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Consummatory Successive Negative Contrast Induces Hypoalgesia

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Rats received training in the consummatory successive negative contrast (cSNC) situation in which access to 32% sucrose solution during ten daily trials is followed by a downshift to 4% sucrose. Separate groups were exposed to the hot plate test for pain sensitivity immediately after either the first or the second downshift trial (Trials 11 and 12, respectively). Rats exhibited hypoalgesia after Trial 12 downshift, but not after Trial 11. These results suggest that cSNC induces the release of endogenous opioids that cause hypoalgesia, but only after some experience with the downshifted solution. This interpretation is supported by experiments demonstrating that opioid agonists reduce cSNC, whereas opioid antagonists enhance it.

Surprising reward omissions or reductions have a variety of consequences on the behavior and physiology of the organism (Papini & Dudley, 1997). In one preparation, rats receive daily access to a 32% sucrose solution for ten 5-min trials and are subsequently shifted to a 4% solution. Compared to an unshifted control group that always receives access to the 4% solution, downshifted rats typically exhibit a sharp suppression of consummatory behavior on Trial 11, followed by a recovery during the following trials (Flaherty, 1996). This phenomenon, called consummatory successive negative contrast (cSNC), is modulated by a variety of pharmacological agents, including opiate peptides. For example, administration of morphine (a nonselective opioid agonist) and DPDPE (a selective δ-receptor agonist) before Trial 11 (the first postshift trial) significantly attenuate cSNC (Rowan & Flaherty, 1987; Wood, Daniel, & Papini, 2005). Interestingly, the same experiments demonstrate that whereas morphine also attenuates cSNC when administered before Trial 12 (the second postshift trial), the more selective agonist DPDPE is not effective. Thus, the δ-receptor subsystem appears to be more selectively involved in the initial response to incentive downshift, whereas the μ- and/or κ-receptor subsystems, the other two major branches of the opioid system, may be responsible for modulating performance during Trial 12, after the rat has acquired some experience with the downshifted incentive.

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The effects of morphine are reversed by the nonselective opioid antagonist naloxone (Rowan & Flaherty, 1987), which itself also enhances cSNC when administered before Trials 11 and 12 (Pellegrini, Wood, Daniel, & Papini, 2005). cSNC is also enhanced on Trial 11 by administration of the δ-receptor antagonist naltrindole (Pellegrini et al., 2005). The effects of opioid agonist administration indicate that cSNC can be modulated by artificially activating the opioid system. However, the effects of opioid antagonists suggests that the opioid system is normally activated by the incentive downshift manipulation.

These results add to a growing body of evidence that suggests a connection between psychological pain (induced by loss and rejection) and physical pain (see Eisenberger & Lieberman, 2004; Papini, 2003). Whereas the opioid studies support such a connection indirectly (i.e., because the opioid system is known to be involved in physical pain; Bodnar & Klein, 2004), the present experiment attempted to illustrate this relationship more directly by assessing changes in physical pain as a function of whether or not the rat had experienced reward loss immediately before. If an incentive downshift event activates the opioid system, then it should be possible to demonstrate that immediately after a 32→4 downshift a rat behaves in a manner analogous to that of an animal that has been injected with opioid agonists.

One consequence of opioid activation is hypoalgesia—a reduction in the ability to respond to nociceptive stimuli. Interestingly, rats exhibit hypoalgesia after expossure to a variety of stressors, including pain induced by electric shock (Terman, Lewis, & Liebeskind, 1983), rotation (Hayes, Bennett, Newlon, & Mayer, 1978), defeat by aggressive conspecifics (Williams, Worland, & Smith, 1990), exposure to natural predatores (Kavaliers, 1988), loud noises (Helmstetter & Bellgowan, 1994), expossure to nociceptive heat (Hawkranko, Monroe, & Smith, 1994), and novelty (Netto, Siegfried, & Izquierdo, 1987). The role of the opioid system is implicated by experiments that demonstrate that hypoalgesia is eliminated by the administration of opioid antagonists (e.g., Helmstetter & Bellgowan, 1994). The present experiment was designed to test the hypothesis that hypoalgesia is also induced by a 32→4 incentive downshift. Separate groups of rats were exposed to the hot plate test, a standard test for assessing pain sensitivity (e.g., Hawkranko et al., 1994), immediately after either Trial 11 or Trial 12 (the first and second postshift trials, respectively). Separate groups were included to avoid carry-over effects from the painful experience in the hot plate test to the consummatory situation.

