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R.F. Westbrook & J.D. Greeley (Eds.)
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Preface

Research involving the pharmacological manipulation of responsiveness to pain has contributed significantly to our understanding of how endogenous opiate systems operate to modulate the perception of, and reaction to pain. The manipulation of endogenous pain control systems is of particular interest to researchers from a variety of areas: those interested in the control of pain, those interested in adaptive behaviour, and those interested in the mechanisms of opiate tolerance and dependence.

In recent years, investigation of the role of learning processes both in drug tolerance and pain control has combined with physiological and pharmacological research to provide some provocative and influential theories of how the body responds to external factors such as drug administration and exposures to stress. In particular, research on Pavlovian conditioning of responses to drugs has an important role to play in elucidating the adaptive capacity of physiological systems.

Morphine and naloxone are the opioid agents most prominent in the study of both pain modulation and opioid tolerance and dependence. Morphine is employed as the prototypical opioid agonist (a drug which acts on the opioid receptor to produce morphine-like effects) which, at sufficient dosages, induces significant analgesia. In contrast, naloxone is seen as a "pure" opioid antagonist (a drug which blocks the effects of opioids at the receptor level but produces no effect itself) which is capable of blocking the potent opioid effects of morphine, and can itself induce the contrary effect of hyperalgesia (increased sensitivity to pain).

The experiments presented here add to a relatively new body of knowledge which indicates that chronic treatment with the opioid antagonist, naloxone, can activate endogenous pain control systems and produce analgesia in rats. This is a rather paradoxical finding since naloxone is known for its ability to reverse the analgesic effects of opioids and to itself produce hyperalgesia.

There are two general types of endogenous pain control systems, that is, systems within the body which are activated to reduce pain produced by external and internal causes. The best studied of these systems is the endogenous opioid system. As the name indicates, the 'endogenous' opioids are substances which are produced within the bodies of lower animals (such as rats and mice) and in humans which produce effects similar to those produced by the external opiates, morphine and heroin. A number of different endogenous opioids have been identified as well as a number of different opioid receptor types. The opioids, both endogenous and exogenous forms, produce their effects by attaching to receptors in the body. The drugs naloxone and naltrexone prevent the opioids from attaching to the receptor and thereby prevent them from producing their effects. They can also remove other opioids from the receptor and thus reverse their effects. It is thought that naloxone produces hyperalgesia by removing endogenous opioids from their receptors.

In the following studies it is shown that naloxone can produce analgesia in rats and that this analgesic response can be learned such that environmental stimuli that have been associated with the drug's injection can themselves come to elicit analgesia in the absence of the drug. It is also of interest that naloxone does not appear to produce this analgesic effect unless the repeated drug administrations are followed by exposure to a painful stimulus (in this case, exposure to a heated surface; the degree of heat applied is sufficient to be uncomfortable for the rat but not so intense as to produce physical damage). It is suggested that the analgesic effect produced by naloxone is due to its blockade of the endogenous opioids. By preventing the opioids from acting to relieve pain, a second,

non-opioid pain control system is activated. Other research has shown that the endogenous opioid and non-opioid pain control systems seem to interact in this way - when one is active the other is inhibited.

We further assessed our hypothesis that the analgesic effect we were seeing in naloxone-treated rats was non-opioid in nature by conducting two standard assays for opioid involvement in a behaviour: blockade by naloxone and cross-tolerance with morphine. As stated earlier, endogenous opioids are blocked by naloxone, thus if a response cannot be reversed or blocked by naloxone, there is a reasonable chance that the response is not produced by an opioid mechanism. Second, opioid-mediated responses, especially those controlled by a particular type of receptor known as the *mu* receptor tend to show cross-tolerance with morphine. Tolerance to morphine is the reduction in effect of a given dose of morphine seen after repeated administrations of the drug. Cross-tolerance, as the name suggests, describes the phenomenon whereby if an organism is tolerant to the effects of one drug, it will also show tolerance to similar effects of similar-acting drugs. It has been demonstrated by other researchers that cross-tolerance exists between endogenous opioids and morphine. Since neither of these two criteria were met by the rats who acquired naloxone-induced analgesia, we reasoned that the analgesic effect we were seeing was mediated by a non-opioid mechanism. Furthermore, the analgesia acquired over repeated naloxone dosings in no way diminished the analgesic effect of an initial dose of morphine, but rather summated with this effect to produce a "superanalgesia".

By this stage, you may be saying, "Well this is all very interesting, but what does it have to do with drug dependence?" One example of the possible implications of these findings is in explaining certain types of drug interactions. For example, prior experience with one drug may influence an animal's or a person's reactions to other drugs. Take the case of polydrug abuse. It may be that if a person is very experienced with the effects of a depressant drug such as alcohol and he or she takes an opposite-acting stimulant drug such as cocaine in the place where alcohol is expected, the impact of the dose of cocaine may be increased. Such a finding has been reported for rats expecting pentobarbital, a barbiturate, and given cocaine (Hinson, Poulos & Cappell, 1982).

Drug tolerance is thought to result from adaptation to drug-induced changes in physiological states and behaviour. One model that has been proposed to explain these adaptive changes is the acquisition of compensatory or drug-opposite responding through Pavlovian conditioning (Siegel, 1983). The elicitation of compensatory responses while a drug is in the body acts to counteract the drug's effect and will result in tolerance (i.e., a reduction in the observed drug effect). However, the occurrence of these responses in the absence of drug administration might be perceived as withdrawal, a defining feature of physical dependence. It has been postulated that the elicitation of withdrawal-like symptoms in the presence of environmental stimuli previously associated with drug-taking may result in "craving", which, in turn, mediates further drug-taking. Extinction of these conditionally elicited "withdrawal symptoms" has been proposed as an important consideration in the effective treatment of drug dependence (Wikler, 1980).

The results from the experiments in which naloxone was employed as the unconditional stimulus (UCS) indicate that it is possible to establish conditional responses which mimic the effects of opioids. It is possible that these responses may effectively counteract the responses elicited in withdrawal. It would seem more efficacious to acquire a response opposite to that which is thought to promote "craving" than to simply extinguish that response. If opioid-like responses other than analgesia can be induced by naloxone, then it may be possible to effectively prevent the occurrence of their opposite counterparts in withdrawal through conditioning.

Wikler (1980) has recommended the use of naltrexone (a long-acting opioid antagonist) in the treatment of opioid dependence to facilitate

extinction. According to Wikler's view, in the presence of naltrexone, opioid administration will be devoid of its reinforcing effects. Through repeated failed attempts to get "high" on opioids while under naltrexone, extinction of cue-elicited craving should occur as well as extinction of drug-acquisitive and drug-taking behaviours.

The work of Greeley, Poulos and Cappell (submitted) suggests a further benefit of antagonist treatment - the possible acquisition of responses which may counteract withdrawal. Other evidence which supports this hypothesis comes from experiments in which naloxone and naltrexone have been used to facilitate withdrawal from methadone (Charney et al., 1982; Riordan & Kleber, 1980). The withdrawal syndrome which accompanies abstinence from long-term methadone use encompasses an extended period of discomfort. It is the duration of this syndrome rather than its intensity that often leads to resumption of opioid use. Clinical studies have shown that the withdrawal period induced by abstinence from methadone can be decreased by chronic administration of naloxone or naltrexone (Charney et al., 1982; Riordan & Kleber, 1980). Although the intensity of the symptoms may be increased, the period over which they occur is significantly reduced. Concomitant administration of the -adrenergic agonist clonidine alleviates some of the withdrawal symptoms without provoking dependence itself.

The faster recovery under antagonist-precipitated withdrawal suggests that naloxone or naltrexone may enhance recovery of the endogenous systems which have been inhibited by repeated opioid administration. In addition to facilitating extinction of conditionally acquired responses, opioid antagonists may elicit their own adaptive responses which counter the withdrawal syndrome and enhance recovery to a drug-free, withdrawal-free state. These conclusions are speculative and require further research.

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General Introduction

The observation that awake rats were rendered analgesic through electrical stimulation of the medial brain stem (Mayer, Wolfe, Akil, Carder & Liebeskind, 1971; Reynolds, 1969) combined with the subsequent discovery of endorphins (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) to suggest that there was an opioid-based mechanism in the brain which modulated pain by descending influences on the spinal cord (cf. Mayer & Watkins, 1981). A role for this system in mediating an organism's interactions with environmental stimuli was provided by the demonstrations that a variety of stressors could produce an analgesia which was cross-tolerant with morphine and reversed by the opioid antagonist naloxone (e.g., Akil, Madden, Patrick & Barchas, 1976; Hayes, Bennet, Newlon & Mayer, 1978).

However, the application of these assays for an opioid involvement in stress-induced analgesia has revealed inconsistent findings. Depending upon their severity, duration and whether or not they are controllable, stressors, such as foot shock, have also been observed to cause an analgesia which is not cross-tolerant with morphine nor reversed by naloxone (e.g., Lewis, Cannon & Liebeskind, 1980; Maier, Drugan & Grau, 1982; Terman, Shavit, Lewis, Cannon & Liebeskind, 1984). Consequently, several investigators have proposed the existence of both opioid and non-opioid mechanisms of pain control (cf. Watkins & Mayer, 1982a).

