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Naloxone facilitates appetitive extinction and eliminates escape from frustration

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ABSTRACT

Two experiments tested the effects of opioid receptor blockage on behavior. In Experiment 1, rats reinforced for lever pressing with either sucrose or food pellets received treatment with saline, 2, and 10 mg/kg naloxone, i.p. (within-subject design). Naloxone 10 mg/kg increased response latency, but 2 mg/kg had no effect. When shifted to extinction (between-group design), naloxone (2 and 10 mg/kg) facilitated extinction relative to saline animals, after reinforcement with either sucrose or food pellets. In Experiment 2, after 10 sessions of access to 32% sucrose or an empty tube (between-group design), all rats were exposed to the empty tube while allowing them to jump over a barrier into a different compartment. Escape latencies were shorter for downshifted saline than for saline rats always given access to the empty tube. This escape-from-frustration effect was eliminated by naloxone (2 mg/kg, i.p.). Opioid blockage appears to reduce the value of alternative incentives.

1. Introduction

Opioid receptors have been implicated in the modulation of physical pain and conditioned fear (McNally and Akil, 2002; McNally and Cole, 2006), but much less is known about their role in situations involving incentive downshift (Papini et al., 2006). The approach taken here and in previous research (Papini, in press) involves the systemic administration of opioid compounds either before or after training in situations involving incentive downshift, including successive negative contrast (SNC), appetitive extinction, and escape from frustration. Systemic drug administration contributes to identifying the role played by various opioid compounds, thus paving the way to microinjection analyses targeting specific brain areas. For example, the nonselective opioid antagonist naloxone has been found to enhance consummatory successive negative contrast (cSNC) when administered before the first and second downshift trials (after a 32% to 6% sucrose downshift), but not when similarly administered to rats in a nonshifted control condition (always exposed to 6% sucrose; Pellegrini et al., 2005). In the cSNC situation, the consummatory behavior of rats exposed to a downshift from a larger incentive to a smaller incentive (usually sucrose solutions) is suppressed relative to the performance of a group always exposed to the smaller incentive (Flaherty, 1996). Under such conditions, at least three mechanisms could explain naloxone's effects on cSNC: motor impairment, reduced sucrose palatability, and increased aversive motivation. That naloxone could lead to a variety of behavioral effects is not surprising considering the diffuse localization of opioid receptors in the brain (Mansour et al., 1995). There is independent evidence supporting each of these effects of opioid blockage.

Consider the effects of naloxone on motor behavior. Monkeys chronically treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a neurotoxin that induces symptoms analogous to those of Parkinson's disease) and subsequently treated with L-Dopa (a dopamine precursor used in therapy for Parkinson's disease) exhibit dyskinesias—involuntary movements of the head and arms. Such dyskinesias are enhanced by concurrent treatment with naloxone (Samadi et al., 2003) and reduced by the k-opioid receptor agonist U50,488H (Cox et al., 2007). Interestingly, U50,488H also has an effect in the cSNC situation and is also opposite to that of naloxone: U50,488H attenuates cSNC (Wood et al., 2008). The motor effects are thought to depend on the presence of opioid receptors in the basal ganglia (Aubert et al., 2007; Johansson et al., 2001). This *motor impairment hypothesis* could account for the effects of naloxone and U50,488H in rats subjected to an incentive downshift, but it incorrectly predicts that unshifted controls should also exhibit correlated changes (Pellegrini et al., 2005; Wood et al., 2008).

A second possibility is that opioid blockage reduces the incentive value of the downshift solution independently of the downshift manipulation (*reduced sucrose palatability hypothesis*). There is considerable evidence suggesting that naloxone treatment suppresses intake of sucrose solutions and other types of food and solutions (see Olszewski and Levine, 2007). In two-bottle tests, naloxone reduced sucrose intake in wild-type and in β-endorphin knockout mice, but not in enkephalin and in dynorphin knockouts (Hayward et al., 2006). Naloxone's (1 mg/kg, ip) suppressive effects were observed also in preweanling rats starting at 11 days of age (Philopena et al., 1996) and in rats with open gastric fistulas exposed to so-called sham feeding (Rockwood and Reid, 1982). The sham-feeding results suggest that

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naloxone devalues the palatability of sucrose solutions, rather than its postingestional effects, an interpretation confirmed by further experiments manipulating sucrose concentration in rats with gastric fistulas. In rats exposed to 10% sucrose, naloxone reduced their intake to the level of a saline condition exposed to 5% sucrose, whereas rats exposed to 20% sucrose and treated with naloxone exhibited an intake similar to saline rats exposed to 10% sucrose (Kirkham and Cooper, 1988). Thus, naloxone can have an effect on sucrose intake that is equivalent to diluting the solution to half its concentration. Accordingly, naloxone-treated rats exposed to a 32-to-6% sucrose downshift may be consuming as much of the 6% sucrose as normal rats exposed to a 32-to-3% sucrose downshift, which is similar to the 32-to-4% sucrose downshift reported by Pellegrini et al. (2005).

