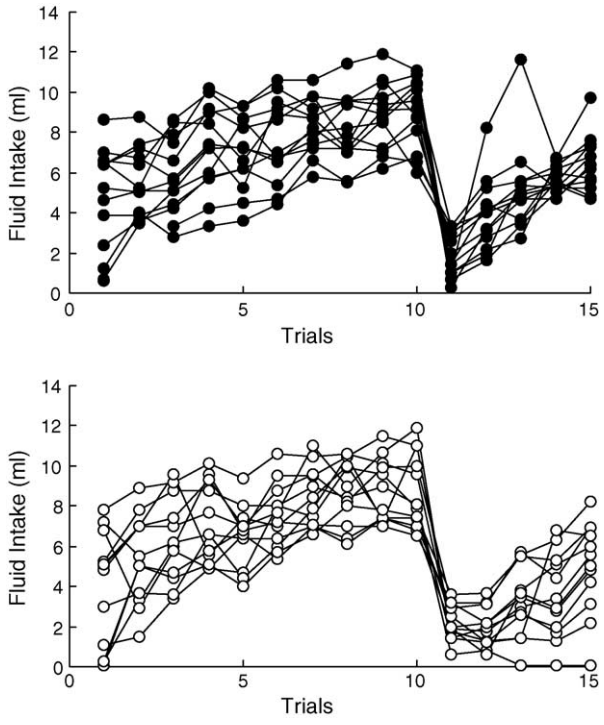

Fig. 3. Individual performance of rats ($N = 25$) trained under a 32 → 4 reward downshift protocol similar to that used in Experiment 3. The dependent measure was the amount of fluid intake per trial. Rats exhibiting relatively rapid recovery are plotted in top panel, whereas rats exhibiting slow recovery are plotted in the bottom panel (see text for details).

incentive downshift procedure. A locomotor activity test was chosen because it shows sensitivity to treatment with naloxone [9,29], and because the testing situation can be modified so as to differentiate it from the reward downshift situation. Different testing parameters between the reward downshift and activity situations also make it is less likely that prior testing in the reward downshift situation may affect behavior in the activity test.

The following are the more relevant differences between the two testing procedures. First, unlike reward downshift testing, activity tests do not involve any obvious form of reward loss. Second, whereas rats were deprived of food before reward downshift testing, they were placed on a free-food schedule before and during activity testing. Third, no extrinsic reinforcers were administered in the activity test, unlike the access to sucrose solutions that prevails during reward downshift testing. Fourth, the activity test was adjusted so as to eliminate the presence of open areas, as in the elevated plus maze or the open field apparatus [33], and to eliminate sources of illumination [26], both of which are known sources of anxiety.

4.1. Methods

4.1.1. Subjects

The subjects were 42 male rats similar to those used in the previous experiments and maintained under the same conditions.

4.1.2. Apparatus

Three main testing procedures were used in this experiment. In chronological order, they were daily water intake, reward downshift, and activity. Water intake was measured by inserting a calibrated bottle into the cage for exactly 24 h and recording the intake (ml) at the end of each day. Reward downshift was measured using the same four conditioning boxes described in Experiment 1. Activity was assessed in a rectangular wooden box measuring 15.5 cm in width, 160.4 cm in length, and 21.5 cm in height, with wooden lids, and without any light source. This box was equipped with three photocells located 65.2 cm apart from each other. One photocell was located in the middle of the box, whereas the others were located 15 cm from the end wall, one in each extreme of the box. The effects of reward downshift on consummatory behavior (goal tracking time, measured in 0.05 s units) and activity (counts of photocell interruption) were recorded by a computer located in an adjacent room.

4.1.3. Procedure

Because rats were scheduled to be assigned according to their performance in the reward downshift test (described below), an effort was made to disregard two main potential confoundings: differences in weight and in water intake. Rats were bred in the TCU colony from parents purchased from Harlan. After weaning at postnatal day (PND) 21, rats were kept in equal-sex groups for about 22 days. All rats were then transferred to individual wire-bottom cages at PND 43–48 and thereafter weighed daily to assess their growth. Because of long-term maintenance in wire-bottom cages, each cage was equipped with a 8 cm × 13 cm plastic plate to provide for a smooth surface on which the rat could stand. At PNDs 60–77, three daily water consumption measurements were taken for each rat to determine potential individual differences in drinking behavior. Graduated bottles were placed in each cage at 10:00 a.m. and withdrawn the next day at the same time. Food was continuously available during these tests. The amount of water (ml) consumed was then recorded.