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This proposal concerning the endogenous pain control system leads naturally to the question of how its opioid and non-opioid components are related to each other. One of the possible answers to this question has been that of collateral inhibition: that is to say, activation of one component by a stressor is assumed to inhibit the other (Akil & Watson, 1980; Kirchgessner, Bodnar & Pasternak, 1982). An interesting property of this hypothesis is its ability to explain the analgesia which accrues in rats exposed to pairings of naloxone and a heat stressor. This analgesia has been observed when the opioid receptors were blocked either intermittently, by administrations of the drug (Rochford & Stewart, 1987), or chronically, by an implanted pellet containing an opioid antagonist (Greeley, Le, Poulos & Cappell, 1988). This analgesia is apparently paradoxical, since naloxone is an antagonist whose lack of agonist properties (Blumberg, Dayton, George & Rapaport, 1961; Blumberg, Dayton & Woolf, 1966) has recommended its extensive use in behavioural as well as simple tissue and single neuron studies of opiate and enkephalin functions (e.g., Hill, 1981). Why, then, should animals become more analgesic in the presence of a drug whose occupation of opioid receptors diminishes or precludes an opioid contribution to the stress-induced analgesia?

In order to account for naloxone-induced analgesia, the collateral inhibition model needs to assume that exposure to the heat stressor provoked the release of endogenous opioids, whose analgesic action normally inhibits the recruitment of the non-opioid component of the endogenous pain control system. However, naloxone's occupation of receptor sites would serve to block any opioid analgesia and thus remove its inhibitory influence upon non-opioid forms of analgesia. According to this hypothesis, therefore, pairings of naloxone with the stressor came to produce a non-opioid form of analgesia, because the drug freed this com-

ponent of the endogenous pain control system from opioid inhibition.

A further characteristic of the endogenous pain control system and of its response to naloxone concerns its recruitment by conditioning processes. Several investigators (e.g., Chance & Rosecrans, 1979a & b; Fanselow, 1986; Watkins, Cobelli & Mayer, 1982) have observed a decreased sensitivity to a stressor, when animals have been exposed to a signal for pain or to the place in which they had previously encountered nociceptive stimulation, so-called conditioned "autoanalgesia" (Chance, 1980). Moreover, since the analgesia thus observed to a classically conditioned stimulus (CS) can be reversed by naloxone, even when the analgesia produced by the unconditioned nociceptive stimulus (US) has been naloxone-resistant, Watkins and Mayer (1982b) have suggested that conditioned autoanalgesia is always opioid-mediated (but cf. Chance, 1980). This suggestion has been extended by Rochford and Stewart (1987) to account for their finding of contextually controlled analgesia, when rats were administered with saline and tested in the place where they had been repeatedly exposed to naloxone-stressor pairings. Although allowing that the analgesia observed in the presence of the drug may have been non-opioid in nature, these investigators argued that the conditioned analgesia may have been opioid mediated. The basis for this argument is the hypothesis that the stressor unconditionally activated the opioid system, whose analgesic effect, of course, was blocked by the action of naloxone. Nevertheless, conditioning would have imbued antecedent cues with the ability to trigger just that system and its analgesic effect would have been detected in the absence of naloxone. Such contextual control over opioid release would constitute a further example of Pavlovian conditioning of responses whose unconditioned expression had been prevented by some means or other (cf. Eikelboom & Stewart, 1982).

The general aim of the present experiments was to provide a further investigation of naloxone-induced analgesia. More specifically, they were aimed at three questions: first, what are the conditions under which the effect occurs?; second, does naloxone interact with conditioning processes to provoke an opioid analgesia in the absence of the drug?; third, what is the relation between the conditioned analgesia occasioned by naloxone and opiate analgesia?

Experiment 1

The present experiment had three aims. The first of these was to document the analgesic effects produced by repeated pairings of naloxone and a heat stressor (Greeley et al., 1988; Rochford & Stewart, 1987). The second aim was to determine the dose-response relation between the acquisition of analgesia and naloxone across a wider range of doses than that used previously (Rochford & Stewart, Experiment 1, 1987). The final aim of the experiment was to examine the persistence of the analgesic reaction to the stressor when the naloxone was discontinued and replaced with administrations of saline.

Method

Subjects. The subjects were 80 experimentally naive, male Wistar rats with an average body weight of 350 g. They were obtained from the colony maintained by the University of New South Wales and were housed in plastic boxes (65 cm x 40 cm x 22 cm) across the course of the experiment. There were five rats to a box and food and water were continuously available. The boxes were kept in a colony room that was maintained on a 12:12 h, light:dark cycle.

Apparatus. The hot plate apparatus consisted of a 24 cm x 48 cm (diameter x height) Plexiglas chamber with a copper floor (1 mm thick) affixed 12 cm above the base of the chamber. The portion of the chamber below the copper floor was perforated with 3 cm diameter holes to permit the circulation of water under the copper floor. The chamber stood in a water bath whose temperature was maintained at 52°C (+ or - 0.5°C) by a Haake D1 Immersion/Open Bath Circulator. The apparatus was located in a laboratory adjacent to the colony room. The laboratory was illuminated by fluorescent lights

on the ceiling. Naloxone hydrochloride or saline was administered by subcutaneous (sc) injection into the dorsal area of the neck. The naloxone was dissolved in 0.9% saline and the volume injected was 1.0 ml/kg. Saline injection consisted in the administration of an equivalent volume of 0.9% saline.

Procedure. The rats were handled and weighed on each of four successive days. They were then assigned randomly to a saline control condition (N=20) or to one of six naloxone-treated groups (N=10 per group). These groups differed in the naloxone dose which they received: 0.07, 0.15, 0.63, 1.25, 2.5, or 5.0 mg/kg. On each of six training days, the rats were weighed and transported in their boxes to the laboratory. Fifteen minutes after arrival, they were given the appropriate dose of naloxone or saline. Fifteen minutes after the injection, each rat was placed for 30 sec on the hot plate. Two observers, one of whom was unaware of the subject's group designation, used push-buttons connected to a microprocessor to record the latencies with which the animal licked its paws (front and back). If the animal failed to lick its paws, then latencies of 30 secs were recorded. On days 7-12, all of the subjects were administered with saline and tested on the hot plate in the manner described.

Results and discussion

For statistical purposes as well as convenience of exposition, the subjects who received adjacent doses of naloxone were combined together so as to yield 3 naloxone-treated groups (N=20 per

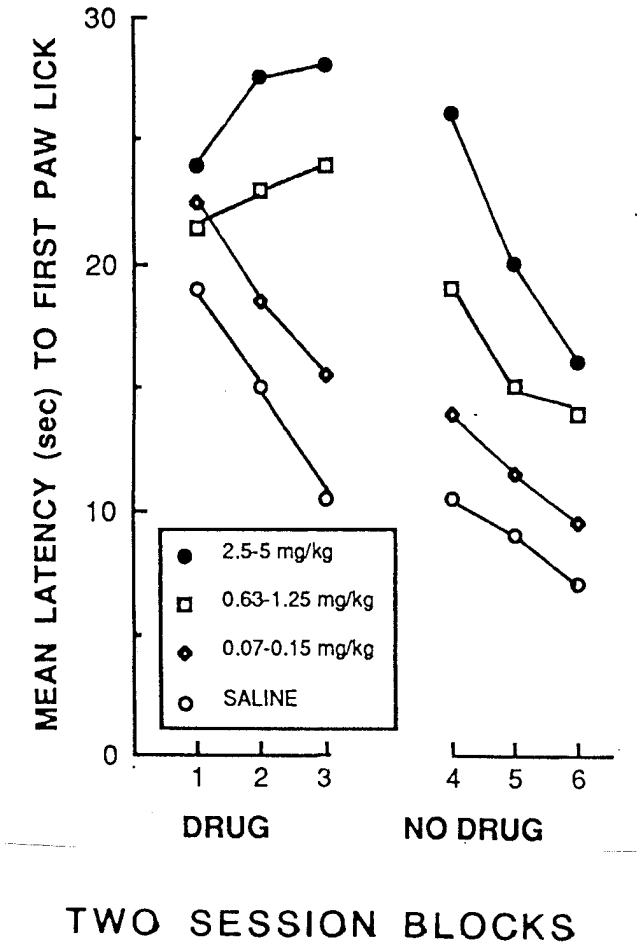

Figure 1. Mean latency to first paw -lick across blocks of two sessions for naloxone doses and saline in Experiment 1.

group). Further, a preliminary inspection failed to reveal any differences in the latencies to lick the front or the back paws. Accordingly, in order to conserve statistical power, a single paw-lick latency was obtained from each subject. This consisted in the latency to the first paw-lick, either front or back. The latencies recorded by each of the two observers were averaged, but they rarely differed by more than 0.5 sec and there was virtually complete agreement about the occurrence of the designated responses.

Figure 1 shows the mean latencies to the first paw lick for each of the three experimental groups and for the control condition. An inspection of this figure suggests that naloxone administration came to enhance the latencies to the first paw lick relative to saline injections and that the drug did so in a dose-dependent manner. Further, this drug-induced enhancement appeared to be long-lasting, since the previously drugged rats continued to display longer paw-lick latencies when given saline, although, to be sure, these differences were reduced by the end of training.

