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Conditioned Fear Exacerbates Acute Morphine Dependence

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ABRAHAMSEN, G. C., B. J. CALDARONE, H. S. STOCK, A. D. SCHUTZ AND R. A. ROSELLINI. *Conditioned fear exacerbates acute morphine dependence.* PHARMACOL BIOCHEM BEHAV 51(2/3) 407–413, 1995.—A variety of physical stressors have been shown to enhance reactivity to opioid drugs. Few studies have examined the effects of nonphysical stressors on opioid drug reactivity. In this regard, it has previously been shown that animals administered morphine in the presence of shock-associated cues demonstrate increases in hypoalgesia relative to nonshock control animals. These findings have typically been viewed as being mediated by the activation of endogenous pain inhibition systems via conditioned fear. In this series, we further examined the nature of these effects by assessing the effects of conditioned fear on acute morphine dependence. Experiment 1 revealed that animals administered 3 mg/kg morphine in the presence of context fear cues demonstrated an enhanced withdrawal response when removed and administered 3 mg/kg naloxone. Because it is known that conditioning effects do not diminish over time, a second experiment examined whether the enhancement of acute dependence by context fear would still be evident 72 h postconditioning. As in Experiment 1, animals administered morphine in a context associated with shock demonstrated an enhancement of acute dependence. Experiment 2b revealed that the shock parameters used in these studies can induce a hypoalgesic response on the test that is opioid mediated. These findings are discussed with regard to the neuroanatomy of fear systems as they relate to the neuropharmacological study of opioid withdrawal.

Acute dependence Conditioned fear Footshock Withdrawal

IT HAS BEEN well established that rats will display a general decrease in pain sensitivity/reactivity following the exposure to a stressor—stress-induced hypoalgesia. Similar changes in responsivity to a painful stimulus can also be observed following the exposure to contextual (e.g., environmental) cues that have been paired with a stressor (7,15). This phenomenon, referred to as conditioned hypoalgesia, has been argued to be mediated by the induction of a central state of fear by cues associated with a stressor. For example, exposure to a context associated with shock can produce a variety of fear-related behavioral changes including defecation, urination, freezing, and passive avoidance [for examples see (2,3,14,42,56)], whereas anxiolytic compounds will simultaneously decrease both freezing and hypoalgesia (18). Moreover, manipulations of the amygdala, either through the direct administration of anxiolytic drugs (22) or lesions (21), have also been shown to disrupt both defensive freezing and hypoalgesia. These findings are consistent with a fear-based interpretation of conditioned hypoalgesia because it is known that the amygdala is necessary for the establishment and maintenance of a number of defensive CRs thought to be associated with fear or anxiety [see (10) for a review]. To maintain consistency with this literature, we refer to an environmental stimulus complex that was paired with shock as the "conditioned fear context."

A number of reports have implicated endogenous opioid systems in the modulation of conditioned fear-induced hypoalgesia (14,15). Although a variety of experimental considerations have been shown to be critical for observing opioid- or nonopioid-conditioned hypoalgesia [see, for example (31,52)], a number of studies have demonstrated that high doses of the relatively nonspecific opioid antagonists naloxone (51), naltrexone (13,15), or specific antagonists to the mu (16) and delta (17) opioid receptors can attenuate conditioned hypoalgesia.

Consistent with the findings of opioid antagonist reversal of conditioned fear-induced hypoalgesia are the observations of increases in hypoalgesia when morphine is administered in the presence of cues associated with shock (1,41,48). It is