Method

Subjects

The subjects were 39 male Wistar rats, all experimentally naïve and bred at the vivarium of the Instituto de Investigaciones Médicas Lanari, Argentina. None of the male rats assigned to this experiment were discarded. The average ad libitum weight was 281.3 g (range: 162-424 g). Ten days before the start of the experiment, at about 90 days of age, rats were transferred to individual wire-bottom cages with water freely available. The daily amount of food was gradually reduced until individual weights reached an 85% of the ad libitum weight. Rats were thereafter kept at the 85% deprivation level by posttrial feeding administered at least 20 min after the end of the trial. The colony room was maintained under a 12:12 h cycle, with lights on at 07:00 h. Ambient temperature in testing and colony rooms was kept constant throughout the experiment at 23 °C.

Apparatus

Rats received training in 4 identical contrast boxes (MED Associates, Vermont, U.S.A.). Each box measured 24.1 cm in length, 29.2 cm in width, and 21 cm in height. The floor was made of aluminum bars (0.4 cm in diameter, 1.1 cm apart). In the center of a lateral wall there was a 5-cm hole, 3.5 cm deep, 1 cm above the floor level, through which a sipper tube could be introduced from the outside. When fully inserted, the sipper tube protruded 2 cm into the box. Goal-tracking time (measured in 0.01-s units) was automatically recorded by a computer that measured the cumulative amount of time that a photocell located in front of the drinking tube was activated during the trial. Each box was enclosed in a sound and light-attenuating cubicle equipped with a source of white noise and diffuse house light.

An Ugo Basile hot plate (Model 7250, Comerio, Italy) was used to test the response to heat-induced pain. The hot plate was set to a temperature of 51.5 ºC. The square plate measured 25 cm on its side. An acrylic cylinder, measuring 18.9 cm in diameter and 20 cm high, was placed on the plate and the animal was positioned inside. A digital clock was used to measured latencies (in 0.1-s units).

The sucrose solutions were prepared (w/v) according to the following procedure. For each solution (32% and 4%), every 32 g or 4 g of commercial sugar were mixed with 100 ml of tap water. Solutions were prepared approximately 24 h before being used and were presented at room temperature.

Procedure

Animals were matched for weight and each pair member was randomly assigned to one of the following groups: 32/Trial 11 (n = 9), 4/Trial 11 (n = 8), 32/Trial 12, (n = 11), and 4/Trial 12 (n = 11), depending on the reinforcer magnitude received during preshift trials 1-10 (either 32% or 4%), and the postshift trial after which they were tested in the hot plate (either Trial 11 or 12). Each trial was administered daily and lasted 5 min from the first recorded interruption of the photocell. During the postshift trials (either Trial 11 or Trials 11 and 12, depending on the group), all animals received access to the 4% solution.

The day before the start of the experiment, all subjects received access to the training solution in their home cage (32% or 4%, depending on group assignment). A drinking bottle with 20 ml of solution was placed in the cage during 20 min. Pretraining exposure to the solution was intended to habituate any neophobic reaction to the solutions. Preshift trials started the following day and lasted for 10 trials. Either one or two postshift trials were administered, depending on the group (see above). Animals were run in squads of four. The running order of the squads was randomized across days, but each squad involved always the same four rats. Each box was cleaned with a wet towel after each trial. A computer recorded the cumulative time a rat spent in contact with the drinking spout in the contrast box. Under the conditions used in this experiment, goal tracking time yields less variable data than the more conventional licking frequency measure. Previous research has shown significant positive correlations between goal tracking time and amount of fluid intake (Mustaca, Freidin, & Papini, 2002).

Immediately after the last postshift trial, whether Trial 11 or 12 depending on the group, rats were manually transported to another room for the hot plate test. Transport took approximately 10-15 s. As a rat was placed on top of the hot plate, a switch was pressed to start the latency count. The test was started when the temperature of the plate was exactly 51.5 ºC. The latency clock was stopped whenever the rat lifted a hind paw from the plate. The hot plate test was administered such that two rats from the 32→4 condition were alternated with two from the 4→4 condition. The plate was cleaned with a wet towel after each test. The experimenter recording hot plate latencies was blind to the treatment assignment of the animals.

Goal tracking times and hot plate latencies were subjected to conventional statistical analyses. Analyses of variance and Pearson's correlation coefficients were interpreted with an alpha value set at the 0.05 level. For brevity, specific p values were omitted in the text.

Results

All animals consumed sucrose solution during the first training trial, exhibiting no clear evidence of taste neophobia. Figures 1a and 1b show the goal track-

ing time for each pair of groups given the hot plate test immediately after Trial 11 or Trial 12. Preshift performance shows the usual increase in goal tracking across trials, with slightly higher scores for animals receiving 32% solution than 4% solution. Separate Group (4%, 32%) x Trial (1-10) analyses indicated significant acquisition effects for the Trial 11 groups, $F(9, 135) = 13.31$, and for the trial 12 groups, $F(9, 180) = 9.72$. The group effect fell short of significance for Trial 11 groups, $F(1, 15) = 3.31$, but was significant for Trial 12 groups, $F(1, 20) = 7.51$. The interaction effects were nonsignificant in both cases, Fs < 1.