The critical value of F (df=1,76, α =0.025) using Hays' technique is 5.25 (Hays, 1972). There were significant differences between paw-lick latencies in the saline- and naloxone-treated groups, F=40.07, and among the naloxone-treated groups, F=23.72 (groups 0.07 and 0.15mg/kg vs groups 0.63, 1.25, 2.5 and 5.0 mg/kg) and F=7.3 (groups 0.63 and 1.25 mg/kg vs 2.5 and 5.0 mg/kg). There were also reliable interactions between these contrasts and quadratic trend, F=19.51 (trend x saline vs naloxone) and F=10.8 (trend x groups 0.07 and 0.15 mg/kg vs groups 0.63, 1.25, 2.5 and 5.0 mg/kg). From inspection of the figure, these interactions confirm that the higher doses of naloxone (0.63 - 5.0 mg/kg) increased the latency to paw-lick and that the substitution of saline for naloxone caused these latencies to decrease.

The present results confirm previous reports that repeated pairings of naloxone and a heat stressor progressively produce analgesia in rats (Greeley et al, 1988; Rochford & Stewart, 1987) and that long paw-lick latencies were maintained when saline was substituted for the drug (Rochford & Stewart, 1987). However, the finding that this analgesia was a function of the dose of naloxone, contradicts the results of a previous experiment by Rochford and Stewart (Experiment 1, 1987). They failed to see an analgesic effect of naloxone when it was administered at doses of 0.5 and 2.0 mg/kg. The discrepancy between their findings and those reported here may be attributed to any number of differences in experimental procedure: drug dosage, time from injection to hot plate test, illumination of the test room, or hot plate temperature. Any attempt to account for these differing results, based on what is currently known about the phenomenon of naloxone-induced analgesia would be entirely speculative. Later experiments in this series, however, may shed some light upon this issue.

Experiment 2

The previous experiment provided evidence that the latencies with which rats licked their paws in response to a heat stressor were positively related to the dose of naloxone. The present experiment constitutes a further examination of this relation by manipulating the temporal interval between administration of the drug and exposure to the stressor. In Experiment 1, doses of 5 mg/kg of naloxone produced greater analgesic reactions to the stressor than doses of 1.25 mg/kg or 0.15 mg/kg. Since estimates of the half-life of naloxone in the blood and brain range between 20 and 35 min (Ngai, Berkowitz, Hempstead & Spector, 1976; Weinstein, Pfeffer & Schor, 1974), the administration of 5 mg/kg should yield functional doses of 1.25 mg/kg and 0.15 mg/kg after approximately 75 and 135 min. In the present experiment, animals were injected with 5 mg/kg of naloxone and were then tested on the heat stressor after 15, 75 or 135 min, in order to determine whether the level of analgesia was related to the functional dose of the drug at the time of exposure to the heat stressor. A further set of rats was injected with saline and exposed to the stressor after comparable intervals of time.

Method

Subjects and apparatus. The subjects were 60 experimentally naive, male Wistar rats with an average weight of 345 g. The animals were

obtained from the same source and kept under the conditions described in Experiment 1.

Procedure. The rats were weighed and handled across 4 days and were assigned randomly to six groups (N=10 per group). On 12 successive days, the animals were transported to the laboratory and injected (sc) 15 min later with 5 mg/kg of naloxone or an equivalent volume of saline. Each rat was then placed for 30 sec on the copper plate whose surrounding water temperature was 52°C. Since there was no difference between front and back paw-lick latencies in Experiment 1, only latency to the first paw-lick, either front or back, was measured in this test. The interval of time between injection and placement on the hot plate was varied across the groups such that one naloxone-treated and one saline-treated group was tested at each interval. These intervals were 15, 75, and 135 min.

Results and discussion

A preliminary inspection of the three groups of subjects given saline failed to reveal any differences on their latencies to paw-lick. Accordingly, the data from these subjects were combined into a single group for convenience of exposition and for statistical purposes. Figure 2 shows the mean latencies to the first paw-lick for the three naloxone-treated groups and for the saline control group. An inspection of this figure indicates that subjects injected with saline progressively decreased the latencies with which they paw-licked, as did those which were administered with naloxone and placed on the hot plate 135 min later. In contrast, the animals exposed to the shorter intervals between naloxone and the heat stressor progressively increased the latencies with which they paw-licked. Further, this increase appeared to be maintained in the subjects exposed to the 15-min interval, but not in those tested 75 min after naloxone administrations.

The critical value of F (df=1,56, α =0.025) is 5.3. There was a significant difference between paw-lick latencies in the saline- and naloxone-treated groups, F=126.63, and a significant interaction with the linear component of sessions, F=20.1.

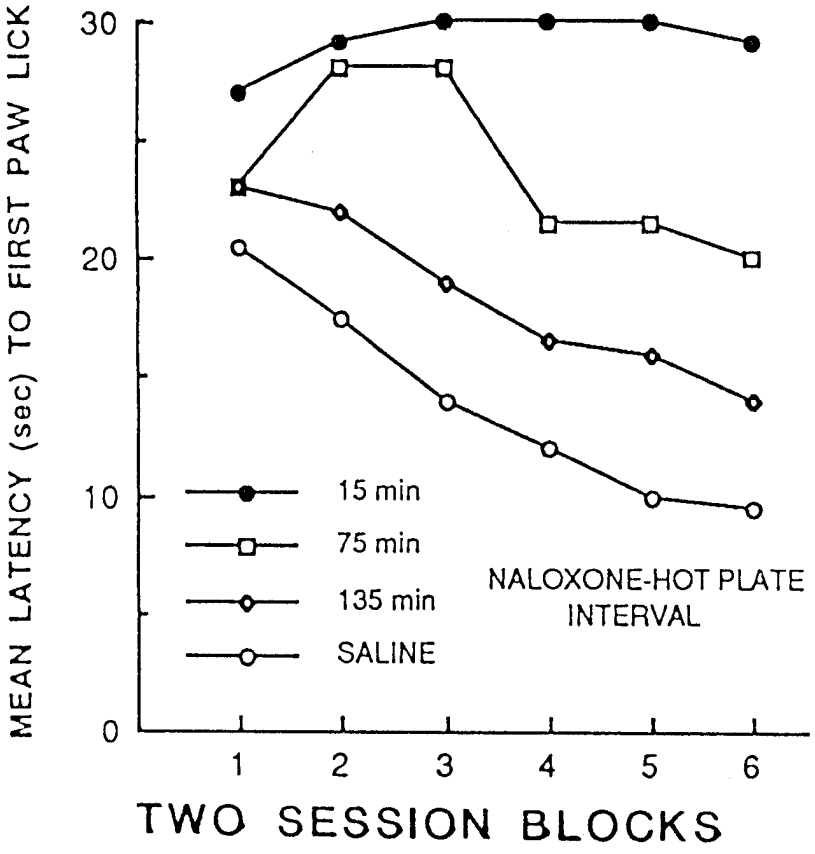

Figure 2. Mean latency to paw-lick across blocks of two sessions for naloxone-stressor intervals and for saline in Experiment 2.

There were also significant differences among the naloxone-treated groups, F=52.8 (15- and 75-min intervals vs 135-min) and F=11.5 (15-min vs 75-min intervals). The latencies of naloxone-treated groups also showed a significant interaction between linear trend and the differences between the shorter (15-min and 75-min) and longest (135-min) intervals, F=22.5. These interactions confirm that unless rats were treated with naloxone and tested shortly after injections, they displayed progressively faster paw-lick latencies over repeated hot plate exposures.

The present results confirm that naloxone exerts a dose-dependent effect upon the latencies with which rats licked their paws when subsequently exposed to a stressor. Further, since these differences were observed after the administration of the same dose of naloxone, the analgesic effects observed in this and in the previous experiment cannot be attributed to some non-specific effect that occurred from the rats having been treated with the drug *per se*. Rather, as was predicted on the basis of the half-life of naloxone (Ngai et al., 1976; Weinstein et al., 1974), the analgesia appears to be due to the concentration of the drug which is present at the time of exposure to the stressor.

Experiment 3

The results of the previous experiments have shown that rats acquire an analgesic reaction when repeatedly exposed to pairings of naloxone and a heat stressor. Further, once established, this reaction to the heat stressor persisted when saline was substituted for naloxone in Experiment 1. Such a result combines with those reported by other investigators (Greeley et al., 1988; Rochford & Stewart, 1987) to suggest a role for conditioning processes in modulating the effects of naloxone upon the animal's reactions to the heat stressor. The present experiment used a within-subject design to determine whether rats were selectively analgesic when tested in the place where they had been exposed to pairings of the naloxone and the heat stressor.

Although naloxone reduced the tendency for rats to lick their paws it was noted that it did not decrease their level of activity on the hot plate. Unlike rats who are analgesic following a dose of morphine, naloxone-treated rats tended to move about and rear shortly after being placed on the hot plate. Other studies reporting an increase in paw-lick latency over repeated naloxone administrations (Greeley et al., 1988; Rochford & Stewart, 1987) have not assessed other behaviours of rats on the hot plate. It is important to examine these other behaviours as they may have an impact on the dependent variable of interest. In the following experiment latency to the first rear was recorded as a measure of the rats' general activity on the hot plate. From casual observation rearing was found to be a common and readily identifiable response exhibited by rats when placed on the hot plate apparatus. It also appeared to discriminate between drugged and nondrugged groups.