At PND 87–92, food intake was restricted until each rat reached an 85% level of its free-food body weight. When rats reached this level, reward downshift training started and continued as described in Experiment 1. Reward downshift involved the same conditions of 32% and 4% sucrose solutions described in Experiment 2, except that no injections were administered. All 42 rats received 10 preshift trials of access to 32% sucrose followed by 5 postshift trials of access to 4% sucrose. Goal tracking time was measured as the dependent variable.

The following criteria were used to segregate the rats into fast-recovery and slow-recovery groups. First, rats that exhibited a trial 11 performance that was 90% or greater than that of trial 10 were discarded; the sample was reduced from 42 to 36 rats. Because the goal was to select rats in terms of the recovery speed, it was necessary that they had exhibited some degree of suppression. Second, quadruplets matched in terms of trial 11 performance were established so that the selected groups would not differ statistically in terms of their initial reaction to reward downshift. Third, a difference score for goal tracking was calculated for each rat by subtracting trial 12 from trial 11. This 12 minus 11 difference score was used to assess speed of recovery. Extensive previous analyses of published and unpublished data collected under the same training conditions used in this experiment had shown that individual differences are particularly evident in the transition from trials 11 to 12. Fourth, for each animal in the quadruplet, the two with the highest difference scores were randomly assigned to the Fast/Nlx and Fast/Sal groups, whereas the two with the lowest difference scores were assigned to the Slow/Nlx and Slow/Sal groups. Finally, each of the four groups was culled to an $n = 8$ by eliminating the rat with the lowest difference score for each of the two Fast groups, and the rat with the highest difference score for each of the two Slow groups.

After reward downshift testing ended, animals were put again on a free-food schedule (PNDs 106–111). After about 10 days on free food, rats received a single activity test trial (PNDs 121–126). Their drug treatment differed according to groups established on the basis of their performance in the reward downshift test. Rats were injected with naloxone (2 mg/kg, i.p.), 15 min before the start of the activity test. They were placed in the center of the activity box, facing always the same end wall, and left undisturbed in the box for 15 min. Each activation of any of the three photocells was added to yield a general activity score; no distinction was made among the photocells. Activity scores were integrated in terms of the first versus the last 5 min of the session for statistical analysis.

4.2. Results

One rat escaped from the activity apparatus by jumping and pushing the lid. Thus, the scores obtained for this rat were eliminated from all the analyses presented below (e.g., activity, reward downshift, weights, and water intake). Group Slow/Nlx was left with $n = 7$ ($n = 8$ for the other three groups).

4.2.1. Reward downshift

Fig. 4 depicts the performance of the four groups in reward downshift situation, segregated according to the speed of recovery as indexed by the trial 12 minus trial 11 difference scores and according to the group assignments for the activity test. As expected, given the criterion used for selection, trial 11 performance was very similar across groups, whereas fast-recovery and slow-recovery groups differed mainly on trial 12 performance. Interestingly, consummatory performance was relatively similar during the rest of the trials for all four groups. A Recovery (Fast, Slow) by Drug (Nlx, Sal) by Trial (11 and 12) analysis

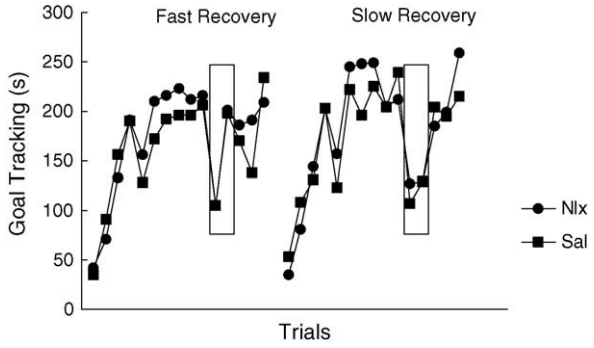

Fig. 4. Performance of rats exposed to a 32 → 4 sucrose downshift and later segregated according to a recovery criterion based on the performance of trials 11 and 12 (see text for details of this criterion). The rectangle encloses the two critical trials. Rats were matched for performance on trial 11, but assigned to different groups depending on whether they recovered fast or slow on trial 12. Data from Experiment 3.