Both pairs of groups exhibited evidence of a cSNC during the postshift trials, with the 32→4 downshifted rats undershooting the performance of the 4→4 unshifted controls. In the Trial 11 groups (Figure 1a), there was a single postshift trial and the performance of downshifted rats was significantly lower than that of unshifted controls, $F(1, 15) = 10.14$. In the case of Trial 12 groups (Figure 1b), the downshifted rats performed significantly below unshifted controls in both postshift trials. A Group (4%, 32%) x Trial (11-12) analysis indicated a significant effect for groups, $F(1, 20) = 11.40$, but nonsignificant trial and group by trial effects, Fs < 3.22.

The main results of this experiment are presented in Figures 1c and 1d. There, the hot plate latencies measured immediately after Trial 11 or Trial 12 were plotted for the downshifted and unshifted conditions. When the assessment of pain was carried out after Trial 11, there was no evidence of a group difference in hot plate latency. This was supported by a one-way analysis, $F < 1$. However, after Trial 12, the hot plate latency of downshifted rats was significantly higher than that of unshifted controls, $F(1, 21) = 6.94$. Thus, sensibility to heat-induced pain was reliably reduced when assessed immediately after the second postshift trial. Notice that the hot plate latencies of unshifted groups was very similar across conditions. An analysis of these latencies for both 4→4 groups yielded a nonsignificant difference, $F(1, 18) = 1.56$. Therefore, the evidence suggests that hot place latencies were selectively lengthened when measured after the second postshift trial.

An alternative possibility is that, after the initial experience with the downshift on Trial 11, early components in the daily routine of training before Trial 12 trigger an opioid response that could cause hypoalgesia before Trial 12 actually starts. This possibility is consistent with the results reported by Mitchell and Flaherty (1998). In one of their experiments, plasma corticosterone levels were elevated in a group that had received a 32→4 downshift, when assessed immediately before Trial 12, relative to an unshifted control. This is consistent with both Flaherty's (1996) multistage model and frustration theory (Wood et al., 2005), provided one assumes that the stress response or secondary frustration conditioned after the Trial 11 experience is triggered by cues consistently paired with trial events. Before Trial 12, such cues would elicit both an expectation of the high sucrose solution drank during preshift trials, as well as an expectation of reward loss conditioned as a result of the Trial 11 downshift. As a cautionary note, however, this interpretation rests on the unproven assumption that the pretrial elevation of corticosterone is specifically connected to the downshift experience of Trial 11 (i.e., that it would not occur, for example, before Trial 11). These possibilities can be easily tested, for example, by administering the hot plate test before Trials 11 and 12, and by delaying the onset of training trials once the animal is placed in the box (so as to unpair pretrial cues with trial events).