Method

Subjects. The subjects were 40 experimentally-naive, male Wistar rats, with an average weight of 350 g. They were obtained from the same source and kept under the conditions described previously.

Apparatus. Two distinctive environments (E1 and E2) were used as conditioned stimuli. E1 consisted in 10 plastic buckets (26 cm x 26 cm, diameter x height) with air holes drilled in the lid and in the sides and whose floors were layered with tissue paper to a depth of 1 cm. This paper was changed before each session and then sprayed with 2 ml of almond essence. E2 consisted in 10 wooden chambers (30 cm x 30 cm x 30 cm) with wire-mesh floors. The litter tray below the wire-mesh was filled with sawdust to a depth of 1 cm and sprayed with 2 ml of coconut essence before each session. The buckets and chambers were located in two different rooms in the laboratory. The hot plate was moved from room to room as required.

Procedure.

Training phase. The animals were weighed and handled for 4 days and were then randomly assigned to two groups (N=20 per group). These

groups differed in terms of the environments correlated with naloxone and saline. Each day, the rats were taken from the colony room to the laboratory where training and testing took place. Subjects in one of the groups (Group E1 N /E2 S) were placed in E1 for 15 min, removed and injected sc with 5 mg/kg of naloxone, replaced in E1 and were then exposed 15 min later to a 52°C hot plate for 30 sec. On other days, these animals were put into E2 for 15 min, removed and injected sc with saline, returned to E2 and were then exposed 15 min later to the hot plate for 30 sec. Subjects in the second group (Group E1 S/E2 N) received naloxone-stressor pairings in E2 on some days and saline-stressor pairings in E1 on others. The intervals between placement in the environments, injections and exposures to the hot plate were those described for the first group. Whether training trials were in E1 or in E2 on any given day was randomly determined across the 20 training sessions, with the constraint that an equal number of trials were conducted in the two environments. Latency to the first paw-lick and to the first rear were the dependent variables. A rear was defined as the rat removing both front paws off the surface of the copper plate.

Test phase. At the end of discrimination training, the animals were tested. One half (N=10) of the rats from each of groups E1 N/E2 S and E1 S/E2 N were placed in the environment that had been paired with naloxone injections and were given either naloxone ("expected") or saline ("unexpected"). The remainder of the rats in each group were placed in the environment where they had previously received saline injections and were given either naloxone ("unexpected") or saline ("expected"). The intervals of time between exposure to the environment, injection and subsequent hot plate test were the same as those described for the training phase.

Results and discussion

Since a preliminary inspection of the data failed to reveal any effect of the environment per se, the data for the two groups across training were collapsed into a single group. The mean latencies to paw-lick for blocks of two naloxone and of two saline sessions across the 20 days of discrimination training are shown in the left panel of Figure 3, while the mean latencies to rear on these sessions are shown in the right panel.

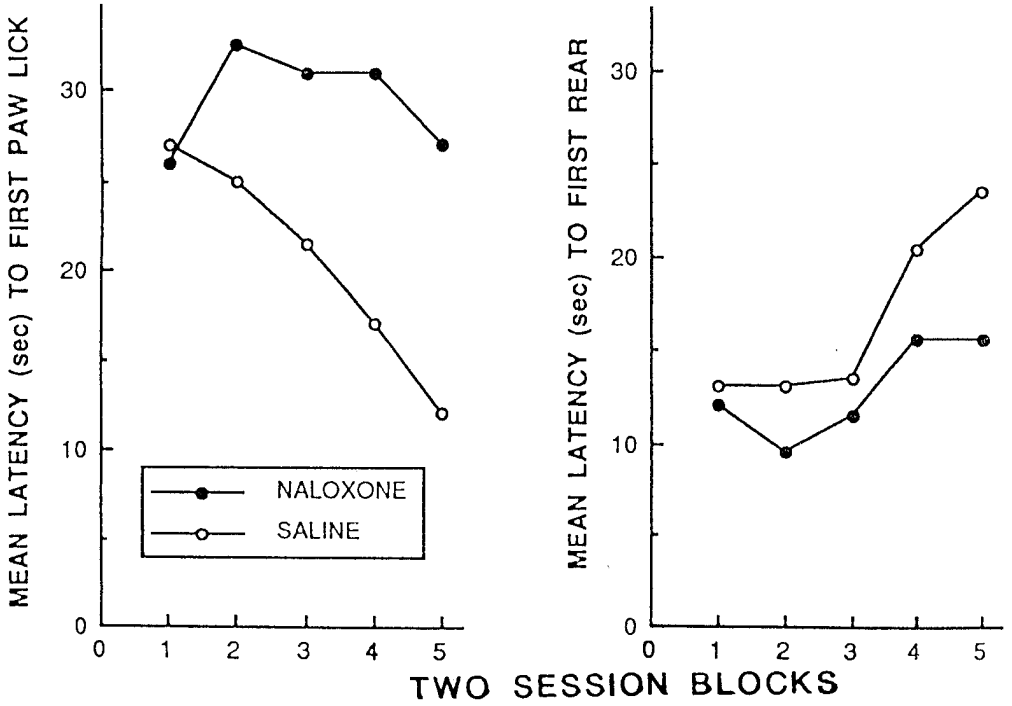

Figure 3. Mean latencies to paw-lick (left panel) and to rear (right panel) across blocks of two naloxone and saline sessions during discrimination training in Experiment 3.

An inspection of the left panel of this figure indicates that subjects maintained long latencies to paw-lick when treated with naloxone, but progressively decreased these latencies across saline sessions. The figure also suggests that this difference was reversed on the rear, since the rats persistently reared with shorter latencies when given naloxone than when treated with saline. These observations concerning paw-licking and rearing were confirmed by the analysis, which showed that there were statistically reliable differences between naloxone and saline sessions on the latencies to paw-lick, F=57.0, and to rear, to form the four major groups. The mean latencies to paw-lick and to rear for each of these groups are shown in the left and right panels, respectively, of Figure 4.

It is clear that the subjects who were tested after exposure to the place in which they had received naloxone-stressor pairings took longer to paw-lick but less time to rear than those tested in the place where they had been given saline-stressor pairings. Further, when the subjects were tested in the place associated with naloxone-stressor pairings, those given the drug took longer to paw-

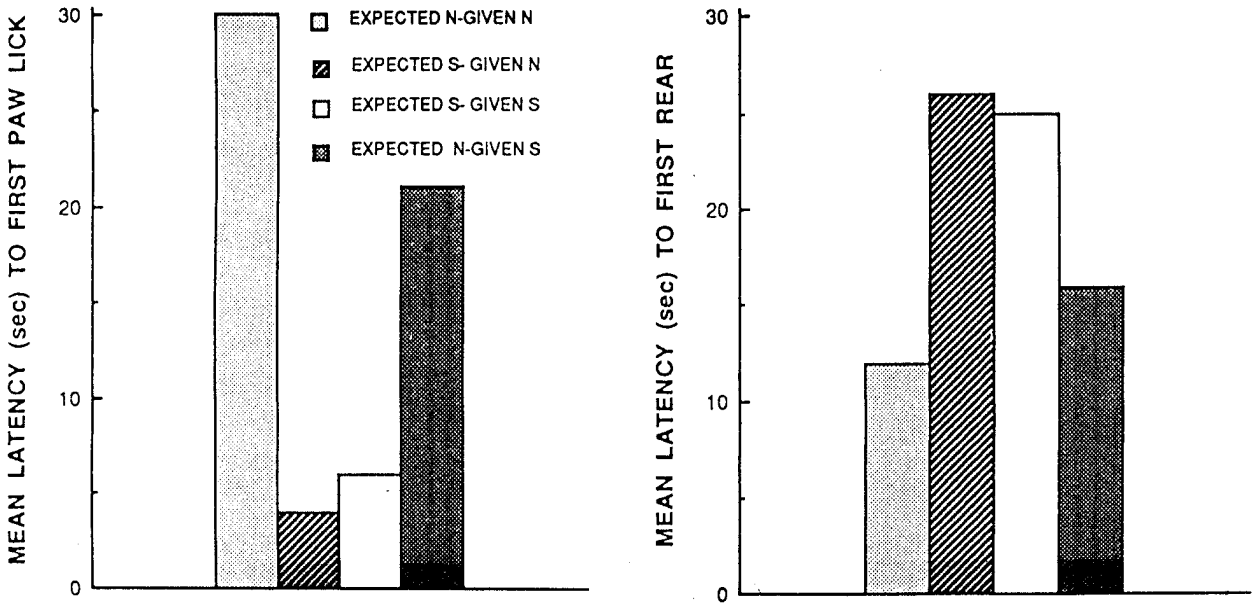

Figure 4. Mean latencies to paw-lick (left panel) and to rear (right panel) on test in Experiment 3. Separate groups of subjects received naloxone or saline either in theplace associated with naloxone-stressor pairings or in the place associated with saline-stressor pairings.

F=13.2. Although they were not licking their paws as rapidly as control rats, naloxone-treated rats initiated rearing with a greater rapidity than did saline-treated controls. The significance of the rearing response will be considered in the next experiment.

