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ORIGINAL INVESTIGATION

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Effects of contextual or olfactory cues previously paired with morphine withdrawal on behavior and pain sensitivity in the rat

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Abstract *Rationale:* Pavlovian conditioning processes have been accorded an important role in maintaining persistent opiate administration. At least one locus for this contribution is during opiate withdrawal. These experiments studied the contribution of Pavlovian conditioning processes to morphine withdrawal. *Objectives:* To determine whether exposure to a distinctive context or odor paired with morphine withdrawal would provoke a withdrawal syndrome, defensive behaviors (e.g., freezing) and pain modulatory (e.g., hypoalgesia) responses similar to those produced by exposure to stimuli signaling other sources of aversive stimulation (e.g., footshock), or whether both withdrawal and fear-like responses would be provoked. *Methods:* Rats were used in four experiments to study the effects on defensive behavior and pain sensitivity of naloxone-precipitated morphine withdrawal or exposure to a distinctive context or odor previously paired with such withdrawal. *Results:* Injection of 2.5 mg/kg naloxone in morphine-dependent rats precipitated a withdrawal syndrome characterized by whole body shaking, diarrhea, ptosis, and postural abnormalities (experiment 1). Exposure to either a distinctive context (experiment 2) or odor (experiments 3) previously paired with morphine withdrawal provoked the species-typical defense response of freezing but not signs of withdrawal. Exposure to an odor previously paired with morphine withdrawal also provoked hypoalgesia in the formalin test, which was mediated by activity at opioid receptors (experiment 4). *Conclusions:* These results show that opiate withdrawal supports the conditioning of defensive and hypoalgesic responses consistent with the arousal of a fear motivational system. The emergence of fear in these experiments, and the relationship between the freezing observed here and the learned avoidance and suppression observed in other withdrawal conditioning preparations, is discussed with reference to dual representation accounts of Pavlovian conditioning.

Keywords Morphine withdrawal · Fear · Pain · Formalin test · Conditioning

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Introduction

Pavlovian conditioning processes have been accorded an important role in the maintenance of persistent opiate administration. Accounts of this role are frequently framed with reference to the contingent relations existing between environmental stimuli, such as the sight of a loaded syringe and tourniquet, and the acute rewarding effects of opiates such as morphine or heroin (e.g., Siegel 1989; Robinson and Berridge 1993). However, just as a restricted set of environmental stimuli often precede administration of a drug, so too do certain stimuli often precede and accompany the absence or withdrawal from a drug. For example, the time since last injection of an opiate or an environment where the drug is not readily available could be viewed as reliable predictors of a withdrawal syndrome. Despite their relevance for understanding persistent opiate administration, these withdrawal-related conditioning processes have received considerably less empirical attention than those related to the acute rewarding effects of a drug. In particular, the contents of the learning which occurs when a stimulus is established as a predictor of opiate withdrawal, and the mechanisms by which this learning influences behavior, remain unclear.

The answer to these questions has typically been approached in two distinct ways. The first approach has been to suggest that a conditioned stimulus (CS) paired with opiate withdrawal will itself come to elicit a withdrawal syndrome (Wikler 1965; O'Brien 1976; Baldwin and Koob 1992). According to this line of reasoning,

Pavlovian conditioning processes simply imbue antecedent stimuli with the ability to provoke responses similar to those observed during acute withdrawal from opiates. Indeed, there have been reports based on both human and animal subjects that presentation of a stimulus previously paired with morphine withdrawal provokes responses (e.g., yawning, tearing, and teeth chattering) commonly observed during withdrawal from morphine (e.g., Wikler and Pescor 1967; O'Brien 1976; Schnur 1992).