A third account, the *increased aversive motivation hypothesis*, explains the effects of naloxone on cSNC is based on the notion that opioid blockage increases the intensity of aversive states. Relevant evidence comes from studies of fear conditioning. Extensive research on Pavlovian fear conditioning in which a tone or a context is paired with pain induced by electric shock shows that systemic opioid blockage facilitates fear acquisition (Fanselow, 1981) and can impair fear extinction (McNally and Westbrook, 2003; but see Vigorito and Ayres, 1987). In these experiments, fear is assessed in terms of freezing behavior; because the acquisition and extinction effects both involve an increase in freezing, it is plausible that these results could be explained in terms of motor interference. For example, naloxone is known to interfere with exploratory behavior in mice (Katz and Gelbart, 1978). However, these effects of naloxone have been interpreted as involving an increase in the functional intensity of the aversive state of shock-induced pain during fear acquisition or CS-induced fear during extinction (McNally and Westbrook, 2003). The extensive behavioral, pharmacological, and neural similarities between pain–fear and frustration (see Papini and Dudley, 1997; Papini et al., 2006) suggests that this hypothesis could be extended to incentive downshift situations. Accordingly, it could be argued that naloxone increases the aversive intensity of the state of frustration induced by the incentive downshift manipulation, which, in turn, increases avoidance and/or rejection of the downshifted solution thus leading to lower goal-tracking times. Alternatively, the presumed aversive state induced by naloxone could support a conditioned taste aversion to the relatively novel downshifted solution, thus leading to an apparent enhancement of cSNC. This later possibility can be dismissed on the basis of data indicating that naloxone has no effect on consummatory behavior in the absence of an incentive downshift (Daniel et al., in press).

Each of these hypotheses accounts for some aspect of the available evidence, but does not explain all the available evidence. There are two aspects of the available evidence from the consummatory situations involving incentive downshifts that must be explained. First, that naloxone increases the cSNC effect; this effect involves a reduction in the consumption of the incentive in animals treated with naloxone before downshift trials. This effect appeared after downshifts from 32% sucrose to 12%, 6%, and 4% sucrose, but not after downshifts from 16% sucrose to 6% or 3% sucrose (Daniel et al., in press; Pellegrini et al., 2005). Thus, the effect is not an automatic consequence of the incentive downshift manipulation. Naloxone also accelerated consummatory extinction after training with 4% sucrose (Norris et al., 2008). Second, naloxone has no measurable effect in unshifted controls exposed only to 6% sucrose (Pellegrini et al., 2005). As they stand, the motor impairment and reduced sucrose palatability hypotheses cannot account for any instance when rats failed to exhibit a disruption of consummatory behavior. Reduced sucrose palatability cannot account for the consummatory extinction effect because no sucrose is administered in these trials (i.e., rats encounter an empty sipper tube). The aversive motivation hypothesis requires that naloxone acts only in situations involving a substantial downshift in incentive value, thus accounting for the pattern of results described above, including the consummatory extinction data.

2. Experiment 1

Experiment 1 was designed to test the effects of opioid blockage under two phases of instrumental training: asymptotic acquisition performance and extinction performance. Lynch and Clark (1983) reported that naloxone (1 and 10 mg/kg, sc, in different experiments) facilitated extinction of a food-reinforced runway task in rats. At 10 mg/kg, naloxone also disrupted reinforced running performance, before extinction (the 1 mg/kg dose was not tested in acquisition). The present experiment was designed to test the effects of naloxone (2 and 10 mg/kg, ip) in a lever-pressing instrumental task, during both reinforced and nonreinforced (extinction) trials, and reinforced with either food pellets or sucrose pellets. The effects of naloxone on reinforced performance were determined in a within-subject design. Thus, when animals were tested during extinction, they had all received the same exposure to the drug during acquisition. Therefore, performance differences in extinction could not be attributed to differential drug exposure during acquisition. With respect to acquisition, the motor impairment hypothesis predicts the slowing down of performance under both incentives, whereas the reduced sucrose palatability hypothesis predicts a greater suppressive effect on performance for sucrose pellets than for food pellets, and the increased aversive motivation hypothesis predicts no effect since there are no apparent sources of aversiveness. With respect to extinction, the motor impairment and increased aversive motivation hypotheses predict accelerated extinction under both incentive conditions, whereas the reduced sucrose palatability hypothesis predicts no effect of naloxone treatment during extinction since no incentives are administered during these trials.

2.1. Method

2.1.1. Subjects

The subjects were 34 experimentally naïve, Long–Evans hooded male rats derived from Harlan (Indianapolis, IN), approximately 100 days old at the start of the experiment. Rats were housed under a 12:12 h light: dark cycle (lights on at 07:00 h) and were deprived of food to 81–84% of their free-food weight. Water was continuously available in each individual wire-bottom cage. Animals were trained during the light phase of the daily cycle.

2.1.2. Apparatus

Four standard operant chambers (MED Associates, St. Albans, VT) each enclosed in a sound-attenuating chamber were used. Each box was 20.1-cm wide, 28-cm long, and 20.5-cm high, with a grid floor consisting of stainless steel bars 0.4 cm in diameter and spaced 1.6 cm apart. Underneath the grid floor was a pan filled with corncob bedding. The food cup was located on the front wall of the chamber 2 cm above the floor. Two retractable levers were located 1 cm to the right and left of the feeder, and 6 cm above the floor. Pellet dispensers delivered 45-mg food pellets (Bio-Serv #F0165, Frenchtown, NJ) or sucrose pellets (TestDiet #1811251, Richmond, IN). Food pellets contained protein (18.8%), fat (5.0%), carbohydrate (61.5%), fiber (4.6%), ash (4.4%), and moisture (<5.0%), and provided 3.68 kcal/g. Sucrose pellets contained sucrose (94.5%), protein (0%), fat (0%), carbohydrate (61.5%), fiber (4.7%), and ash (0.1%), and provided 3.41 kcal/g. The sound-attenuating chambers were equipped with a light (GE 1820) that provided diffuse illumination, a speaker that administered white noise, and a fan for air circulation. Background masking noise (speaker and fan) registered 75 dB (SPL, scale C).