A preliminary examination of the test results again failed to reveal any effects of counterbalancing across the environments. Accordingly, the data for each of the sub-groups were collapsed lick than the ones who received saline. In contrast, when the animals were tested in the place associated with saline-stressor pairings, subjects given naloxone took marginally less time to paw-lick than those who received saline.

The critical value of F (df=3,36, α =0.025) using Scheffe is 10.5. There were significant differences between paw-lick latencies in the groups which received the drug, F=77.4, and in the groups which received saline, F=26.9. The difference

between the subjects who "expected" and received naloxone and those who "expected" the drug and received saline approached the critical value, F=10.3, while the difference between those who "expected" and received saline and those who "expected" saline and received naloxone was F<1. There were also significant differences between rearing latencies in the groups which received the drug, F=23.7, and in the groups which received saline, F=16.3. There were no significant differences in rearing latencies between groups given saline or naloxone and tested where they "expected" the drug, F<1, or between groups given saline or naloxone and tested where they "expected" saline, F<1.

The results of this experiment have documented that conditioning processes modulate the effects of naloxone's action upon the rat's reaction to a heat stressor. In the absence of contextual cues associated with naloxone, administration of the drug produced latencies to paw-lick and to rear which were similar to those occasioned by saline. Further, in the absence of the drug, the contextual cues associated with naloxone were capable of supporting longer latencies to paw-lick and shorter latencies to rear than those produced by saline. Although the present experiment provided a single test of the ability of the context to support a conditioned analgesic reaction, the comparable results in Experiment 1 suggest that the context maintains this ability across several extinction sessions.

Experiment 4

The previous experiments have documented that naloxone-treated rats eventually take longer to paw-lick in response to a heat stressor than animals given saline. In those experiments, the development of this analgesia was assessed across repeated pairings of naloxone and the stressor. Consequently, their design confounded the use of the stressor as an assessor of naloxone's analgesic properties and the role which the stressor may have played in mediating the analgesic effects of the drug. This confounding was also seen in a number of the experiments by Rochford and Stewart (1987). In an experiment in which they attempted to disentangle the analgesic effects of naloxone *per se* from that contributed by novelty and heat stress, Rochford and Stewart (Experiment 5) found that contingent exposure to a heat stressor, "contributes to naloxone-induced analgesia" (p.98), but is not essential to the development of the effect. Even rats exposed to an ambient plate after naloxone showed analgesia. Although the between groups design of this experiment did allow an evaluation of drug-stress interactions, it did not afford all groups equivalent experience with the test apparatus (i.e., the hot plate) prior to the test. Lack of experience with the hot plate may have affected the outcome independently of whether the rats received drug-stressor pairings.

In the present experiment a discrimination procedure was employed in which all groups received equivalent experience with the hot and ambient plates as well as with naloxone and saline injections prior to the test. For one group the drug was paired with the heat stressor, while for the others hot plate exposure followed saline injections.

Experiment 3 showed that naloxone-treated rats tended to rear quite soon after placement on the hot plate. In other studies, where heat stress has not been employed, researchers have reported that naloxone reduces exploratory (File, 1980) and motor activity (Amir, Solomon & Amit, 1979) in rats who have been exposed to a novel environment. The following experiment thus allowed a comparison between the effects of naloxone on the rats' motor activation through an examination of the rearing latency both in the presence and in the absence of pain.

Method

Subjects and apparatus. The subjects were 30 experimentally-naive, male Wistar rats with an average weight of 350 g. The rats were obtained from the colony maintained by the University and were housed, fed and watered in the manner described in Experiment 1. The apparatus used was that described in Experiment 3.

Design. The design of the experiment is presented in Table 1.

Table 1
Design for Experiment 4.

	Environment		
	E1	E2	HOME
Condition	N HOT	S AMBIENT	S
	N AMBIENT	S HOT	S
	S HOT	S AMBIENT	N

Procedure. The rats were weighed and handled for 4 days and then randomly assigned to three groups (N=10 per group). Every 3 days, subjects in Group E1 N HOT, E2 S AMBIENT, S HOME, were placed in E1 and injected sc with naloxone (5 mg/kg) 15 min later. Fifteen minutes after the injection, each rat was placed for 30 sec on a plate whose surrounding water temperature was 52°C (termed HOT). On the other two days, these animals were either injected with saline in their home cages or were placed in E2 and injected with saline 15 min later. Fifteen minutes after the injection, the rats were placed on a plate, whose water temperature was maintained at 22°C (termed AMBIENT). Subjects in the other two groups were likewise placed in E2, every third day, injected with saline and exposed to the HOT plate. On the other two days, subjects in Group E2 S

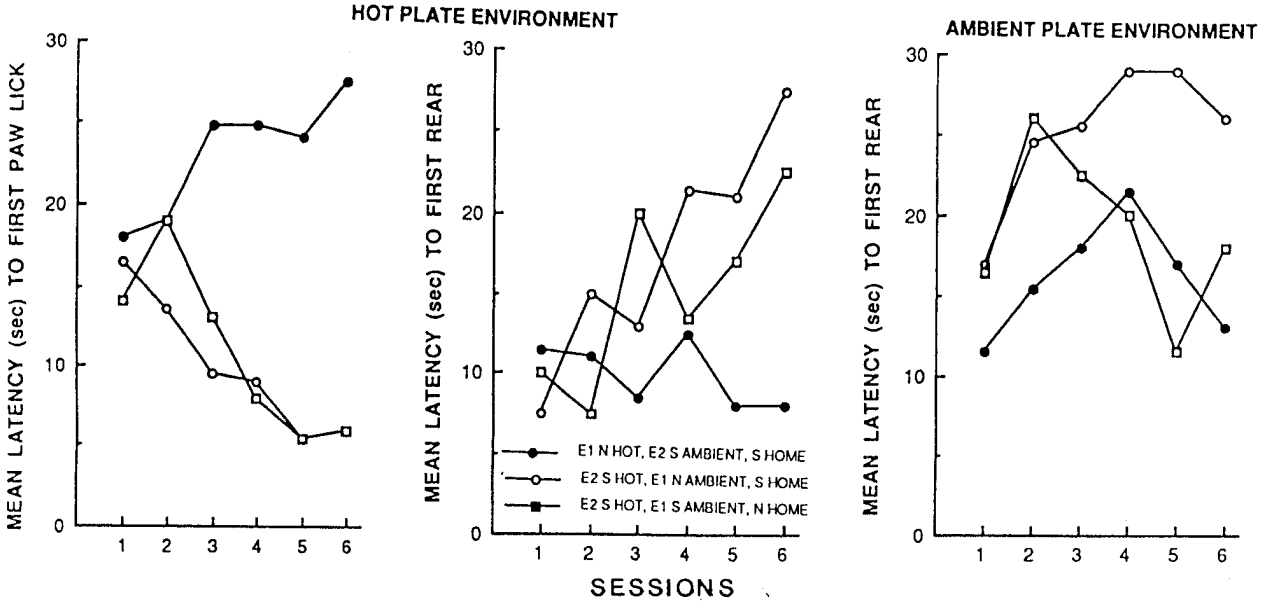

Figure 5. Mean latencies to paw-lick (left panel) and to rear (centre panel) on the hot plate as well as the latencies to rear on the ambient plate (right panel) for the three groups across discrimination training in Experiment 4.

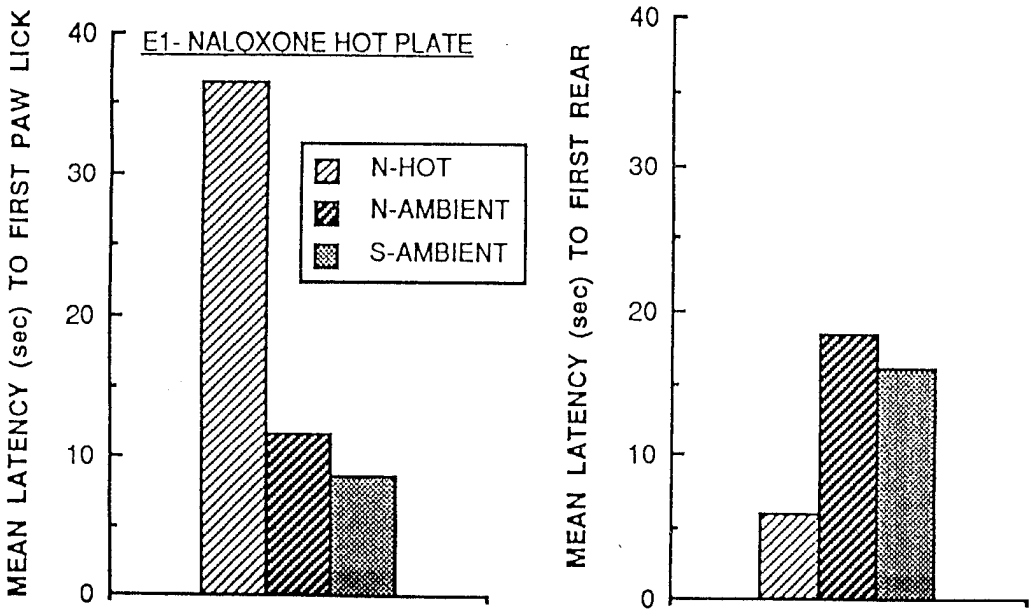

Figure 6. Mean latencies to paw-lick (left panel) and to rear (right panel) when the subjects were challenged with naloxone and the hot plate in the environment correlated with naloxone and the stressor, or naloxone and the ambient plate, or saline and the ambient plate.