of goal tracking behavior confirmed that the selection was effective in terms of a significant trial by recovery effect, $F_{1,32} = 9.72$, $p < 0.005$, coupled with nonsignificant effects for all main effects and interactions involving the drug factor, Fs < 1. The nonsignificant drug effect confirms that the two subgroups in each of the fast- and slow-recovery groups to be tested for sensitivity to naloxone in the activity situation were not different in their reward-downshift performance.

4.2.2. Litter effects

Because the litter of each animal was known, it was possible to estimate the probability that siblings used in the present experiment were assigned to the same recovery condition. There were 13 litters with two or three males (2 litters had only a single male and were thus discarded for this analysis). Since there were two recovery conditions, fast and slow, the probability of siblings exhibiting either one by chance was 0.5. The average observed probability for assignment to the same condition was 0.74. A one-sample, two-tailed *t*-test calculated over the 13 litters. Indicated a significant deviation from chance, $t_{12} = 3.50$, $p < 0.005$. This litter effect may reflect the influence of genetic factors, early experience, or their interaction, over recovery from cSNC.

4.2.3. Activity

The main results, presented in Fig. 5, indicate that naloxone had a greater effect on activity in the slow-recovery group than in the fast-recovery group. This was detected in terms of an activity block by drug by recovery triple interaction, $F_{1,27} = 4.97$, $p < 0.05$. The analysis also indicated a significant decrease in activity, $F_{1,27} = 89.30$, $p < 0.001$, and a significant suppression of activity by naloxone, $F_{1,27} = 11.02$, $p < 0.004$. Additional analyses for each set of recovery groups demonstrated that the activity block by drug interaction was significant for the slow-recovery groups, $F_{1,13} = 13.80$, $p < 0.004$, but not for the fast-recovery group, $F < 1$.

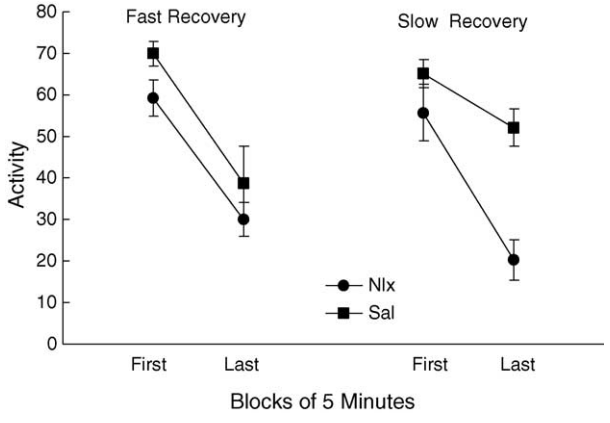

Fig. 5. Performance of rats in the activity test. Fast-recovery and slow-recovery groups were established according to the rats' performance in a reward downshift test (see Fig. 3 and then administered either saline solution (Sal) or naloxone (Nlx) before the activity test. Data from Experiment 3.

Table 1
Growth, deprivation weight, and water consumption in Experiment 3

Fast/Nlx	Fast/Sal	Slow/Nlx	Slow/Sal
Weights before incentive downshift test (85% deprivation)			
336.0 (±31.3)	326.9 (±27.3)	320.8 (±15.4)	310.0 (±13.6)
Weights before activity test (nondeprived)			
431.4 (±40.9)	421.1 (±28.4)	411.6 (±25.7)	400.4 (±18.0)
Water intake (ml) at PND 60–77			
27.6 (±9.8)	27.8 (±9.8)	25.8 (±9.7)	26.1 (±9.2)

Note: Weight (g) and water intake (ml) means (±S.D.) for the rats ultimately assigned to the four conditions in the activity test. Fast and Slow refer to recovery speed in the reward downshift test (see text for selection criterion). Naloxone (Nlx) and saline (Sal) refer to the drug treatment in the activity test. PND: postnatal day.