Naloxone hydrochloride in desiccate form (stored at 2–8 °C) and isotonic saline solution were purchased from Sigma-Aldrich (St. Louis, MO). Naloxone was mixed with isotonic saline within 48 h of use to produce two doses, 2 mg/kg and 10 mg/kg, and stored in a sealed, air tight container at the appropriate temperature. Saline controls received an equal-volume injection of isotonic saline. All injections were

intraperitoneal, administered 15 min prior to start of the session, and at a volume of 1 ml/kg.

2.1.3. Procedure

Prior to training, pairs matched by ad libitum weight were randomly assigned to either food pellet or sucrose pellet condition. During training, animals were run in squads of 4; the composition of each squad was constant, but the sequence of squads was randomized across days. Prior to acquisition training, all subjects received two 20-min sessions of habituation to the operant chamber, one per day. Acquisition training started the next day according to a combined Pavlovian-instrumental procedure. Each acquisition session consisted of 5 trials separated by a 180-s variable intertrial interval (range: 140 to 220 s). On sessions 1–5, each trial started with the protraction of the right retractable lever (the left lever was not used in this experiment). Lever presentation lasted until the animal pressed the lever 3 times (fixed-ratio 3 instrumental component) or 10 s elapsed (Pavlovian component), whichever occurred first. This was followed by the delivery of 10 pellets of the appropriate type in rapid succession (one every 0.2 s). On sessions 6–10, the maximum time was increased to 20 s and on sessions 11–15 to 30 s. The same fixed-ratio 3 requirement was enforced throughout all acquisition sessions. This gradual increase in the maximum time to complete the fixed-ratio requirement was implemented after robust responding had developed to minimize a possible ceiling effect in case naloxone were to retard responding.

Sessions 12–14 were assigned to determine the effects of naloxone on instrumental behavior during acquisition training. These effects were studied using a within-subject design according to which each rat was treated with saline, 2 mg/kg, or 10 mg/kg before each of three successive sessions. The order of the doses was counterbalanced across subjects. Animals received no injections before session 15. Thus, before the start of extinction, all rats had received each of the 3 treatments once.

Prior to extinction training, triplets of rats matched in terms of overall acquisition performance within each incentive condition (i.e., food pellets, FP, or sucrose pellets, SP) were randomly assigned to one extinction condition depending on the dose administered. Thus, the effects of naloxone on appetitive extinction were studied using a between-subject design. Each subgroup within a given incentive condition was assigned to saline, 2 mg/kg, or 10 mg/kg naloxone. This gave rise to 6 groups: FP/Sal ($n=6$), FP/2 ($n=6$), FP/10 ($n=5$), SP/Sal ($n=6$), SP/2 ($n=6$), and SP/10 ($n=5$). The appropriate drug treatment was administered 15 min before the start of each extinction session. Each extinction trial (sessions 16–23, 5 trials/session) lasted until the animal completed a fixed-ratio 3 requirement or a maximum of 30 s elapsed from the start of the trial. No pellets were delivered during these trials. In all sessions, the dependent variable was the latency to complete the fixed-ratio 3 requirement (measured in 0.05-s units). Whenever the response requirement was not completed, the animal was assigned a latency corresponding to the maximum time allowed in that stage (i.e., 10, 20, or 30 s, depending on the session; see above). Because latency distributions tend to violate homogeneity of variance, we applied nonparametric tests to the raw data. Mann–Whitney tests were used for pairwise comparisons between independent samples and Wilcoxon signed-ranks test for pairwise comparisons between dependent samples. All tests were two tailed and the alpha value was set to the 0.050 level. Parametric tests using analysis of variance based on $\log_{10}$ transformed scores yielded virtually identical results.

3. Results

All rats consumed both types of pellet in all sessions, including sessions 12–14 in which animals were injected with naloxone. Three statistical analyses were conducted on data from the acquisition phase. First, the acquisition performance of groups trained with SPs or FPs was compared by calculating the mean performance on Sessions 1 to 11 for each animal. The mean (±SEM) latencies were 5.7 s (±0.4) for SP and 5.8 s (±0.4) for FP. A pairwise comparisons indicated a nonsignificant difference between these two conditions, $U(17, 17)=143.0$, $p=0.959$. There was no indication that acquisition was influenced by the type of incentive. Second, the effects of naloxone on asymptotic lever-pressing performance were evaluated by comparing the results for Sessions 12–14, when animals were treated on a within-subject design. These results are presented in Fig. 1. Mann–Whitney tests comparing the performance of groups trained with SP vs. FP, at each drug dose (saline, 2, and 10 mg/kg), indicated nonsignificant effects for incentive type, $Us(17, 17)>132.0$, $ps>0.69$. Wilcoxon tests comparing each naloxone dose to the saline indicated nonsignificant differences either for the SP or FP groups, $Z<1.92$, $ps>0.05$. Because there was no effect of pellet type but a tendency for latencies to increase at the 10 mg/kg dose, Wilcoxon tests were calculated pooling the data from both pellets. The results indicated that saline animals did not differ from those administered 2 mg/kg naloxone, $Z=0.509$, $p=0.611$, but saline latencies were significantly lower than those of animals given 10 mg/kg naloxone, $Z=2.120$, $p=0.034$. It should be noted that all rats completed all the trials in these three sessions. Therefore, the increase in latency in the groups receiving the 10 mg/kg dose, relative to the saline controls, does not reflect a failure to complete the fixed-ratio requirement. Third, an analysis of performance during the last acquisition session (Session 15, see Fig. 2), when no drugs were administered and before the start of extinction, indicated nonsignificant differences across groups trained with SP vs. FP, $U(17, 17)=133.5$, $p=0.705$.