HOT, E1 N AMBIENT, S HOME, were placed on the ambient plate after injection of naloxone in E1, or were injected with saline in their home cages. Across these three days, in addition to saline-stressor pairings in E2, subjects in Group E2 S HOT, E1 S AMBIENT, N HOME received pairings of the saline and the ambient plate in E1 as well as injections of naloxone in the home cages. After 18 days of training, all the rats were taken to E1, administered with naloxone and were tested on the hot plate for 60 sec.

Results and discussion

The mean latencies to paw-lick for each of the groups on the hot plate are shown in the left panel of Figure 5. The centre panel in this figure shows the mean latencies to rear on the hot plate, while the right panel shows the latencies to rear on the ambient plate. The rats were never observed to paw-lick on the ambient plate.

An inspection of the left and centre panels shows that exposures to naloxone-stressor pairings induced long latencies to paw-lick, while maintaining relatively short rearing ones. In contrast, saline-treated subjects progressively decreased their paw-lick latencies but increased their rearing ones across training. The right panel indicates that exposure to the ambient plate reversed the rearing latencies observed on the hot plate. That is to say, by the end of training, naloxone-treated subjects were failing to rear within the 30-sec, while the animals given saline typically reared after 15 sec.

Since the design of the experiment permitted a number of outcomes, it was decided to write a set of post-hoc contrasts. Further, since the results suggested a particular set of interesting comparisons a set of orthogonal contrasts was written and analysed with the technique described by Rodger (1967). With the significance level set at 0.01 to take into account the fact that three dependent variables were used across training, and 2,27 df, this yields a critical F value of 9.08. The analysis revealed that the latencies to paw-lick and to rear on the hot plate differed between the naloxone- and saline-treated subjects, F=103.8, for paw-lick, and F=34.95, for the rear. None of the contrasts which tested for differences between the saline-treated subjects were statistically reliable: F=1.06, for the differences in the latencies to paw-lick, and F=3.6, for the differences in the latencies to rear. The analysis also confirmed that there were differences between the naloxone- and saline-treated subjects in the latencies with which they reared on the ambient plate, F=13.0, and that the two saline-treated groups did not differ from each other, F=2.0.

The mean latencies with which each of the groups paw-licked and reared, when placed in E1, given naloxone and tested on the hot plate, are shown in

Figure 6. The test results can be described succinctly: subjects who had been exposed to pairings of naloxone and the hot plate in E1 took a long time to paw-lick, while rearing shortly after their placement on the plate; subjects who had been exposed to pairing of an ambient plate and either naloxone or saline in E1 paw-licked rapidly and reared slowly.

The statistical analysis of the test data confirmed that exposure to naloxone-stressor pairings in E1 had acted to enhance paw-lick latencies and to decrease rearing ones, since the contrasts which tested for the difference between this and the other two groups yielded F's of 34.4 and 10.2 on the paw-lick and rearing latencies, respectively. The contrast which tested for the differences between rats pre-exposed to naloxone or to saline on the ambient plate in E1 were not reliable, on either paw-licking or rearing, F's<1.

The results of this experiment have provided evidence that the analgesic reactions which have been displayed to the stressor in naloxone-treated subjects were due to the pairings of the drug and the stressor rather than to a history of exposure to the drug. The stressor, then, has not served merely as an assay, revealing naloxone's analgesic properties. Instead, the stressor appears to have been necessary for the drug to have engaged an analgesic mechanism.

These results differ from those of Rochford and Stewart (1987) in that they reported contingent exposure to a heat stressor was not necessary to evoke naloxone-induced analgesia. On the first test following pretraining under hot, cold or no exposure to the plate Rochford and Stewart, did not, however, see a marked enhancement in the analgesic response to morphine in rats pretreated with naloxone and exposed to the cold plate and no plate. Only on the second and subsequent trials in which naloxone was given was there a clear difference between the cold plate pre-exposed groups pretreated with naloxone versus saline. It is possible that had continued testing been employed in the present experiment, a difference may have emerged between the group that had received naloxone followed by ambient plate exposures and saline-pretreated rats.

It is also possible that the differences in experimental design between this experiment and that conducted by Rochford and Stewart can account for the conflicting results. It is well known that rats who are experienced with the hot plate procedure respond differently than naive rats. Whether or not a rat was experienced with the hot plate may have affected the outcome of the Rochford and Stewart study, independently of whether the rats received drug-stressor pairings.

Experiment 5

The results of Experiment 4 have shown that the recruitment of analgesia depends upon pairings of naloxone and nociceptive stimulation. There was also evidence that such pairings imbue the place in which they occurred with the ability to support an analgesic reaction in the absence of the drug (Experiment 3). The present experiment addressed the question of whether this was mediated by environmental activation of the opioid or non-opioid components of the endogenous pain control system. It will be recalled that Rochford and Stewart (1987) have suggested the usefulness of a distinction between the analgesia that accrue to a stressor in the presence of naloxone, and the conditioned analgesia which can be detected in the absence of the drug. Specifically, these investigators have suggested that naloxone comes to provoke analgesia by engaging the non-opioid component of the endogenous pain control system, while the associated environment does so by activating the opioid component. Their argument is that exposure to the stressor releases endogenous opioids whose effects are blocked by naloxone's occupation of receptor sites. This blockade, in turn, is assumed to release the non-opioid component from inhibition by the opioid one (Akil & Watson, 1980). However, naloxone is assumed to block the effects, but not the release of endogenous opioids. Since their release constitutes the neural basis for the primary, or unconditioned, reactions to the stressor, conditioning processes may ensure their activation by associated events. Thus, in the absence of naloxone's blockade of opioid receptors, the environment associated with naloxone-stressor pairings may produce analgesia by activation of the opioid system.

The present experiment examined this account of the conditioned analgesia observed in Experiment 3 by attempting to modify the effectiveness of the opioid component of the endogenous pain control system. Presumably, if that component was altered, then the context that had been associated with naloxone-stressor pairings would be less able to support the hypothesised opioid-mediated analgesia. The strategy adopted was based on the use of cross-tolerance with morphine as a criterion for invoking an opioid involvement in either conditioned or unconditioned analgesic reactions (cf. Watkins & Mayer, 1982a). Four groups of subjects were used in the main experiment. Two of these were given naloxone-stressor pairings in a distinctive environment, and were then administered with morphine or saline in their home cages in the colony room. The other two groups received saline-stressor pairings in the distinctive environment, and were then given morphine or saline in the home cages. Finally, subjects in these four groups were returned to the distinctive environment, injected with saline and tested on the hot plate. The question of interest was whether the conditioned effects, accruing from a history of naloxone-stressor pairings, would be diminished in morphine tolerant animals. In order to show that the morphine was capable of producing analgesia and that the history of morphine exposures were capable of removing that analgesia, two further groups were used. One of these was repeatedly injected with morphine in the home cages and then tested with the drug on the hot plate, while the second group was tested with morphine after a history of exposure to saline.

Method

Subjects and apparatus. The subjects were 48 male Wistar rats with an average weight of 400 g obtained from the colony maintained by the University. The conditions of housing, feeding, watering and the apparatus employed were the same as those described previously.

Procedure. The rats were weighed and handled for 4 days and were then assigned randomly to two major groups. Subjects in one of them were given daily exposures to naloxone-stressor pairings in the room which contained the plastic buckets (N=16), while the remainder received saline-stressor pairings in that environment (N=32). The intervals between placement in the buckets, injection and testing on the 52°C hot plate were the same as those described in Experiment 3, as were the doses of naloxone and saline. However, the duration of each exposure to the hot plate was increased to 60 sec. After 4 sessions of such exposures, the naloxone-treated subjects were assigned randomly to two further conditions. In one of these (N=8), the subjects were given an sc injection of morphine each day for 9 days, while the remainder (N=8) received daily injections of saline. Morphine Hydrochloride was diluted with 0.9% W/V saline solution such that all injections were given in an equivalent volume of 1.0 ml/kg of body weight. The morphine dose was increased after 3 days from 5 mg/kg to 10 mg/kg and after a further 3 days from 10 mg/kg to 20 mg/kg. Four days after the final administration of morphine, these subjects were placed in the plastic buckets, administered with saline and tested on the hot plate.

The subjects who had been trained with saline and the hot plate were also assigned to morphine or saline conditions. Subjects (N=16) were treated in their home cages with the doses of morphine already described, while the remainder (N=16) were given saline. Four days after the last morphine or saline administration, one of the morphine- and one of the saline-treated groups (N=8per group) were placed in the plastic buckets, injected with saline after 15 min, and then tested for 60 sec on the hot plate, 15 min after the injection. The remaining morphine- and saline-treated subjects (N=8 per group) were placed in the buckets and injected with 5mg/kg of morphine 15 min later. Fifteen min after the morphine injection, these rats were tested for 60 sec on the hot plate.

Results and discussion

The mean latencies to paw-lick in the naloxone- and saline-treated subjects across training are shown in the left panel of Figure 7, while their latencies on test are shown in the right panel.