A second approach has suggested that conditioning with morphine withdrawal is similar to conditioning with other kinds of aversive unconditioned stimuli and that the effects on behavior of a stimulus signaling withdrawal from morphine should be similar to those of a stimulus signaling non-pharmacological noxious events (e.g., Mucha 1991). An influential account of conditioning with non-pharmacological noxious events is the Perceptual-Defensive-Recuperative (PDR) model of fear and pain proposed by Bolles and Fanselow (1980). The PDR model predicts that a CS which has been rendered dangerous as a consequence of signaling noxious stimulation will arouse a fear motivational system releasing species-specific defensive behaviors directed towards escape from the source of aversive stimulation. The PDR model also predicts that arousal of this fear- or aversive-motivational system recruits endogenous antinociceptive circuits resulting in a decreased sensitivity to pain (hypoalgesia). This hypoalgesia is accorded a central role in permitting the expression of defensive behavior in the presence of tissue injury (Fanselow 1986). In support of this possibility, Mucha (1991) demonstrated that rats engaged in defensive burying when presented with a small spherical object previously paired with morphine withdrawal. This defensive burying response is typically observed when such a CS is associated with other forms of aversive stimulation such as footshock (Pinel and Treit 1978). Moreover, recent studies indicate that neural mechanisms which mediate aversive conditioning with morphine withdrawal are similar to those mediating aversive conditioning with footshock (Schulteis et al. 2000).

The present experiments studied the impact on behavior and pain sensitivity of exposure to contextual or olfactory stimuli previously paired with morphine withdrawal. Their aim was to determine whether this exposure would provoke a withdrawal syndrome, defensive responses (freezing) similar to those observed following exposure to a stimulus paired with footshock, or whether both withdrawal and fear-like responses would be provoked. In experiment 1, we studied the behavioral effects of morphine withdrawal. The aim of this experiment was to characterize the behavioral responses observed during withdrawal to permit comparison with the remaining experiments. In experiments 2 and 3, we studied the effects on withdrawal and defensive-related behavior of exposure to either contextual (experiment 2) or olfactory (experiment 3) cues associated with morphine withdrawal. Finally, in experiment 4 we studied the effects on pain sensitivity of exposure to an olfactory cue previously paired with morphine withdrawal using the formalin test. The formalin test was chosen for two reasons. First, it is a well validated model of acute tissue damage in rodents (Abbott et al. 1995). Second, it has provided the most consistent evidence that stimuli paired with aversive stimulation provoke hypoalgesic responses (Fanselow 1986).

Materials and methods

Subjects

Subjects were experimentally naive, male Sprague-Dawley rats weighing between 275 and 300 g at the start of the experiments. Rats were obtained from Charles River Laboratories (Portage, Mich., USA) and were housed in plastic cages, two to three animals per cage, in a colony room maintained on a 12:12-h light-dark cycle (lights on 7 a.m.). Food and water were continuously available for the duration of the experiment. These experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, 1996).

Apparatus

Four chambers (30 cm long×26 cm wide×27 cm high or 28.5 cm long×23-cm wide×28.5 cm high) were used as conditioning and test chambers. The floors consisted of stainless steel rods. A 10% ethanol solution was used to clean the chambers between subjects. In experiment 2, a distinctive odor was presented in the chambers by smearing 0.5 g of Vicks VapoRub (Procter & Gamble, Cincinatti, Ohio, USA) on a piece of paper placed beneath the grid floors of the chambers. The behavior of each rat while in the chambers was recorded using a camera facing the chambers. The camera was connected to a videotape and monitor located in the same room. Plastic buckets (24 cm diameter×70 cm height) were used to present the odorant during conditioning in experiments 2 and 3. Specifically, 0.75 g of the odorant (Vicks VapoRub) was smeared on the walls of the plastic buckets 5 cm from their top.

Procedure

Rats were handled for 1 min each day for three days prior to the start of the experiment.

Pre-treatment

Across days 1–6 of the experiments, rats were weighed and injected daily in their home cages with SC morphine. On days 1 and 2, rats were injected with 10 mg/kg morphine in a volume of 2 ml/kg, whereas on the remaining days they were injected with 15 mg/kg morphine in a volume of 2 ml/kg. Rats were maintained on a daily injection of 15 mg/kg morphine across days 6–15 of the experiments.