Extinction performance is shown in Fig. 2. Both doses of naloxone facilitated appetitive extinction independently of the incentive type administered during acquisition. Mann–Whitney tests based on the mean response latency for Sessions 16–25 provided the following results. For the SP groups, Group SP/Sal was significantly lower than both Group SP/2, $U(6, 6)=0.0$, $p=0.004$, and SP/10, $U(6, 5)=2.0$, $p=0.018$. For the FP groups, Group FP/Sal was also significantly lower than both Group FP/2, $U(7, 6)=7.0$, $p=0.046$, and FP/10, $U(7, 5)=3.0$, $p=0.019$. The two naloxone-treated groups were not significantly different from each other whether they received training with sucrose or food pellets, $Us>14$, $ps>0.85$. Similarly, when pairs of groups given the same drug treatment but trained with different incentives were compared with each other, they were not significantly different, $Us>9$, $ps>0.39$. Thus, naloxone facilitated extinction relative to saline control, regardless of the type of incentive.

As latencies increase during extinction sessions, animals may fail to complete the fixed-ratio requirement in some trials. Because a maximum latency was assigned in incomplete trials, it may be the

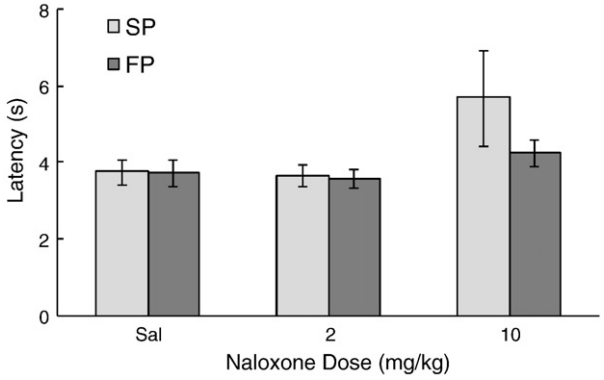

Fig. 1. Latency (in seconds) to complete a fixed-ratio 3 schedule for animals trained with either sucrose pellets (SP) or food pellets (FP) as a function of naloxone dose (0, 2, and 10 mg/kg, ip) during acquisition sessions (0 = saline solution). All animals received all treatments in a counterbalanced manner.

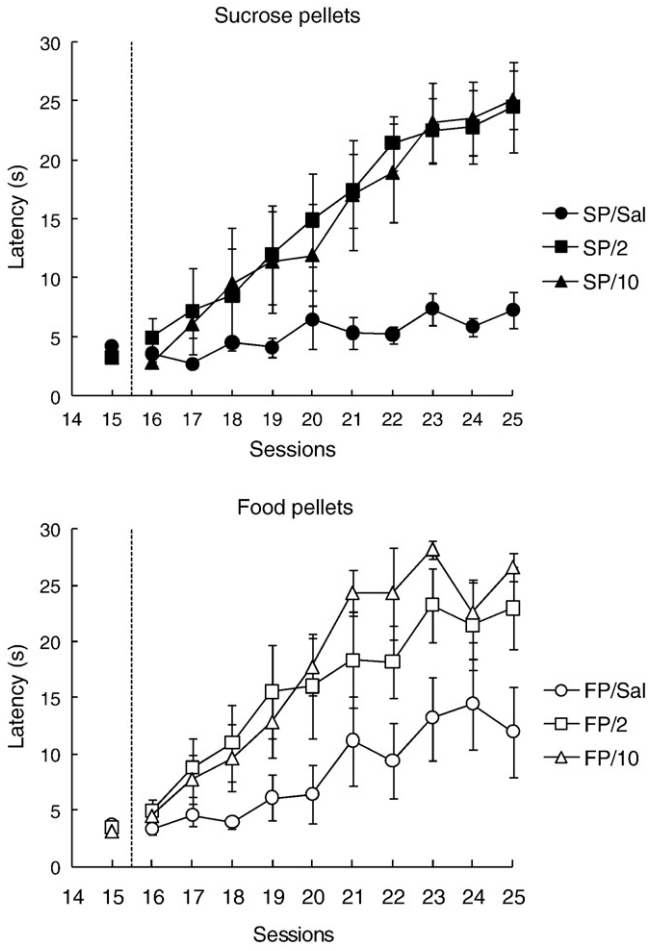

Fig. 2. Latency (in seconds) to complete a fixed-ratio 3 schedule across extinction sessions in groups reinforced with sucrose pellets (SP, top panel) or food pellets (FP, bottom panel). Sal: saline solution. 2: naloxone 2 mg/kg, i.p. 10: naloxone 10 mg/kg, i.p.