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interesting to note that conditioned fear can enhance hypoalgesic responsivity following the administration of morphine even under testing conditions in which conditioned fear hypoalgesia is not observed (1,48). Such findings begin to suggest that the elicitation of conditioned fear may importantly affect an organism's sensitivity to opioid drugs. Although the above findings aid in our understanding of the factors governing pain-inhibition mechanisms, they also suggest that context fear may influence factors that modulate the motivational liability of opiates. For example, fear-eliciting cues present upon the delivery of an opioid drug may serve to enhance the subsequent withdrawal response that is manifested following the removal of the drug from the system. In this regard it is relevant to note that exposure to physical stressors can enhance the withdrawal response seen following exposure to morphine (47,57). Recent work by Shaham (47) has demonstrated that exposure to restraint stress enhances the oral self-administration and withdrawal to opiates. Furthermore, Williams et al. (57) demonstrated that exposure to 2 days of "learned helplessness" inducing tail shock proactively produced an enhanced withdrawal response when naloxone was administered following the administration of a single dose of morphine. Ostensibly, these effects were due to a sensitization of the endogenous opioid system because the tail shock parameters used in that study have been shown to induce opioid-mediated hypoalgesia and produce cross-sensitizations with morphine (20). However, these studies all employed physical stressors. It remains to be determined whether nonphysical stressors could exert the same effect.

The purpose of the present studies was to examine the capacity of context fear present during the administration of morphine to affect the withdrawal response elicited by the administration of naloxone. It is expected that the naloxone-precipitated opioid withdrawal response will be enhanced following morphine administration simultaneous with placement in a conditioned fear context.

EXPERIMENT 1

The administration of the opioid antagonist naloxone rapidly elicits a variety of withdrawal-related behaviors in animals receiving either long-term exposure to morphine (34,45,53,54) or a single dose of morphine (4,29,44,57). We reasoned that the capacity of conditioned fear to influence opioid withdrawal distress might not be revealed in animals receiving long-term morphine exposure because the high frequency of withdrawal behaviors elicited in these animals might preclude us from observing an effect of conditioned fear. Although many of the same symptoms of opioid withdrawal can be seen following both acute and long-term morphine exposure (e.g., mastication, forepaw tremors, teeth chattering), increasing the amount of dependence has been shown to increase the frequency of these behaviors (53). Therefore, an acute dependence technique was used in these studies to increase the likelihood that any effects of conditioned fear on withdrawal would be detected.

In the present experiment animals from a context fear-conditioning group and an unconditioned group received morphine either in the fear-conditioning context or the separate neutral context. Thus, the design for this experiment was a 2 × 2 with conditioning [footshock (FS) vs. no shock (NS)] and morphine exposure context [shock context (SC) vs. neutral context (NC)] as the two factors. Following the administration of morphine, animals were removed from their respective chambers and were administered naloxone to precipitate acute dependence. Animals were observed for signs of opioid withdrawal over a 15-min interval in a separate, and neutral, testing chamber.

METHOD

Subjects

Forty-eight experimentally naive adult male Sprague–Dawley rats (Blue Spruce, Altamont, NY) served as subjects in this study. They were housed under a 12L : 12D cycle, with the light onset at 0700 h. Subjects were provided with ad lib Purina Rat Chow and water throughout the study. All procedures employed in these studies have been reviewed and approved by the University Animal Welfare Committee.

Apparatus

Four operant chambers were used as the shock conditioning context. Each measured 21.0 × 30.5 × 27.9 cm. The walls were constructed of aluminum and the ceiling and door of clear Plexiglas. The floor consisted of stainless steel rods 3.0 mm in diameter and spaced 1.2 cm apart. A 28 VDC houselight was located 29 cm above the grid floor and was centered on the front wall. Scrambled shock (0.90 mA) could be delivered to the grid floor by solid-state shock sources (Coulbourn Instruments Model 13-16). These boxes were housed in chambers equipped with ventilating fans that also provided background masking noise.

Four different chambers were used as the neutral context. Each measured 30 × 30 × 30 cm. The walls were constructed of aluminum and the ceiling and door of clear Plexiglas. The floor consisted of wire mesh. Importantly, lights and ventilating fans were not present in these chambers. These chambers were housed in a room adjacent to the colony room and separate from the room that housed the chambers that served as the shock context.

An observation chamber was used to test the incidence of withdrawal-related behavior in the animals and was placed in a room separate form the other contexts. This chamber was 39.4 × 39.4 × 30.5 cm and was constructed of clear Plexiglas.