Conditioning

The procedure for conditioning in experiments 2–4 is shown in Fig. 1. Briefly, on Conditioning day (day 6), 90 min after daily injection of morphine, rats were transported to the laboratory. Upon arrival rats were placed in the chambers (experiment 2) or plastic buckets (experiments 3 and 4). Ten minutes later, they were briefly removed from the chambers (experiment 2) or buckets (experiments 3 and 4), injected with either 2.5 mg/kg naloxone (group Paired) or saline (group Unpaired), and returned to the chambers

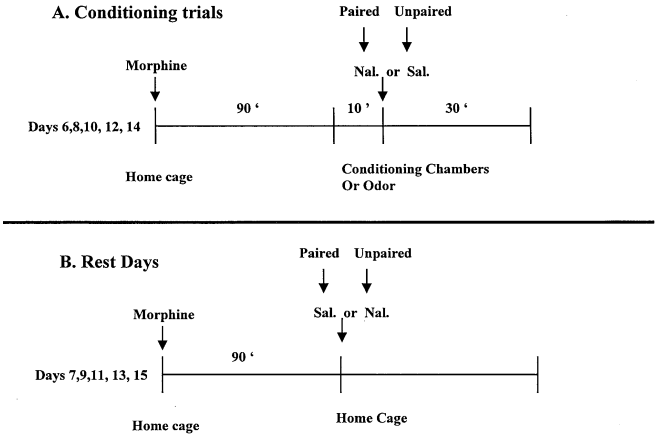

Fig. 1 **A**, **B** Procedure for conditioning in experiments 2–4

(experiment 2) or buckets (experiments 3 and 4) for 30 min. On Rest day (day 7), 90 min after the daily injection of morphine, rats were briefly removed from their home cages, injected, and returned to those home cages. In this instance, rats in group Paired were injected with saline whereas rats in group Unpaired were injected with 2.5 mg/kg naloxone. This 2-day cycle was repeated a further four times. Thus, at the end of conditioning, rats in group Paired had received five paired exposures to the distinctive context or odor and injection of naloxone, whereas rats in groups Unpaired had received five such unpaired exposures. There were 7 days rest between the final day of conditioning (day 15) and test (day 22).

Test

Experiment 1. Across days 4 and 5, rats were transported twice each day to the test laboratory and placed in the test chambers. Rats were familiarized with the test apparatus and handling procedures to prevent the influence of novelty on test. On day 6, 90 min following injection of 15 mg/kg morphine, rats were transported to the laboratory and placed in the chambers. Five minutes later they were injected with SC saline (2 ml/kg) and their behavior was observed for 15 min. Specifically, behavior was observed for freezing (defined as a crouching posture with the absence of all movement other than that required for respiration), signs of withdrawal (wet dog shakes, teeth chattering, ptosis, diarrhea, profuse salivation, chin rubbing (rubbing the chin on the floor of the test chamber), mucous secretions from the eyes or nose, ejaculation or genital grooming, and irritability on handling, as well as rearing, grooming, sniffing/exploration (raising and/or lateral movements of the head accompanied by locomotor activity) and immobility. Extensive pilot studies indicated that morphine dependent rats injected with naloxone displayed characteristic postural abnormalities: lying on the belly or the side with almost complete loss of muscle tone and with head in contact with the floor of the chamber. This response was readily distinguished from freezing: a crouching posture with neither the ventral surface nor head in contact with the floor and clear evidence of muscle tone. Thus, although both postural changes were characterized by a lack of movement they were otherwise readily distinguished. On day 7, 90 min following injection of 15 mg/kg morphine, rats were transported to the laboratory and placed in the chambers. Five minutes later they were injected with SC 2.5 mg/kg (2 ml/kg) naloxone and their behavior was scored for 15 min according to the categories defined above.

Experiment 2. On day 22 rats were transported to the laboratory and placed in the distinctive chambers for 10 min. Rats were observed every 5 s for the behaviors described above.