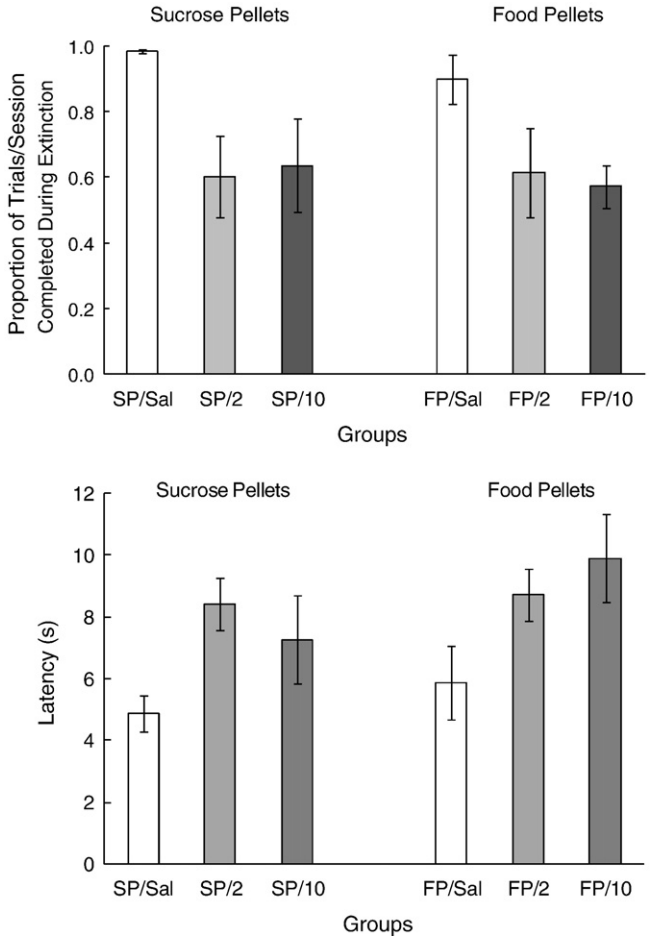

Fig. 3. The top panel shows the proportion of trials per session in which rats from each group completed the fixed-ratio requirement during the 10 extinction sessions. The bottom panel shows the mean response latency (in seconds) for each group calculated only from extinction trials in which rats successfully completed the fixed-ratio requirement.

case that the effects of naloxone have more to do with completing the fixed-ratio requirement than with response initiation per se. As Fig. 3 (top panel) shows, indeed naloxone reduced the number of completed trials during extinction. Mann–Whitney tests confirmed that the proportion of completed trials in extinction was significantly larger in Group SP/Sal than in Groups SP/2, $U(6, 6) = 0.0$, $p = 0.004$, and SP/10, $U(6, 5) = 0.5$, $p = 0.007$, and also larger in Group FP/Sal than in Group FP/10, $U(6, 5) = 3.0$, $p = 0.025$. All other comparisons were nonsignificant, including those across incentives, $Us > 7$, $ps > 0.06$. To determine whether naloxone also had an effect on response latency independently of fixed-ratio failures, overall extinction means were calculated only from trials in which each rat completed the fixed-ratio requirement (Fig. 3, bottom panel). Although the overall mean latencies go in the same direction, only the difference between Groups SP/Sal and SP/2 was significant, $U(6, 6) = 2.0$, $p = 0.010$. All other comparisons were nonsignificant, $Us > 4$, $ps > 0.06$. Thus, the effects of naloxone on appetitive extinction were strongest when failures to complete the fixed-ratio requirement were included in the analyses, as shown in Fig. 2.

4. Experiment 2

A common feature of cSNC and appetitive extinction is that both situations involve response suppression. A previously reinforced behavior (whether consummatory or instrumental) is suppressed following either an incomplete (cSNC) or a complete (appetitive extinction) downshift in incentive magnitude. In the training situation known as escape from frustration, rats are offered an opportunity to engage an active response that allows the animal to move out of the context in which they experienced a downshift in incentive (Daly, 1974). The traditional name of this procedure should not be taken literally; rats may be escaping from an aversive state of frustration (Daly, 1974), approaching an alternative location in search for the missing incentive (Elliott, 1928), or both. In one of the original experiments (Adelman and Maatsch, 1956), rats trained to traverse a runway to obtain food were given the opportunity to escape from the goal box during extinction trials by jumping on a platform located 25 cm above the floor of the goal box. These rats acquired the jumping response faster than a group never reinforced at the goal box and just as fast as a group of rats actually rewarded for jumping on the high platform. In Experiment 2, a similar situation was implemented in which rats were exposed to 32% sucrose in a goal box during 10 daily trials followed by an empty tube during the next 5 trials, in a manner similar to a typical cSNC experiment. During the last 5 trials, a door was opened 30 s after trial onset and the rat could exit the goal box by jumping over a barrier. No explicit reinforcer was provided for jumping. The questions of interest are, first, whether downshifted animals would acquire this escape response faster than unshifted controls, and, second, whether opioid blockage would modulate this type of escape learning. The motor impairment hypothesis predicts that opioid blockage should interfere with the development of the

escape response in both downshifted and unshifted groups. The reduced sucrose palatability hypothesis predicts no effect because no sucrose solution will be available during these trials. Finally, the increased aversive motivation hypothesis predicts faster acquisition of the escape response after naloxone treatment in the downshifted condition, but no effect in the unshifted condition. This hypothesis is consistent with the enhancing effects of naloxone on escape conditioning (Martinez et al., 1984) and passive avoidance (Tomaz et al., 1990), both situations involving shock-induced peripheral pain.

4.1. Method

4.1.1. Subjects

The subjects were 32 experimentally naïve Long–Evans hooded male rats derived from Harlan (Indianapolis, IN), approximately 100 days old at the start of the experiment. All maintenance conditions were as described in Experiment 1, including deprivation regime.