4.2.4. Other variables

The rationale behind this experiment was that normal variability in opioid receptor effectiveness determines both recovery from incentive downshift and the sensitivity to naloxone in an unrelated activity test. Two additional measures were taken in an attempt to provide a more complete description of fast- and slow-recovery rats: growth rates and drinking behavior. Growth functions for the rats that were eventually assigned to the four groups in the activity experiment indicated slightly higher body weight gains for the two fast-recovery groups than for the two slow-recovery groups. An analysis with Recovery (Fast, Slow), Drug (Nlx, Sal), and Age (PND 44–91) as factors indicated a highly significant growth, $F_{47,1269} = 1950.42$, $p < 0.001$. The recovery effect came very close to a significant value, $F_{1,27} = 3.92$, $p < 0.06$, indicating a tendency of fast-recovery rats to weigh slightly more than slow-recovery rats. All other effects failed to reach a significant level, Fs < 1.77. Additional analyses were calculated on the weights of rats before the start of reward downshift testing (under 85% deprivation) and before the start of the activity test (nondeprived). These values are shown in Table 1. Although the tendency for a higher body weight persisted in the high-recovery rats before reward downshift and activity testing, it again fell short of statistical significance, Fs$_{1,27} > 3.61$, ps < 0.07. The drug and recovery by drug interaction for both tests failed to reach a significant level, Fs < 1.39.

Table 1 also shows daily water intake averaged over three days of measurements for each rat. Not surprisingly, there was also a tendency for the slightly smaller rats in the slow-recovery groups to drink somewhat less fluid than the slightly larger, fast-recovery rats. This difference, however, was not reliable, as indicated by nonsignificant effects in a Recovery by Drug analysis, Fs < 1.24.

5. General discussion

These experiments provide the first evidence that opioid antagonists increase the size of the cSNC effect. The effects of naloxone were robust and long lasting, contrasting with the

lack of evidence provided by Rowan and Flaherty [28]) in a previous report. Several differences across experiments may account for these disparate results. For example, 2 mg/kg of naloxone, twice the size of the largest dose used by Rowan and Flaherty, were administered in the current experiments. Furthermore, the postshift concentration was chosen so as to minimize the potentially obscuring effects of a floor effect. A 32 → 6 downshift was implemented in Experiment 1, rather than the 32 → 4 downshift used by Rowan and Flaherty. In Experiment 2, when a 32 → 4 downshift was also used, naloxone was administered before each postshift trial so as to increase chances of observing enhanced consummatory suppression.

Experiment 1 also provided data on the effects of naltrindole on cSNC. The restricted effects of this antagonist fit previous results with the agonist DPDPE–both having selective affinity for the delta opioid receptor. DPDPE was shown to attenuate cSNC on trial 11, but not on trial 12 [32]. In the present Experiment 1, naltrindole was injected before both trials, but had a measurable effect only on trial 11. The fact that this dose increased consummatory suppression on trial 11 suggests that a lack of effect of naltrindole on trial 12 cannot be attributed to an ineffective dosage. Still, a proper demonstration of antagonistic selectivity should be based on a design similar to that used by Wood et al. [32] for DPDPE, in which independent groups of rats were injected before each target trial.

Naloxone injected before trials 11 and 12, in Experiment 1, prolonged the cSNC for three additional trials and still exhibited significant suppressive effects 72 h after being last administered. Such a long-term effect is consistent with an effect of naloxone on recovery from cSNC, rather than with an effect on consummatory behavior per se. This point is further supported by the absence of a naloxone effect on the 6 → 6 unshifted controls. Following a line of evidence started in a previous study [32], we suggest an interpretation of the effects of naloxone on cSNC based on Amsel's [1] frustration theory. According to this interpretation, access to the postshift solution retrieves a memory of the preshift solution that induces a comparison between the current and remembered solutions and triggers an aversive internal response labeled primary frustration. Primary frustration has two main effects: it leads the animal away from the current food source, thus activating alternative behaviors [23,24], and it supports Pavlovian conditioning of an anticipated form called secondary frustration. Whereas primary frustration is assumed to be mainly responsible for consummatory suppression on trial 11, secondary frustration is assumed to be the main source of consummatory suppression during the recovery phase that usually develops after trial 12. Based on the results of Experiments 1 and 2, and on previous experiments [28,32], we suggest that the opioid system is intimately involved in the recovery process that follows surprising reward omissions in the cSNC situation, by affecting one of the following two processes: (1) the intensity of primary frustration, or (2) the acquisition of secondary frustration (for a detailed analysis of these and other theoretical possibilities, see Ref. [32]).