The mean latencies to rear across training and test

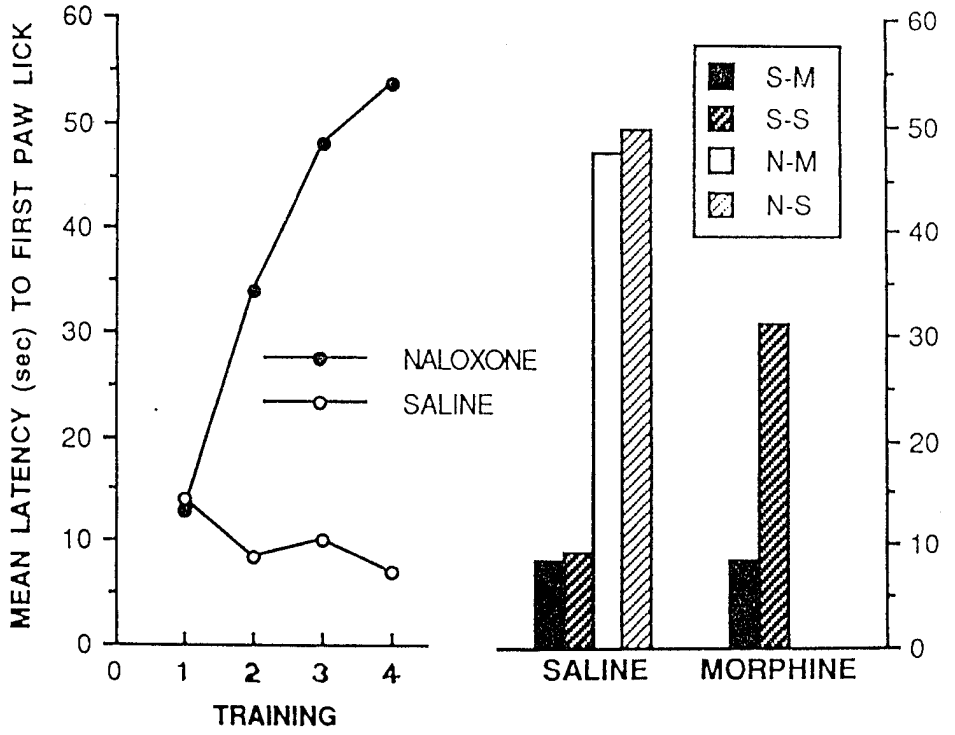

Figure 7. Mean latencies to paw-lick for naloxone (N) - and saline (S) - treated subjects across the four sessions of hot plate training in Experiment 5. Subjects were then treated with morphine (M) or saline (S) and then tested on the hot plate with saline (left portion of right panel). Two other groups were treated with saline and the hot plate (S), exposed to morphine (M) or saline (S) and then tested with morphine on the hot plate.

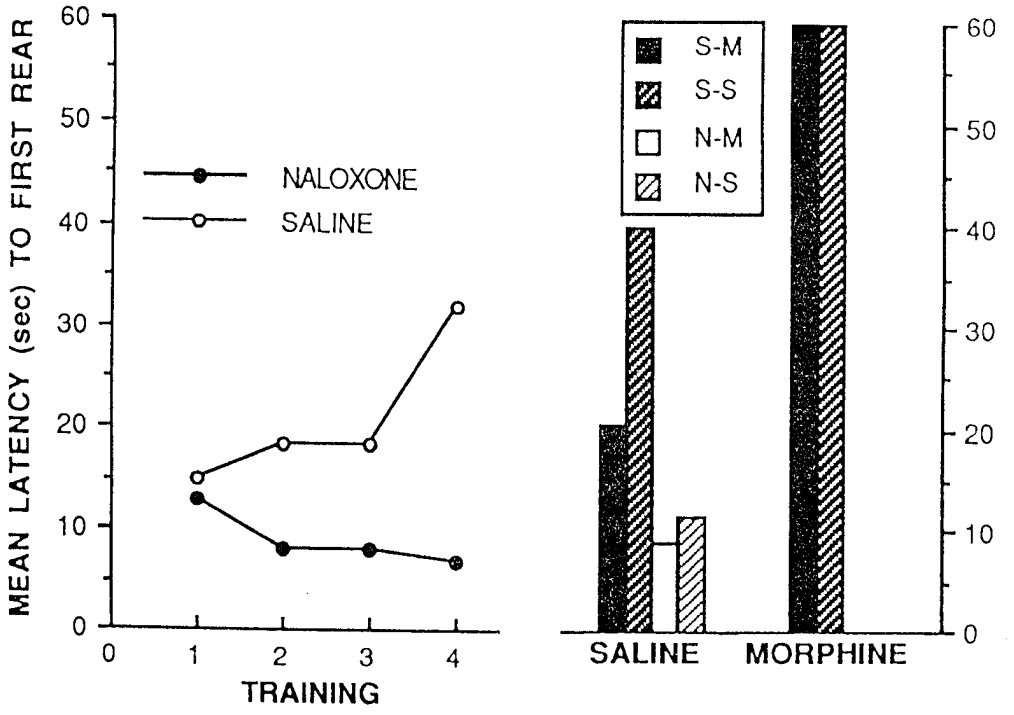

Figure 8. Mean latencies to rear for naloxone (N) - or saline (S) - treated subjects across the four sessions of hot plate training in Experiment 5. Subjects were then treated with morphine (M) or saline (S) and then tested on the hot plate with saline (left portion of right panel). Two other groups were trained with saline and the hot plate (S), exposed to morphine (M) or saline (S) and then tested with morphine on the hot plate.

are shown in the left and right panels, respectively, of Figure 8.

An inspection of the left panels of these figures confirms that the subjects exposed to naloxone-stressor pairings rapidly inhibited paw-licks, while maintaining relatively quick rearing latencies, in comparison to the saline-treated subjects who paw-licked within 10-sec but eventually took around 30-sec to rear. The right panel of Figure 7 shows that the subjects tested under morphine reacted differently to the hot plate, depending upon whether or not they had been pre-treated with the drug. Morphine-naive subjects displayed long latencies to paw-lick, while morphine-experienced ones paw-licked within a few seconds of exposure to the hot plate.

Inspection of the results from the saline test suggests that subjects reacted differently to the hot plate, depending upon whether they had been previously trained with naloxone or saline, but not upon whether they had received morphine or saline interpolated between that training and the test. Subjects displayed long paw-lick and short rearing latencies in the context associated with naloxone-stressor pairings in comparison to those who had been exposed to saline-stressor pairings. However, a history of morphine treatment had not served to diminish the ability of the naloxone-associated context to promote long paw-lick or short rearing latencies. Although such a treatment produced no detectable effect on the paw-lick latencies of the subjects trained and tested with saline, the drug history may have influenced their rearing latencies.

These observations concerning the test results were confirmed by the statistical analysis. The critical value of F (df=3,28, α=0.025) using Scheffe is 10.89. There were significant differences between paw-lick as well as rearing latencies in the groups trained with naloxone and tested with saline and the groups trained and tested with saline, F's =129.5 and 19.77. However, there were no significant differences between paw-lick or rearing latencies in the groups which received morphine versus saline after the naloxone training, F's <1, or between these latencies in the groups which received morphine versus saline after the saline training, F<1, for differences on paw-licking, and F=3.71, for the differences on rearing latencies. Finally, there was evidence that the schedule of morphine administrations was effective in rendering the animals tolerant to the drug's analgesic properties, since there was a significant difference between paw-lick latencies in the groups tested with morphine, F=18.7.

The results of this experiment failed to support the hypothesis concerning an opioid basis for the analgesia that accrues to the place associated with naloxone - stressor pairings (Rochford & Stewart, 1987). Subjects treated with morphine and then tested with that drug displayed faster paw-lick latencies than those who were morphine-naive. In spite of such tolerance, naloxone-trained subjects displayed paw-lick and rearing latencies on test that were similar to those who had been given saline between naloxone training and the saline test.

Experiment 6

The previous experiment used the criterion of cross-tolerance with morphine in an attempt to determine whether the conditioned effects that accrued from naloxone-stressor pairings were opioid-mediated. The present experiment used the other assay, namely, naloxone reversibility, in a second attempt to examine this question. It may seem paradoxical to suppose that naloxone would be capable of reversing the conditioned analgesia which was based upon pairings of naloxone and the stressor. However, it will be recalled that Watkins and Mayer (1982b) provided evidence that naloxone can prevent the conditional recruitment of opioid analgesia but cannot reverse this state once it has been entrained (Watkins, Cobelli & Mayer, 1982, Experiment 5). Recall, too, that the procedures used in the present experiments, for example, Experiment 3, involved placing rats in a distinctive environment and then administering the naloxone. Accordingly, it is possible exposure to the place in which naloxone-stressor pairings occurred triggered an opioid analgesia which was not reversed by the subsequently administered naloxone.