4.1.2. Apparatus

Training was carried out in a custom-made wooden apparatus with two compartments, a goal box measuring 42.5 cm in length, 15.5 cm in width, and 27.5 cm in height, and an escape box measuring 160.4 cm in length, 15.5 cm in width, and 27.5 cm in height. Both boxes were covered with wooden lids. A side-opening door separating the goal and escape boxes remained closed during preshift trials. In the opposite side, inside the goal box, was a house light (GE 1820), approximately 20 cm above the floor. A retractable sipper tube (MED Associates, St. Albans, VT) was mounted on a side wall of the goal box. When fully inserted inside the goal box, this sipper tube protruded 0.5 cm and was located 6.5 cm from the floor. When rats contacted the sipper tube, a circuit involving a stainless-steel floor panel was closed and the signal thus generated was used to measure the cumulative time in contact with the tube (goal-tracking time, in 0.01-s units). The interior of the start box was covered in black paint, except for the aluminum panels protecting the sipper tube and the stainless-steel floor panel.

The adjacent escape box had no light source. The interior of the escape box was covered in gray paint. Inside the escape box was a black hurdle barrier, 15.5 cm in width and 6.5 cm in height, 8 cm from the side door. Next to the hurdle barrier was a single photocell located 15 cm from the door, 3 cm above the floor. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube, the operation of the side door, and recorded goal-tracking times and escape latencies (both in 0.01-s units). Escape latencies were counted from the moment the side door opened to the moment the animal activated the photocell on the other side of the barrier. Outside the apparatus was a speaker that delivered white noise (80.1 dB, scale C).

4.1.3. Procedure

All subjects received a single 5-min session of exposure to the escape box similar to that used by Daly (1969). Animals were placed inside the escape box with the lid closed. Rats were then randomly assigned to one of two groups ($n=16$) depending on the incentive condition during preshift sessions. One group received access to 32% sucrose (w/w; e.g., 32 g of commercially available sugar for every 68 g of distilled water) during acquisition sessions, whereas the other was exposed to an empty sipper tube during acquisition sessions. Acquisition training lasted 10 daily sessions. In each session, animals were placed in the goal box with the door closed. After a variable interval of 30 s (range: 15–45 s), the sipper tube was automatically inserted. Detection of the first contact with the sipper tube initiated a 5-min session. The trial ended with the retraction of the sipper tube. After a variable interval of 30 s (range: 15–45 s), the animal was placed back in its home cage. Goal-tracking time was recorded during the 10 acquisition sessions.

At the end of the preshift phase, rats in the 32% sucrose and empty sipper tube conditions were matched in terms of overall acquisition performance and randomly assigned to one of two drug conditions, thus generating four groups ($n=8$): 32/Sal, 32/Nlx, 0/Sal, and 0/Nlx. Naloxone (2 mg/kg) or saline (equal volume) were prepared and administered as described in Experiment 1. Animals were injected 15 min prior to each escape session. Only the 2 mg/kg dose of naloxone was used in this experiment because there was no evidence that it would impair motor performance in the results of Experiment 1. These escape sessions started in a manner similar to the acquisition sessions, except that the sipper tube was empty for all animals. Rats were placed inside the goal box with the side door closed and the empty sipper tube was inserted after a variable interval of 30 s (range: 15–45 s). The side door remained closed during the initial 30 s after the empty sipper tube was inserted. Goal-tracking times were recorded during this initial interval. At the end of this period, the side door opened and the animal could move over the barrier and enter the escape box. The main dependent variable in Experiment 2 was the latency to exit the goal box and walk over the barrier. Activation of the photocell initiated a variable interval of 30 s (range: 15–45 s) and, at the end of this interval, the animal was removed from the escape box and returned to its home cage. Other aspects of the procedure were as described in Experiment 1.

5. Results

Two animals from Group 32/Sal received the appropriate training experience, but the data from sessions 3 and 8 were lost; for the purpose of statistical analyses, these data were replaced with the group average for their respective session (Kirk, 1968). By session 10, the average (±SEM) goal-tracking times were 258.5 s (±11.5 s) for animals exposed to 32% sucrose and 23.8 s (±3.8 s) for animals exposed to the empty sipper tube. A comparison of the overall means for Sessions 1–10 indicated that animals with access to 32% sucrose had significantly higher goal-tracking times than those exposed to an empty tube, $U(16, 16)=0$, $p=0.000$.

The results from the escape phase are shown in Fig. 4. The escape response developed only in Group 32/Sal, was not present in the first trial, and was also transient—all expected from previous results under similar conditions (Daly, 1974). Importantly, naloxone treatment

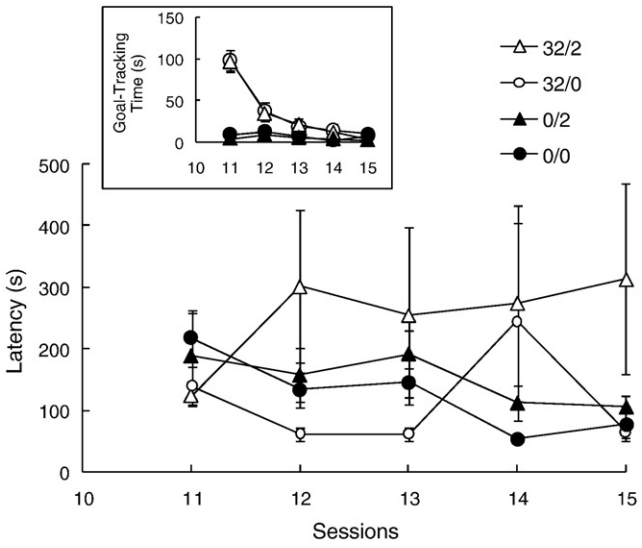

Fig. 4. Latency (in seconds) to break the photocell in the escape box across extinction sessions for groups previously given access to 32% sucrose (32) or an empty tube (0) during acquisition sessions, and treated with either naloxone (Nlx, 2 mg/kg, i.p.) or saline solution (Sal) during extinction sessions. The inset box shows the goal-tracking time (in seconds) during the initial 30 s of each extinction session, before the opening of the door allowing for an escape response.

eliminated the escape-from-frustration effect. Mann–Whitney pairwise comparisons among the two groups treated with saline indicated that Group 32/Sal exhibited significantly lower latencies on Sessions 12 and 13, $Us(8, 8) < 10.0$, $ps < 0.020$, but did not differ significantly on Sessions 11, 14, and 15, $Us(8, 8) > 19.0$, $ps > 0.20$. By contrast, Groups 32/Nlx and 0/Nlx were not different from each other in any of the sessions, $Us(8, 8) > 18.0$, $ps > 0.17$.