Experiment 3. On day 22, rats were transported to the laboratory and were placed in the test chambers. Thus, rats were tested in the same chambers described for experiments 1 and 2, which were distinct from the chambers used to present the odor during conditioning (plastic buckets). The distinctive odor was not presented. Rats were observed every 5 s, for 10 min, for freezing and signs of withdrawal. On day 22, rats were transported to the laboratory and were placed in the chambers. The distinctive odor was presented by smearing 0.75 g of the odorant on a piece of paper placed beneath the floor of the chambers. Rats were observed every 5 s, for 10 min, for freezing and signs of withdrawal. The two tests in this experiment (odor absent and odor present) were separated by a period of 4 h and the order of testing was counterbalanced.

Experiment 4. On day 22, rats were removed from their home cages and injected SC with 50 µl of a 2.5% formalin solution into the plantar surface of the right hindpaw. For formalin injection rats were loosely wrapped in a padded cloth and a 30-gauge needle was inserted between the distal tips of the two basal tori. Immediately following formalin injection rats were returned to their home cages. Ten minutes later, rats were briefly removed from their home cages, injected SC in the dorsal neck region, and returned to their home cages. Half of the rats in groups Paired and Unpaired were injected with 3.0 mg/kg naloxone, whereas the remainder were injected with saline. Fifteen minutes later, rats were transported to the laboratory and placed in the test chambers. Thus, rats were tested in the same chambers described for experiments 1 and 2, which were distinct from the chambers used to present the odor during conditioning (plastic buckets). Five minutes later, the amount of time spent responding to the formalin-injected paw was recorded for 5 min (30–35 min post-formalin). The distinctive odor was then presented by smearing 0.75 g of the odorant on a piece of paper placed beneath the floor of the chambers. Formalin responding was scored for a further 5 min (35–40 min post-formalin injection). The behavioral response to formalin is stable during this 10-min period (Abbott et al. 1995).

The two tests in this experiment (odor absent and odor present) were conducted during the same experimental session to avoid repeated tissue damage associated with multiple formalin injections. The test without the odor was always conducted first (30–35 min post-formalin) to avoid contamination of the data by persistence of the odor.

Statistical analysis

Withdrawal behaviors were converted into a global withdrawal score as described by Gellert and Holtzmann (1978). The number of observations spent in other behavioral categories (freezing, rearing, etc.) were converted to a percentage of total observations prior to analysis. The data were then analyzed by means of a planned orthogonal contrast testing procedure which preserved the factorial nature of the experimental designs (Hays 1972). The behavior of rats in these experiments was scored by two observers, one of whom was unaware of group allocations. The inter-rater reliability for these ratings was 0.95 ($P<0.001$).

Results

Experiment 1

The aim of this experiment was to determine the effects on behavior of an episode of morphine withdrawal thereby allowing comparison with the remaining experiments studying the effects on defensive behavior of a withdrawal-associated stimulus. The top left-hand panel of Fig. 2 shows the mean and SEM percentage of observations that rats spent in each of the behavioral categories