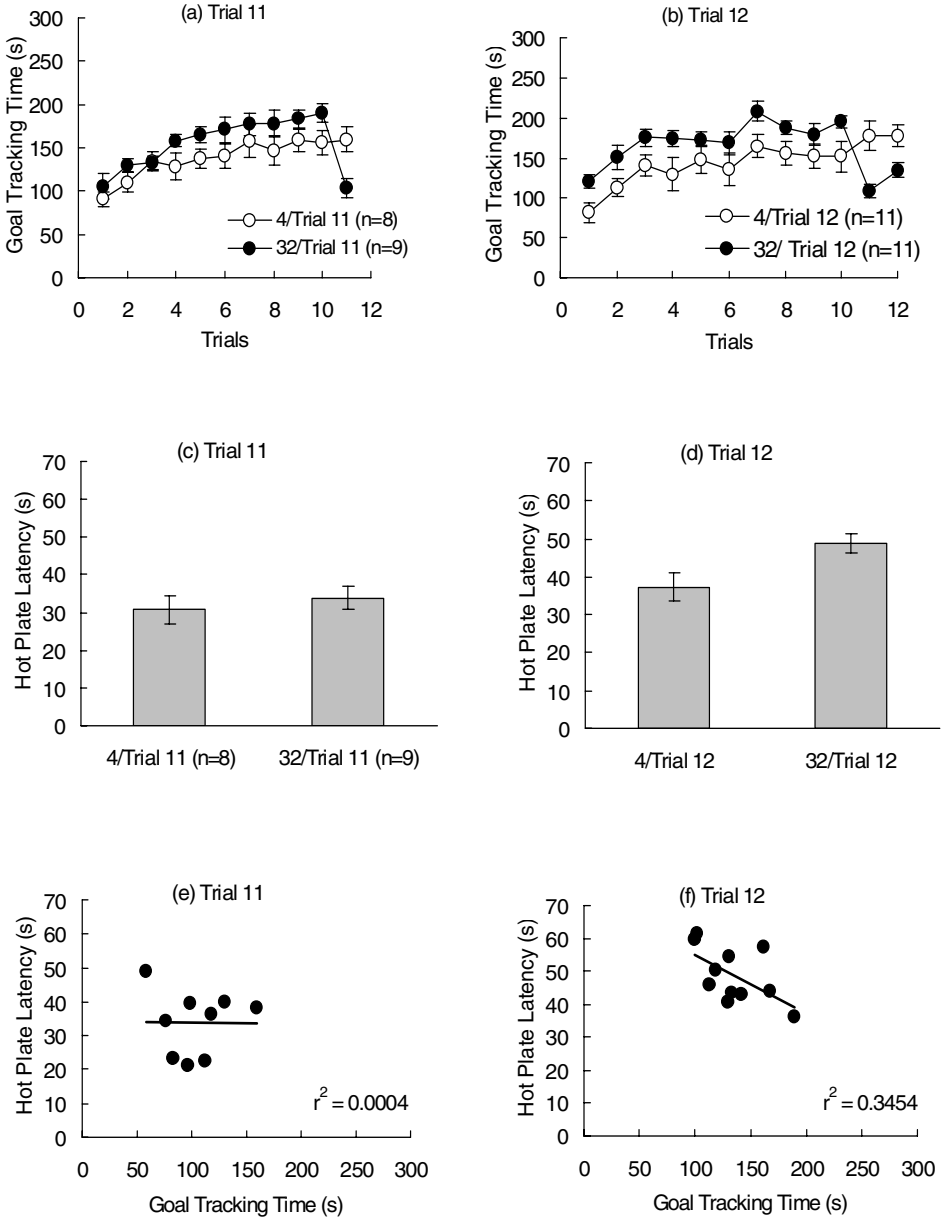

Figure 1. **(a)** cSNC in groups exposed to either 32% sucrose solution for 10 daily trials and then shifted to 4% solution for one final trial (n = 9), or exposed to 4% sucrose for 11 daily trials (n = 8). **(b)** Similar to the previous groups, except that a total of 12 trials were administered either in the 32→4 (n = 11), or in the 4→4 condition (n = 11). **(c)** Hot plate latencies after Trial 11, as a function of treatment in the cSNC situation (either 32→4 downshifted or 4→4 unshifted groups). **(d)** Similar to the previous results, except the hot plate test was administered after Trial 12 in the cSNC situation. **(d)** Correlation of goal tracking times obtained in Trial 11 of the cSNC and hot plate latencies obtained immediately after Trial 11. These rats received a 32→4 downshift. **(e)** Similar to the previous figure, except that these data come from Trial 12. Coefficients of determination (r^2) are provided in the last two figures.

Table 1
Summary of Opioid Effects on cSNC.

Drug	Opiate Receptor (function)	Effect on Trial 11	Effect on Trial 12	Reference
Morphine	μ, δ, κ (agonist)	Reduces	Reduces	Rowan & Flaherty (1987)
DPDPE	δ (agonist)	Reduces	No effect	Wood et al. (2005)
Naloxone	μ, δ, κ (antagonist)	Enhances	Enhances	Pellegrini et al. (2005)
Naltrindole	δ (antagonist)	Enhances	No effect	Pellegrini et al. (2005)

Based on previous results, it is hypothesized that this hypoalgesia response is mediated by activation of μ-opioid receptors. This hypothesis is based on three sources of evidence. First, the development of pain-induced hypoalgesia is blocked by μ antagonists, but not by δ or κ antagonists (Bellgowan & Helmsetter, 1998; Foo & Helmstetter, 1999). This is relevant to the cSNC situation on the assumption that there is a correspondence between frustration and the pain-fear system (Papini, 2003). Second, the nonselective agonist morphine reduces cSNC when administered before Trial 12 (Rowan & Flaherty, 1987), and the nonselective antagonist naloxone enhances cSNC when administered before Trial 12 (Pellegrini et al., 2005). Both morphine and naloxone act on all opioid receptors, including the μ receptor. Third, the effects of morphine and naloxone on Trial 12 are probably not mediated by their actions on the δ-receptor subsystem. Neither the δ-receptor agonist DPDPE nor the antagonist naltrindole affect cSNC on Trial 12 (Pellegrini et al., 2005; Wood et al., 2005). Morphine, which does attenuate cSNC on Trial 11 (Roway & Flaherty, 1987), could exert this effect via its ability to influence the δ receptor; as mentioned above, δ activation is ineffective to generate hypoalgesia or attenuate cSCN. These results are summarized in Table 1. A test of this hypothesis will require treatment with selective opioid antagonists.

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