Groups were also compared in terms of their goal-tracking times during the initial 30 s of Session 11–15, before the opening of the door that connected the goal and escape boxes (Fig. 3, inset box). Mann–Whitney pairwise tests calculated on the overall mean for the five sessions indicated that Group 32/Nlx differed significantly from 0/Nlx, $U(8, 8) = 2.0$, $p = 0.002$, and Group 32/Sal differed significantly from 0/Sal, $U(8, 8) = 2.0$, $p = 0.002$. More importantly, naloxone did not affect responding to the empty sipper tube, either among the two downshifted groups, $U(8, 8) = 29.0$, $p = 0.753$, or among the two nonshifted controls, $U(8, 8) = 26.0$, $p = 0.529$. Thus, there was no evidence that changes in escape latencies were related to an initial suppression of consummatory behavior caused by naloxone.

6. General discussion

The present results indicated that opioid blockage (1) increased response latency during asymptotic (reinforced) performance at the 10 mg/kg dose, but had no effect at 2 mg/kg (Experiment 1); (2) increased response latency during extinction (nonreinforced) performance at both doses (Experiment 1); and (3) increased escape latencies, eliminating the escape-from-frustration effect (Experiment 2). Table 1 summarizes these results and others obtained in experiments involving various incentive downshift procedures and pretrial naloxone administration. How could these results be explained in terms of the alternative hypotheses outlined in the Introduction?

In the initial experiment involving opioid blockage (Pellegrini et al., 2005, Experiment 1), naloxone administered before the first and second downshift sessions increased consummatory suppression after a 32-to-6% sucrose downshift. To explain this reduction of consummatory behavior, it could be argued that naloxone interfered with the motor components of licking (see Philopena et al., 1996), that it increased aversive motivation induced by the incentive downshift (see McNally and Westbrook, 2003), or that it caused the 6% sucrose solution to be less palatable (see Kirkham and Cooper, 1988). However, naloxone failed to induce any detectable changes in consummatory behavior for a group given access to 6% sucrose throughout the experiment. Unlike a previous report in which an unshifted control was not included (Lynch and Clark, 1983), the results reported by Pellegrini et al. are difficult to explain for the motor impairment and sucrose palatability hypotheses, both of which assume that the effects of naloxone on consummatory behavior are independent of the incentive downshift operation. Unlike these two hypotheses, the increased aversive motivation hypothesis applies only when animals are exposed to a source of aversiveness, such as incentive downshift. Thus, naloxone should not affect the consummatory behavior of unshifted rats because they experience no downshift in incentive conditions.

Experiments manipulating the degree of the downshift indicate that opioid blockage affects consummatory behavior when the absolute difference between the pre- and postshift sucrose concentrations is large (Daniel et al., in press). Thus, naloxone enhances consummatory suppression in 32-to-6% or 32-to-12% sucrose downshifts, but it has no effects in 16-to-3% and 16-to-6% sucrose downshifts, relative to saline controls. The absolute difference between these pre- and postshift concentrations was, from smaller to larger, 10, 13, 20, and 26% sucrose. Thus, the difference threshold for naloxone to reduce consummatory behavior is somewhere between 13 and 20 percentage units of sucrose concentration. Such dependence on the pre–post difference between the sucrose concentrations is inconsistent with Kirkham and Cooper's (1988) conclusion that the effects of naloxone are equivalent to diluting the solution. The postshift solutions in Daniel et al.'s experiment varied between 2 and 12% sucrose; any dilution by a common denominator would yield very similar sucrose solution. Indeed, in the 32-to-6% and 16-to-6% sucrose groups, this dilution account would predict the same degree of suppression, when in fact only the former led to significant suppression. It seems that consummatory behavior depends not just on the current solution and not just on the previous solution, but on their relationship (Papini and Pellegrini, 2006). Thus, the reduced palatability hypothesis cannot account for these results. For the same reasons, the motor impairment hypothesis also fails to account for these results, leaving again the increased aversive motivation hypothesis as the most viable of the three.

Consummatory extinction experiments provide further evidence against the reduced palatability hypotheses of the effects of opioid blockage on incentive downshift. Two experiments reported by Norris et al. (2008, Experiments 4 and 5) compared the extinction of consummatory behavior after access to 4% sucrose in naloxone and saline conditions, one with continuous access to the empty sipper tube and the other with a discrete-trial procedure. In both cases, naloxone (2 mg/kg, i.p.) reduced sipper-tube contact in later portions of the session without affecting the initial levels. Obviously, in the absence of sucrose solution (or any solution), palatability effects are irrelevant. The motor impairment hypothesis could account for these results in terms of some cumulative effect of opioid blockage that interferes with licking responses on later sections of the session. Norris et al. (2008) favored an interpretation in terms of the recruitment of an internal aversive state of primary frustration induced by extinction leading to rejection and/or avoidance of the empty sipper tube.