Drugs

Morphine sulfate (Sigma Chemical Co., St. Louis, MO) and naloxone hydrochloride were each dissolved in 0.9% saline solution at concentrations of 10 mg/ml. Injections were administered in a volume of 0.3 ml/kg (3 mg/kg).

Procedure

Animals were habituated to handling and injection procedures for 2 days prior to the beginning of the experiment. In addition, animals were preexposed to the testing apparatus for 5 min during this period. On days 1 and 2, all animals were preexposed for 0.5 h to the neutral context (NC). During days 3 and 4 all animals were placed in the shock context (SC). On each of these days, one-half the animals [group foot shock (FS)] received exposure to 20, 0.9-mA, 5-s foot shocks administered on a RT-90 second schedule (range 60–120 s) whereas the other half were placed in the same chambers for an equivalent time period but did not receive foot shock [group no shock (NS)]. These parameters have previously been shown to condition high levels of fear to a context in our laboratory (41,43). It should be noted that, to minimize the possibility of the generalization of fear from the shock context to the neu-

tral context, different experimenters were used for the context conditioning and the withdrawal test phase. On day 5 (1 day following contextual fear conditioning), one-half the animals were administered morphine (3 mg/kg, SC) and placed in the shock context (groups FS-SC, $n = 12$; NS-SC, $n = 12$), whereas the other half were administered morphine and placed in the neutral context (groups FS-NC, $n = 12$; NS-NC, $n = 12$). None of the groups received shock during this phase. Thirty minutes later, all animals received an injection of naloxone (3 mg/kg, IP) and were placed in the observation chamber and observed for 15 min. An observer blind to treatment condition recorded the frequency of withdrawal behaviors during this period. The behaviors most frequently observed at these doses include bouts of mastication, teeth chattering, and head and body shakes. The frequency of the emission of these behaviors has previously been employed to assess of the severity of opioid withdrawal in rats [see, for example (34)]. Mastication was defined as bouts of vigorous chewing behavior. Teeth chattering refers to bouts of highly audible gnawing and knocking of the teeth often accompanied by facial tremors. A 1-s cessation of the auditory feature of this behavior designated the end of a bout. Body shakes were most frequently observed as instances of vigorous shaking of the front paws and occasional full body or "wet dog shakes." Pilot studies conducted in our laboratory have investigated the effect of morphine dose (either 0, 1, 3, or 5 mg/kg) on withdrawal elicited by a 3-mg/kg dose of naloxone. The results of these studies have shown that the most reliable index of dose–response function using small doses of morphine is the overall incidence of withdrawal-related behavior assessed as the summation of the individual behaviors (withdrawal score = mastication + teeth chattering + body shakes). It should also be noted that there is no difference in the emission of these behaviors between animals administered either saline or 3 mg/kg of naloxone in the absence of morphine. Interrater reliability for the scoring of these behaviors has been demonstrated to be high in our laboratory. Individual assessments of mastication, forepaw tremors, and teeth chattering have yielded respective interrater correlations of 0.84, 0.94, and 0.80 (all $p < 0.001$). (Behaviors associated with high-dose morphine withdrawal are occasionally observed at these doses. These include bouts of swallowing, genital licking, and ptosis. However, due to their infrequent emission and the consequent lower interrater correlation of scoring these behaviors, they were not included in statistical analyses.)

Data Analyses

All data analyses were conducted using analysis of variance (ANOVA). Newman-Keuls post hoc tests ($p < 0.05$) were used to assess the source of significant effects.

RESULTS AND DISCUSSION

Figure 1 shows that animals administered morphine in a context previously associated with shock (group FS-SC) exhibited the largest total opioid withdrawal response relative to shocked animals administered morphine in a neutral context (FS-NC) and nonshocked controls (NS-SC; NS-NC). An ANOVA conducted on these data confirmed this observation. This analysis revealed a significant effect of group, $F(1, 44) = 10.26$, $p < 0.01$, and, more importantly, a significant group × context interaction, $F(1, 44) = 4.98$, $p < 0.05$. Newman-Keuls post hoc tests indicated that the total withdrawal score for group FS-SC was significantly greater than