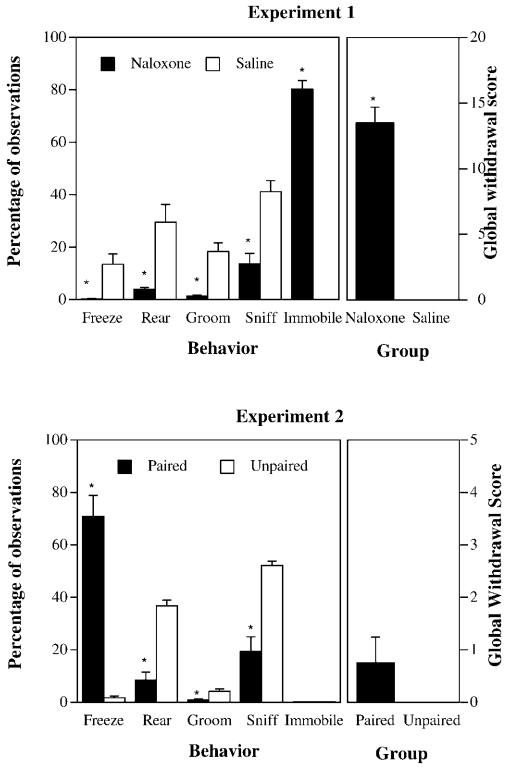

Fig. 2 *Top panels*: mean and SEM percentage of observations in each behavioral category (*left panel*) and global withdrawal scores (*right panel*) in experiment 1 during morphine withdrawal. *Bottom panels*: mean and SEM percentage of observations in each behavioral category (*left panel*) and global withdrawal scores (*right panel*) following exposure to the withdrawal-associated context in experiment 2

measured. Inspection of the panel indicates that the predominant behavioral response among rats injected on day 6 with saline was sniffing/exploration followed by rearing and grooming. By contrast, on day 7 when rats were injected with naloxone, the predominant behavioral responses was immobility. This response was characterized by lying on the belly or the side, with almost complete loss of muscle tone. The mean and SEM global withdrawal scores are shown in the top right-hand panel of Fig. 2.

Inspection of the panel indicates that injection of naloxone on day 7, but not saline on day 6, provoked a withdrawal syndrome. The statistical analysis confirmed these observations. There was a significant difference between injection of saline (day 6) and injection of naloxone (day 7) in the levels of freezing [$F(1,9)=11.7$, $P<0.01$[, rearing [$F(1,9)=13.4$, $P<0.01$], sniffing/exploration [$F(1,9)=20.9$, $P<0.01$], grooming [$F(1,9)=23.9$, $P<0.01$], immobility [$F(1,9)=564.8$], and global withdrawal scores [$F(1,9)=133.9$, $P<0.01$]. It is clear from inspection of the figure that rearing, grooming, sniffing/exploration, and freezing were reduced by injection of naloxone but that levels of immobility and withdrawal behavior were increased by this injection compared to injection of saline.

Experiment 2

Experiment 2 studied the effects on defensive behavior of exposure to a distinctive context previously paired with morphine withdrawal. The purpose of this experiment was to determine if exposure to a context previously paired with morphine withdrawal would provoke defensive behaviors (e.g., freezing) or a withdrawal syndrome similar to that observed in experiment 1.

One rat from group Unpaired died during the induction of morphine dependence. Therefore, there were seven rats in group Unpaired and eight rats in group Paired on test. The bottom left-hand panel of Fig. 2 shows the mean and SEM percentage of observations that rats spent in each of the behavioral categories measured. Inspection of the panel indicates that the predominant behavioral response among rats in group Paired was freezing, whereas rats in group Unpaired displayed very little freezing, and instead displayed equivalent levels of rearing and other exploratory behaviors. The mean and SEM global withdrawal scores for rats in both groups, shown in the bottom right-hand panel of Fig. 2, were low. The statistical analysis confirmed these observations. There was a significant difference between group Paired and group Unpaired in the levels of freezing [$F(1,14)=65.9$, $P<0.01$], rearing [$F(1,14)=55.8$, $P<0.01$], sniffing/exploration [$F(1,14)=28.6$, $P<0.01$] and grooming [$F(1,14)=11.7$, $P<0.01$], but no significant differences in levels of immobility. There was also no significant difference between groups in global withdrawal scores. Indeed, the only withdrawal sign observed was chin rubbing which occurred in two out of the eight animals tested.

Experiment 3

Experiment 3 studied the effects on defensive behavior of exposure to a distinctive odor previously paired with morphine withdrawal. The aim of this experiment was to determine if exposure to an odor previously paired with morphine withdrawal would provoke freezing similar to that produced by the withdrawal-associated context in experiment 2 or whether this exposure would provoke a withdrawal syndrome similar to that observed in experiment 1.