Experiment 3 sought to demonstrate the connection between recovery from reward downshift and the opioid system using an approach based on individual differences. The analysis of published and unpublished data from several experiments carried out in our lab indicated extensive individual differences in the speed of recovery from a 32 → 4 downshift. Individual records from a sample of 25 rats exposed to the same conditions implemented in the present Experiments 2 and 3 are shown in Fig. 3. Interestingly, even rats that show essentially similar levels of consummatory suppression on trial 11 may differ dramatically in the speed of recovery over the subsequent trials. If these individual differences in recovery were related to differences in some property of the opioid system, one would predict a direct correspondence between recovery from reward downshift and general sensitivity to opioid treatments. The structure of the μ opioid receptor, one site of action for naloxone, is a property of the opioid system known to exhibit allelic variation in rodents and humans [19,34]. Such variations differ in the efficacy with which the receptor interacts with opioid agonists. Thus, it was hypothesized that individual differences in some aspect of the opioid system, such as μ receptor efficacy, determine the speed of recovery from cSNC. Locomotor activity was selected as a test arena for this hypothesis mainly because it can be structured to involve different conditions from those operating during reward downshift testing, including particularly a minimization of conflict and a nondeprived internal state (see introduction to Experiment 3 for further details). The results of Experiment 3 indicated that activity tends to decrease more rapidly in fast-recovery than in slow-recovery rats, with naloxone causing no detectable effect on the fast-recovery rats, while causing a sharp decrease in activity scores in the slow-recovery rats.

One advantage of the procedure used in Experiment 3 is that it demonstrates the connection between recovery and the opioid system indirectly (i.e., naloxone was not injected during consummatory testing). Direct drug-behavior influences are open to potential pharmacological effects on sensory-perceptual, motivation, and motor processes [32]. Although some such alternatives may be safely discarded, they cannot be eliminated in any single experiment. The indirect effect reported in Experiment 3 strengthens the hypothesis that surprising reward loss activates the opioid system. Still, these results merit further thought. There are two potential problems with the results reported in Fig. 5. First, it may be argued that naloxone had different suppressive effects on activity in fast-recovery rats than in slow-recovery rats because of a floor effect affecting the data in the fast-recovery rats. The low activity scores of Fast/Sal rats during the last 5-min block could have made it difficult to detect a significant difference with the Fast/Nlx rats. This possibility is contradicted by significantly lower scores in Slow/Nlx than in Fast/Sal, $F(1,13) = 9.29$. This difference indicates that there was suffi-

cient room in the activity scale to detect an effect of naloxone on the fast-recovery rats, had the effect been present. Second, some may disagree with the interpretation of the effects of naloxone on activity. Activity was chosen because it does not involve reward loss, it can be implemented under different conditions from those prevailing during reward downshift testing, and it had shown sensitivity to naloxone. Because activity was related in an orderly fashion to recovery from reward downshift, it may be suggested that they share some common underlying mechanism. Several factors affect activity in rats, including exploratory tendencies, hunger motivation, novelty-induced arousal, and intrinsic reinforcing effects of motor activity [2,8,11,16], some of which may also affect adjustment to reward downshift. For example, rats placed in a novel situation exhibit an increase in glucocorticoid levels between 15 and 45 min after session onset; corticosterone levels also decrease after repeated exposure to the same situation [8]. Thus, novelty-induced arousal habituates over trials, a process analogous to recovery from reward downshift. A parallel may be drawn between trials 11 and 12 in the reward downshift test and the first and last 5 min of the activity test: fast-recovery rats exhibited relatively more behavioral change than slow-recovery rats in both tests.

In summary, the results reported here demonstrate an intimate connection between the behavioral consequences of surprising reward loss and the activation of the opioid system. As shown by the present results, this link exceeds whatever effects naloxone and naltrindole may have on consummatory behavior per se. This strengthens the hypothesis that some aspects of the opioid system are intrinsically engaged during an event involving incentive downshift and play a role in the recovery of behavior that follows the experience of reward loss.

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