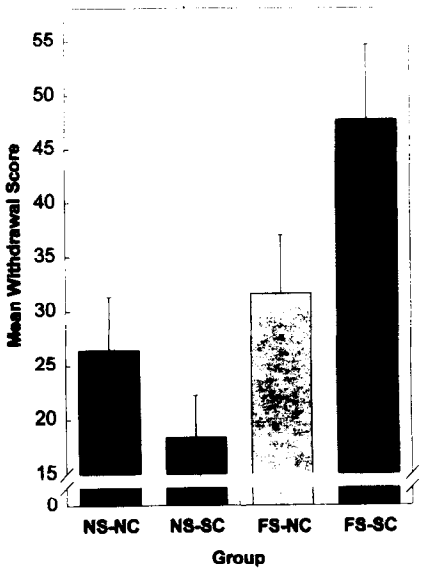

FIG. 1. Mean ± SEM withdrawal score on the naloxone-precipitated morphine withdrawal test in Experiment 1. Animals received fear conditioning to a context (group FS) or context exposure (group NS). Twenty-four hours following conditioning, animals were administered 3 mg/kg morphine either in a context associated with shock (SC) or a neutral context (NC). Thirty minutes later, animals were administered 3 mg/kg naloxone and withdrawal behavior was observed in a neutral testing chamber.

that of each of the other three groups (FS-NC; NS-SC; NS-NC), which did not differ from one other.

These findings suggest that the frequency of withdrawal-related behavior is increased following the administration of morphine in a fear-conditioning context. This result is consistent with the existing literature on stressful events and opioid drug reactivity. In this regard, others have shown that exposure to more extended shock parameters (e.g., 1 or 2 days of 80–100 trials of 1-mA shock), such as those capable of producing helplessness, can enhance morphine responsiveness 24 h following shock exposure (20,51). The fact that previously shocked animals administered morphine in the neutral context (group FS-NC) did not differ from nonshocked controls (NS-SC; NS-NC) implies that this effect is most likely a conditioned fear effect and not an effect of exposure to a shock stressor per se. Therefore, the above findings support the notion that context fear may enhance acute morphine dependence. However, it could also be argued that an enhanced withdrawal response in group FS-SC could be attributable to the interaction of stress and/or sensitized fear systems, and the context fear present during morphine exposure. Previous research has shown that extended exposure to shock stressors can produce a sensitization of fear-related processes demonstrable by an enhancement of shock-induced freezing (32) or neophobia (36) 24 h following shock exposure. Thus, it is possible that context fear might interact with these other effects to exacerbate acutely precipitated withdrawal.

EXPERIMENT 2

It has long been known from the pavlovian conditioning literature that conditioned fear effects are temporally robust and, therefore, in the absence of extinction training, diminish little with the passage of time (24,25). On the other hand,

shock stress effects induced by substantially greater amounts of shock exposure (e.g., 100 trials administered in 1 day) have been shown to decrease relatively rapidly with the passage of time (33,36). For example, exposure to uncontrollable shock is known to sensitize neophobia (36), opioid drug effects (20,57), and fear-conditioning processes (32). It may be possible then that prior exposure to shock in Experiment 1 may have enhanced the modulatory role of context fear. It is relevant to note that the aforementioned sensitizations induced by uncontrollable shock have previously been shown to diminish within a 72-h period (32,33,36). Given this consideration, a second experiment was conducted to replicate the findings observed in Experiment 1 and to assess the effects of an imposition of a 72-h interval between context fear conditioning and the withdrawal test. Prior work in our laboratory (1) has shown that the hypoalgesia observed following the administration of morphine is enhanced in the presence, but not absence, of shock context cues, suggesting that our shock parameters per se do not enhance the subsequent response to morphine. If context fear enhances acute morphine dependence induced by naloxone, it is expected that these effects should be evidenced even with the imposition of a 72-h interval between conditioning and test.

METHOD

Subjects and Apparatus

Forty-one experimentally naive Sprague–Dawley rats were used in Experiment 2. Housing conditions, apparatus, and drugs were identical to those used in Experiment 1.