One animal died during the induction of morphine dependence. Therefore, there were 18 rats in group Paired and 17 rats in group Unpaired on test. The mean and SEM percentage of observations spent freezing on test are shown in the left hand panel of Fig. 3. Inspection of the panel indicates that whereas freezing for rats in both groups was low when tested in the absence of the odor, rats in group Paired displayed a greater increase in freezing when tested in the presence of the odor compared to rats in group Unpaired. These observations were confirmed by the statistical analysis. There was a significant main effect for conditioning history [$F(1,33)=16.1$, $P<0.01$]. There was also a significant main effect for presence of the odor [$F(1,33)=32.9$, $P<0.01$]. Important-

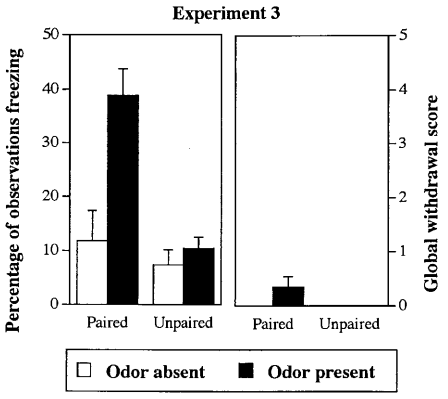

Fig. 3 Mean and SEM percentage of observations freezing (*left panel*) and global withdrawal scores (*right panel*) in the presence and absence of the withdrawal-associated odor in experiment 3

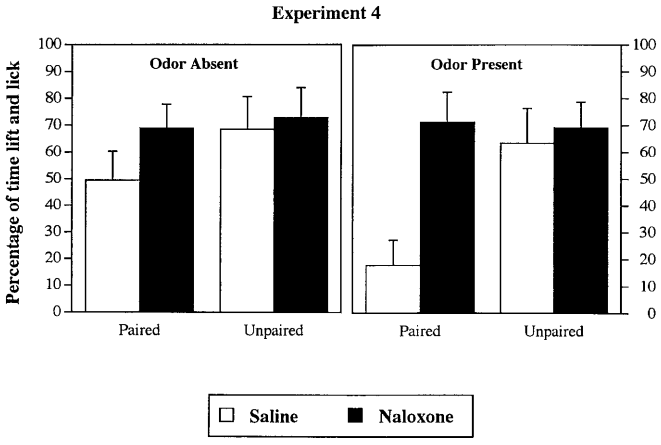

Fig. 4 Mean and SEM percentage of time spent responding to the formalin-injected paw before (*left panel*) and after (*right panel*) presentation of the withdrawal associated odor in experiment 4

ly, there was a significant 2×(2) interaction [$F(1,33)=20.9$, $P<0.01$]. It is clear from inspection of the figure that this interaction reflects the greater increase among rats in group Paired compared to group Unpaired in freezing from the test conducted in the absence of the odor to the test conducted in the presence of the odor.

The mean and SEM global withdrawal scores for rats in this experiment are shown in the Fig. 3. Inspection of the panel indicates that there was little evidence for withdrawal behaviors in either group. Although withdrawal scores increased in group Paired following presentation of the odor, this increase reflects the occurrence of teeth chattering in three out of the eighteen animals tested. There was no significant main effect for conditioning history, no significant main effect for presence of the odor, and no significant 2×(2) interaction.

Experiment 4

Experiment 4 studied the effects on pain sensitivity of exposure to a distinctive odor previously paired with morphine withdrawal. According to the PDR model (Bolles and Fanselow 1980; Fanselow 1986), hypoalgesia and freezing can be viewed as responses to the conditioned arousal of a fear or aversive motivational system. It follows from this model that animals should be hypoalgesic during the display of defensive behaviors. Experiment 4 also studied opioid receptor involvement in any pain modulatory response observed. Specifically, rats in groups Paired and Unpaired were injected with naloxone or saline prior to exposure to the distinctive context. Demonstrations that conditioned alterations in pain sensitivity can be reversed by injection of opioid receptor antagonists provide convincing evidence that these responses are a consequence of activation of endogenous pain control circuits rather than secondary to motoric deficits or response competition.