None of the hypotheses described above can explain all the results of Experiment 1. In asymptotic acquisition performance, when animals were responding for food or sucrose pellets, opioid blockage caused a dose-dependent increase in response latencies. Under such conditions there are no clear sources of aversive motivation, providing evidence against the increased aversive motivation hypothesis (see Lynch and Clark, 1983), although such behavioral outcome is consistent with either a motor impairment or a reduced palatability hypothesis. However, the reduced palatability hypothesis does not apply to the extinction results because no incentive was delivered in

Table 1
Incentive downshift and opioid blockage: summary of results.

Preparation	Group comparison	Effect of naloxone
iSNC[a]	5-1 vs. 1-1 pellets, Nlx vs. Sal	Increased iSNC, no 1-1/Nlx control
cSNC[b]	32-6 vs. 6-6, Nlx vs. Sal	Increased cSNC
	6-6/Nlx vs. 6-6/Sal	No effect
	32-4/Nlx vs. 32-4/Sal	Increased suppression
cSNC[c]	32-6/Nlx vs. 32-6/Sal	Increased suppression
	32-12/Nlx vs. 32-12/Sal	Increased suppression
	16-3/Nlx vs. 16-3/Sal	No effect
	16-6/Nlx vs. 16-6/Sal	No effect
iE[a]	5-0/Nlx vs. 5-0/Sal, pellets	Facilitated extinction
cE[d]	4-0/Nlx vs. 4-0/Sal	Facilitated extinction
Instrumental conditioning[e]	0 vs. 2 mg/kg, FP & SP	Acquisition: no effect
	0 vs. 10 mg/kg, FP & SP	Acquisition: increased latency
	0 vs. 2 or 10 mg/kg, FP & SP	Extinction: increased latency
Escape from frustration[f]	32-0 vs. 0-0, Nlx vs. Sal	Eliminated escape from frustration

Note. cSNC: consummatory successive negative contrast. cE: consummatory extinction. Nlx: naloxone, 2 mg/kg unless otherwise stated, i.p. pretrial administration. Sal: equal-volume saline injection. FP: food pellets. SP: sucrose pellets.

[a] Lynch and Clark (1983).
[b] Pellegrini et al. (2005).
[c] Daniel et al. (in press).
[d] Norris et al. (2008).
[e] Present Experiment 1.
[f] Present Experiment 2.

these sessions. Facilitated extinction could be accounted for in terms of either motor impairment or increased aversive motivation. The motor impairment hypothesis also explains the increase in escape latencies observed in Experiment 2, whereas, again, palatability was not an issue given that animals were tested with empty tubes. Interestingly, the aversive motivation hypothesis also failed, since it predicted a facilitation of the escape-from-frustration effect, rather than the observed elimination. This prediction was based on previous experiments that tested the effects of naloxone in conventional escape-avoidance situations. For example, pretraining opioid blockage facilitates shock-induced escape conditioning (Martinez et al., 1984), whereas posttraining blockage facilitates retention of passive avoidance (Tomaz et al., 1990). Thus, opioid modulation of frustration and fear provides some leads against the otherwise successful view that equates these two emotional states (Gray, 1987; Papini et al., 2006).

The first conclusion out of this discussion is that none of the three hypotheses considered here can account for all the results. Whereas these hypotheses explain a wide variety of other data (see Introduction), none of them provides a comprehensive explanation of data involving incentive downshift manipulations, either incomplete downshifts (as in SNC) or complete downshifts (as in extinction). This may indicate that (1) opioid blockage has more than one effect—a plausible hypothesis given the diffuse brain distribution of opioid receptors (Mansour et al., 1995)—or (2) opioid blockage affects behavior in incentive downshift situations via a fourth mechanism that has not been considered thus far. One candidate is *reduced incentive value* (Lynch and Clark, 1983). Many of the effects of opioid blockage can be interpreted in terms of the loss of appetitive value by the available incentive. For example, mice exposed to progressive ratio schedules that demand an increasingly larger number of responses for successive reinforcers quit significantly earlier when treated with naloxone (Brennan et al., 2001). Naloxone is also known to reduce the secondary reinforcing value of a conditioned stimulus previously paired with water (Rudski, 2007). The results of Experiment 2 suggest that naloxone may have reduced the reinforcing value of escaping to a neutral compartment. In fact, such "escape" behavior may be seen as searching for the missing reward (Elliott, 1928). Interestingly, lactating rats treated with naloxone reduced the time spent hunting for insects, but increased maternal behavior relative to saline controls (Sukikara et al., 2007), suggesting that opioid blockage reduced the incentive value of prey. In such a scenario, escape-from-frustration failed to develop because naloxone reduced or eliminated the incentive value associated with foraging search. This mechanism would not operate in the unshifted controls because they are not exposed to an aversive situation, such as incentive downshift, and thus have no reason to tag the escape compartment as motivationally appetitive. Similarly, naloxone may increase cSNC (Pellegrini et al., 2005) by reducing the appetitive value of the downshifted sucrose solution and enhance instrumental extinction (Experiment 1) by reducing the secondary incentive value of the discriminative stimulus. Thus, the underlying assumption of the incentive value hypothesis is that sources of aversive motivation (e.g., incentive downshift) trigger a compensatory opioid-based response that functions to enhance sources of appetitive reinforcement and thus redirect behavior toward alternative goals. Opioid blockage would then reduce such appetitive compensatory response freeing the aversive state from interference. The extent to which the incentive value hypothesis can account for the effects of naloxone in situations involving fear conditioning remains to be determined.

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