Procedure

Foot shock exposure and withdrawal testing procedures were identical to those of Experiment 1. The primary difference between Experiments 1 and 2 was the imposition of a 72-h interval between the second day of fear conditioning and the acute morphine dependence test. As in Experiment 1, one of the foot shock groups received morphine in the shock context (group FS-SC) and one other in the neutral context (group FS-NC). Because in Experiment 1 the two NS groups did not differ from each other, a single NS group was used in the present study. One half of this group received morphine in the shock context and the other half received it in the neutral context.

RESULTS AND DISCUSSION

Figure 2 illustrates the results of this study. It can be seen that animals administered morphine in the presence of context cues associated with shock (group FS-SC, $n = 13$) displayed the highest frequency of withdrawal related behaviors relative to the other groups (groups FS-NC, $n = 12$; NS, $n = 16$). These latter two groups did not appear to differ from one another. A one-way ANOVA confirmed these observations in revealing a significant main effect of group, $F(2, 38) = 3.69$, $p < 0.05$. A post hoc contrast revealed that group NS did not differ from group FS-NC, $F(1, 38) = 0.41$, whereas the combination of these groups differed significantly from group FS-SC, $F(1, 38) = 6.66$, $p < 0.05$.

These findings replicate those of the prior study in suggesting that conditioned fear can enhance acutely precipitated morphine withdrawal. Other studies that have utilized more intense shock parameters have shown stress effects including sensitizations of the fear system(s) to diminish over a 48-h interval (32,36). Therefore, in addition to replicating the results of Experiment 1, these data indicate that conditioned fear is a primary mediator of these effects.

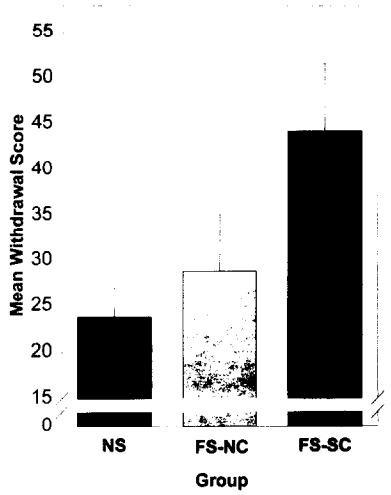

FIG. 2. Mean ± SEM withdrawal score on the naloxone-precipitated morphine withdrawal test in Experiment 2. Seventy-two hours following conditioning, animals were administered 3 mg/kg morphine. Thirty minutes later, animals were administered 3 mg/kg naloxone and withdrawal behavior was observed in a neutral testing chamber. Group FS-SC received morphine in the context in which shock was administered whereas group FS-NC received morphine in a neutral context. Half of group NS received morphine in the shock context whereas the other half received morphine in the neutral context.

EXPERIMENT 2b

The findings of Experiments 1 and 2 indicate that exposure to a fearful context can exacerbate acutely precipitated morphine withdrawal. A likely explanation for this effect is that conditioned fear may sufficiently activate endogenous opioid systems and that this activation may enhance or summate with the effects of morphine. Indeed, a number of studies have shown that conditioned fear can produce hypoalgesia that is attenuated by a variety of opioid antagonists (16,17). Alternatively, other studies have shown that the fear conditioned with more severe stressor parameters can produce hypoalgesic responses that are not modified by the administration of opioid antagonists (31). Thus, we sought to assess whether our context fear-conditioning parameters were sufficient to produce opioid-mediated hypoalgesic effects. Therefore, 1 day following fear conditioning, animals received baseline tail flicks in the colony room and subsequent tail flick tests 10 and 20 min following placement in the shock-conditioning context. If these parameters produce opioid-mediated hypoalgesia, then animals administered saline should display enhancements in tail flick latency relative to their own baseline and relative to animals administered naloxone.

METHOD

Subjects

Fifteen adult male Sprague–Dawley rats served as subjects in this study. All housing conditions were identical to the prior studies.