Six rats died during the induction of morphine dependence. Therefore, on test there were seven rats in group Paired-Saline, six rats in group Paired-Naloxone, seven rats in group Unpaired-Saline, and six rats in group Unpaired-Naloxone. The mean and SEM percentage of time spent responding to the formalin-injected paw on test is shown in Fig. 4. Inspection of the figure indicates that whereas there was little difference between levels of formalin responding among rats in group Paired and Unpaired when tested without the distinctive odor, presentation of the distinctive odor produced a decrease in formalin responding among rats in group Paired, but not group Unpaired, and that this decrease was prevented by pretreatment with 3.0 mg/kg naloxone.

These observations were confirmed by the 2×2×(2) analysis. There was no main effect for conditioning history, no main effect for injection of naloxone versus saline, or an overall interaction between these manipulations. However, there was a significant main effect for presence of the odor [$F(1,22)=14.5$, $P<0.01$], which interacted significantly with the main effect of injection of naloxone versus saline [$F(1,22)=12.2$], but not the main effect of conditioning history. Importantly the three-way interaction was also significant [$F(1,22)=10.5$, $P<0.01$]. This final result confirms what is obvious from the figure: there was conditioned hypoalgesia among rats in group Paired which was prevented by pre-treatment with naloxone.

Discussion

These experiments studied the effects on defensive behavior and pain sensitivity of exposure to withdrawal-associated contextual or olfactory cues. Their results can be summarized succinctly. Rats exposed to either a distinctive context or odor paired with naloxone-precipitated morphine withdrawal displayed few signs of morphine withdrawal. Instead, their behavior was characterized by the species-typical defense response of freezing. Rats were also hypoalgesic when tested for pain sensitivity in the presence of the withdrawal-associated odor us-

ing the formalin test. This hypoalgesia was mediated by the release of endogenous opioid peptides because it was prevented by a moderate dose (3.0 mg/kg) of the opioid receptor antagonist naloxone. There was evidence here that freezing and hypoalgesia were mediated by associative learning processes because they were only observed among rats with a history paired exposures to either the context or odor and morphine withdrawal. Moreover, freezing and hypoalgesia were selectively observed among rats in group Paired when tested in the presence, but not absence, of the distinctive odor. Together, these results provide compelling evidence that the behavioral and pain modulatory alterations observed in these experiments were the products of associative learning and were not non-specific consequences of a history of morphine or naloxone injections.

These experiments show for the first time that exposure to either a withdrawal-associated context or an odor provokes the species-typical defense response of freezing and opioid receptor-mediated hypoalgesia in rats. The occurrence of freezing and hypoalgesia in these experiments adds considerable strength to the proposal that the role of Pavlovian conditioning in morphine withdrawal is similar to conditioning with non-pharmacological noxious stimuli such as footshock (Mucha 1991). Specifically, freezing and hypoalgesia could be viewed as responses to the conditioned arousal of a fear or defensive motivational system by the context or odor previously with morphine withdrawal. Nonetheless, it is not immediately clear how to reconcile the emergence of defensive behaviors with two classes of experiments frequently taken as evidence for an important role for Pavlovian conditioning processes in regulating withdrawal from morphine. First, as reviewed above, it has been reported that environmental stimuli signaling the occurrence of morphine withdrawal provoke withdrawal-like responses (often only a subset of those observed during withdrawal) (e.g., Wikler and Pescor 1975; O'Brien 1976; Schnur 1992). Second, it has frequently been reported that presentations of a withdrawal-associated stimulus elicit avoidance responses and serve as conditioned punishers of instrumental responding (e.g., Goldberg and Schuster 1967; Pilcher and Stolerman 1967; Baldwin and Koob 1993).