Apparatus

The foot shock boxes were identical to those used in the prior study. Algesia testing was conducted using a modified tail-flick apparatus (9) that measured 65 × 23 × 11.25 cm. The top of the apparatus measured 1.25 cm in thickness. A Sylvania 150-W projection bulb (Sylvania Inc., Winchester, KY) was mounted 5.6 cm below the top of and was centered 23 cm from the long end of the apparatus. A 1.1-cm diameter hole was centered in the top of the apparatus directly above the light source. The intensity of the light was regulated by use of a variable autotransformer (Staco Inc., Dayton, OH) and was adjusted such that the baseline tail flick response occurred with a latency of about 10 s in naive animals. This apparatus was mounted on a cart so that it could be transported to different experimental rooms as necessary.

Drugs

Naloxone (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline solution at concentrations of 10 mg/ml. Injections were administered IP in a volume of 0.7 ml/kg (7 mg/kg).

Procedure

All animals received context conditioning using the same shock parameters used in the prior studies. Twenty-four hours later, animals received three baseline tail flicks in the colony room. The last two of these flicks were averaged to yield a baseline score. Following baseline, one group of animals was administered saline (group FS-saline, $n = 9$), whereas the other group was administered a 7-mg/kg dose of naloxone (group FS-nalox, $n = 6$). To minimize the number of animals used, and because others have not observed changes in baseline pain sensitivity with similar doses of naloxone (8) or naltrexone (19,31), we did not use a nonshock control group in this study. Approximately 2 min following this injection, all animals were placed in the fear-conditioning context. Each animal was removed from the apparatus and administered one algesia test at 10 (and subsequently returned to the apparatus) and 20 min following placement.

RESULTS AND DISCUSSION

Animals that received saline demonstrated about a twofold increase in tail flick latencies from baseline to the two test times. In contrast, animals administered naloxone did not demonstrate a change in tail flick latency. An ANOVA revealed a significant effect of group, $F(1, 13) = 5.19$, $p < 0.05$, and a significant group × trial interaction, $F(2, 26) = 4.40$, $p < 0.05$, indicating that animals in the saline condition increased their latency to tail flick following placement in the shock context relative to those in the naloxone condition.

These results imply that the fear conditioning parameters used in these studies were sufficient to produce conditioned fear-induced hypoalgesia. Further, this response appears to be mediated by endogenous opioid activity because animals administered the opioid antagonist naloxone did not display hypoalgesia. Parenthetically, we should note that although others (55) have reported decreases in pain sensitivity following repeated exposure to thermal stimulation using the hot plate test, we have not observed similar effects with the tail flick test. Indeed, pilot data from our laboratory and the baseline data from the current experiment indicated there was no detectable change in tail flick latencies across repeated tests. Therefore, the most parsimonious explanation for these data is that it is context conditioned fear that produced decreases in pain reactivity.

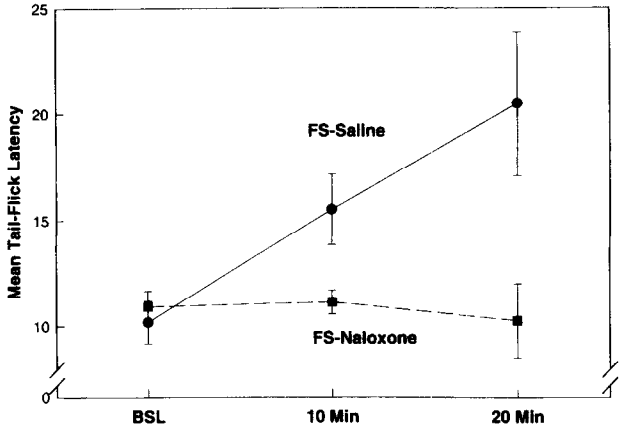

FIG. 3. Mean ± SEM score for the conditioned fear-induced hypoalgesia test in Experiment 2b. All animals received conditioning of fear to a specific context. Twenty-four hours following conditioning, animals received baseline algesic testing in the colony room. Group FS-Nalox received a 7-mg/kg injection of naloxone whereas group FS-Saline received an injection of saline. One minute following injection animals were placed in the shock context and their algesic sensitivity was tested at 10 and 20 min following placement.

GENERAL DISCUSSION

The present experiments imply that the exposure to the opiate morphine in a context associated with shock (but in the absence of a shock stressor) can exacerbate the subsequent withdrawal syndrome precipitated by naloxone. This effect was evidenced both 24 (Experiment 1) and 72 (Experiment 2) h following conditioning. These findings are consistent with previous research, which has demonstrated that context fear can produce opioid-mediated analgesic effects (13,15) and can enhance analgesia when morphine is administered in the presence of shock-associated cues (1,41,48). The present results are the first to document that acute morphine dependence can be enhanced when morphine is administered in the presence of contextual stimuli associated with shock. As noted above, this finding is consistent with other reports that have shown that *physical* stressors can enhance the withdrawal to opioids (47,57). Our findings further suggest that *aversive conditioning* factors can enhance the withdrawal response to opioids. Further, the finding that the shock parameters used in this study can condition a naloxone-reversible hypoalgesic response suggests that endogenously released opioid peptides may interact with exogenous opioids to exacerbate the acute withdrawal syndrome. This conclusion must be entertained with caution because the observation of opioid-mediated effects induced by conditioned fear does not unequivocally imply that endogenously recruited opioids per se exacerbate acute dependence.

The finding that acute morphine dependence is enhanced following the administration of morphine in a context associated with shock is consistent with a number of pharmacological and neuroanatomical reports concerning both opioid withdrawal and conditioned fear effects. The withdrawal from opioids is thought to be a ubiquitous neurobiological phenomenon mediated by numerous neuroanatomical loci (27). Indeed, a prior report has shown that many of the classic

symptoms of opioid withdrawal can be elicited in morphine-dependent animals by the infusion of the hydrophilic opioid antagonist methylnaloxonium into a variety of brain regions (28,34). Among the many brain areas responsive to the withdrawal-inducing properties of methylnaloxonium are the locus coeruleus (LC), amygdala, and periaqueductal gray (PAG), all of which demonstrate light to heavy labeling for tritiated mu-, delta-, and kappa-opioid receptor ligands in autoradiographic studies (35). These brain areas have been implicated in the learning and generation of conditioned fear responses, including opioid-mediated effects. For example, lesions of the amygdala disrupt the acquisition (2,11,26,38) of conditioned fear responses and application of anxiolytics in this area disrupt conditioned fear-induced hypoalgesia (22). Efferents from the central nucleus of the amygdala are thought to transmit fear-relevant information to brain areas that modulate the behavioral expression of conditioned fear effects [see (30)]. One primary efferent is the PAG (12), a region that has long been thought to be implicated in opioid-mediated hypoalgesic effects (5,49). Interestingly, it has been shown that the hypoalgesia induced by conditioned fear is attenuated by the administration of naltrexone in the ventral portions of this region (23). The locus coeruleus, the major source of afferent noradrenergic input in the rat brain, is another region that receives input from the amygdala (50). This region is thought to be a primary mediator of opioid withdrawal (28,34,37) and has also been demonstrated to be responsive to conditioned fear stimuli (6,39). The firing rates of LC cells are known to be increased by exposure to conditioned fear stimuli (39) and also demonstrate increases in firing that parallel the time course of the behavioral symptoms of withdrawal (37,40). It is, then, important to note that cellular activity in this region is also enhanced in the presence of conditioned fear stimuli (39). Therefore, an enhancement of acute dependence by conditioned fear stimuli could possibly be mediated in part by the hyperreactivity of LC cells. In summary, recent behavioral and neurobiological evidence suggests that there is an overlap between the neurobiological substrates underlying opioid withdrawal and conditioned fear. It is possible, then, that increments in opioid withdrawal-related behavior observed following the administration of morphine in a conditioned fear-inducing context may be mediated through one, or all, of these substrates. Specifically, it may be possible that the activation of opioid responsive cells in the amygdala, locus coeruleus, or periaqueductal gray via conditioned fear stimuli may subsequently modify the withdrawal response to morphine induced by naloxone. Additional research must be conducted to assess the validity of this hypothesis.

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