This contrast as well as the emergence of freezing could be explained by considering the dual representation accounts of Pavlovian conditioning offered by Konorski (1967) as well as Wagner and Brandon (1989). These accounts propose that a Pavlovian conditioned stimulus which has been rendered dangerous as a consequence of signaling an aversive event will elicit discrete, consummatory responses emerging from learning about the specific sensory and spatiotemporal (epicritic) properties of that event (e.g., eyeblink to paraorbital shock), as well as diffuse, preparatory responses emerging from learning about its aversive motivational (protopathic) features (e.g., avoidance responses; conditioned suppression). Importantly, these motivational features are shared by all aversive stimuli regardless of their epicritic properties (e.g., footshock, loud noises, illness, etc.). It follows that withdrawal-like responses could be viewed as consummatory responses elicited by the conditioned arousal of the epicritic properties of morphine withdrawal whereas avoidance, suppression, and defensive behaviors could be viewed as preparatory responses elicited by the conditioned arousal of the aversive motivational/protopathic features of morphine withdrawal. Thus, rather than emphasizing a dichotomy between withdrawal-like and unlike responses, this explanation suggests that differences observed in the behavioral and pain modulatory responses following exposure to a withdrawal-associated stimulus could reflect differences in learning about the sensory versus negative affective properties of withdrawal.

Consistent with this interpretation, there is a similarity between the morphine withdrawal syndrome and the effects of other aversive internal states, such as the illness produced by injection of the bacterial endotoxin lipopolysaccharide or the emetic drug lithium chloride. Some signs of morphine withdrawal are similar to those observed during illness and infection (e.g., nausea, diarrhea, autonomic arousal). Moreover, both supporting taste aversion learning accompanied by shifts in the palatability of the target fluid towards distaste (Pilcher and Stolerman 1967; McDonald et al. 1997). Indeed, human opiate addicts frequently describe drug withdrawal in terms a flu-like sickness. Importantly, the present experiments show that this similarity extends to the production of freezing and opioid receptor-mediated hypoalgesia by associated contextual and olfactory stimuli (McNally et al. 1999).

The tests in these experiments were conducted 7 days after last morphine injection because morphine inhibits the expression of fear (e.g., Davis 1979; Good and Westbrook 1995). It follows that the presence of morphine on test may have obscured any fear-related effects of the withdrawal-associated stimulus. However, this design is potentially confounded by shifts in motivational state between conditioning (morphine-dependent) and test (post-dependent) which are worthy of comment. It is unclear whether this change in motivational state impacted significantly upon the production of freezing. Freezing in these experiments was only observed among rats with a history of paired exposures to the withdrawal-associated stimulus and was observed when rats were tested in the presence, but not absence, of that stimulus. It is possible that the interval between conditioning and test is an important factor in pharmacological conditioning experiments. We predict that it could be difficult to detect fear-related responses in rats currently dependent upon morphine because of the inhibitory effects of morphine on the expression of fear and defensive behaviors. However, given that human opiate users are likely to encounter withdrawal-associated stimuli in the weeks to months following last drug exposure this issue is worth further investigation. It is also possible that the results from experiment 3 were confounded by challenging the rats with naloxone in the absence of morphine. However,

we consider this possibility unlikely because there was no evidence from observation of the animals in experiment 3 that injection of naloxone precipitated characteristic withdrawal-like behaviors. This suggests that the effects of that injection were directly related to an antagonism of activity in antinociceptive circuits.

Finally, the present experiments may provide insights into the neural and pharmacological mechanisms for opiate withdrawal. Specifically, we and others have now shown that stimuli associated with opiate withdrawal elicit species-typical defensive responses (e.g., freezing, defensive burying) and opioid receptor-mediated hypoalgesia similar to those produced by stimuli associated with footshock. The analysis offered here indicates that these common responses reflect the conditioned arousal of the same fear or defensive motivational system. It follows from this analysis that there should be significant overlap in the neural structures (e.g., amygdala, bed nucleus of the stria terminalis) and neurotransmitter systems (e.g., corticotropin-releasing hormone) regulating fear or anxiety and those regulating withdrawal from opiates.

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