An implication of this suggestion is that the contextual control over analgesia, observed in Experiment 3, might be reversed, if the rats were injected with naloxone before they were exposed to that context. The present experiment used a within-subject, discrimination arrangement to test this idea. Subjects were exposed to pairings of naloxone and the hot plate in one environment, E1, and to pairings of saline and the hot plate in another, E2. The subjects were then allocated to four groups. In two of these, the animals were injected with saline in their home cages in the colony room and were then transported to either E1 or to E2, given saline and tested on the hot plate. The results of Experiment 3 suggest that subjects tested in E1 would be analgesic in comparison to those tested in E2. If these differences in the reactions to the stressor were mediated by E1's activation of the opioid system, then the administration of naloxone in advance of the animals being placed in that environment should serve to remove the analgesia otherwise observed in E1. Accordingly, two further groups of subjects were given naloxone in the colony room and then transported to E1 or to E2, given saline and tested on the hot plate. A subsidiary experiment was also conducted in order to show that the circumstances of naloxone's administration on the test was capable of reversing morphine's analgesic properties. Two groups of experimentally-naive rats were given sc injections of either naloxone (5 mg/kg) or saline in their home cages, taken to E1 and administered sc with morphine (5 mg/kg) 15 min later. They were then tested on the hot plate 15 min after the morphine injection.

Method

Subjects and apparatus. The subjects were 56 experimentally-naive, male Wistar rats with an average weight of 350 g, obtained from the colony maintained by the University. The conditions of housing, feeding, watering as well as the apparatus used were the same as those described previously.

Procedure. After 4 days of handling and weighing, subjects in the main experiment (N=40) were taken each day to the laboratory where training and testing took place. On any given day, the rats were placed either in the plastic buckets and exposed to naloxone-stressor pairings (E1), or they were put into the wooden chambers and given pairings of saline and the hot plate (E2). The intervals between placement in the buckets or boxes, injections and exposure to the hot plate were the same as those described in Experiment 3. However, the duration of each exposure to the hot plate was extended to 60 sec.

At the end of 20 sessions of discrimination training, the rats were allocated randomly to four groups (N=10 per group). Subjects in two of these groups were injected with naloxone in their home cages, transported to the laboratory and placed either in E1 or in E2, where they were injected with saline 15 min later and then tested on the hot plate 15 min after the saline injection. Subjects in the remaining two groups were treated in an identical manner, except that they were injected with saline in their home cages. In the subsidiary experiment, the animals were allocated randomly to two groups (N=8 per group). Subjects in one of these were injected sc with naloxone in their home cages, transported to the laboratory and placed in either the plastic buckets (N=4) or the wooden chambers (N=4) and administered sc with morphine (5mg/kg) 15 min later. They were then exposed for the first time to the hot plate 15 min after the morphine injection. Subjects in the second group were treated in an identical manner, except that they received an injection of saline in their home cages.

Results and discussion

The mean latencies to paw-lick for blocks of two sessions of naloxone and of two saline sessions across the 20 days of discrimination training are shown in the left panel of Figure 9, while the mean latencies to rear are shown in the right panel.

It is evident that exposure to naloxone in E1 maintained relatively long latencies to paw-lick but short ones to rear, while saline produced short latencies to paw-lick but long ones to rear. These observations were confirmed by the analysis which revealed that there were statistically reliable differences in the latencies to paw-lick and to rear between the naloxone- and the saline session

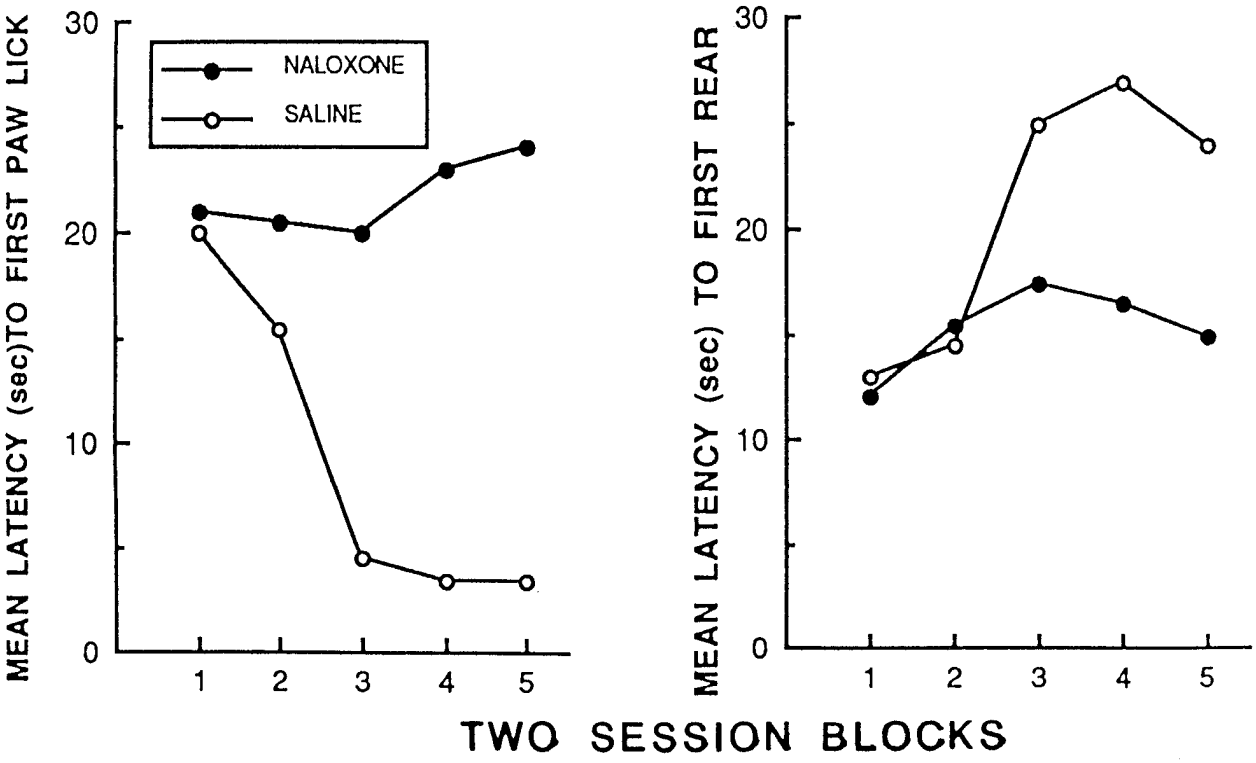

Figure 9. Mean latencies to paw-lick (left panel) and to rear (right panel) across blocks of two naloxone and two saline sessions during the discrimination training in Experiment 6.

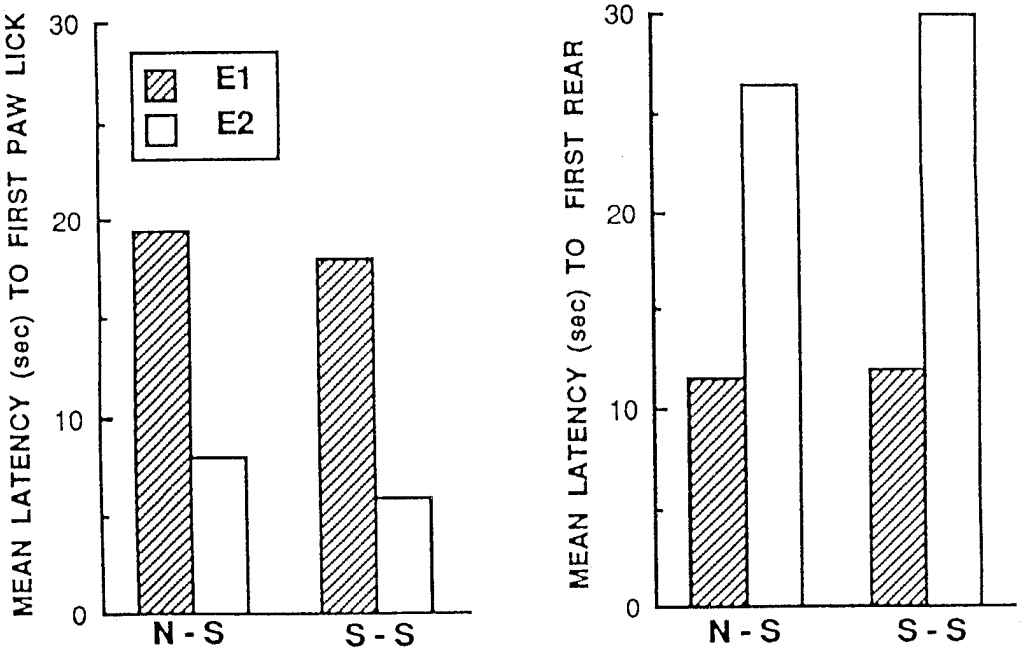

Figure 10. Mean latencies to paw-lick (left panel) and to rear (right panel) on test for the four groups of subjects in Experiment 6. The first letter designates whether subjects received naloxone (N) or saline (S) before exposure to the places associated with pairings of the stressor and naloxone (E1) or saline (E2). The second letter, S, designates the saline which each of the four groups received after exposure to E1 or E2.

F's=185.7 and 59.2 respectively.

However, the results of major interest are the reactions to the hot plate when the subjects were tested with saline in E1 or in E2. The mean latencies to paw-lick and to rear for each of the four groups are shown in the left and right panels, respectively, of Figure 10.

It is clear that the subjects who received saline where they "expected" naloxone took longer to paw-lick and less time to rear than the subjects who "expected" and received saline. It is also obvious that the administration of naloxone in advance of placement in E1 did not serve to reduce the paw-lick latencies nor to enhance the rearing ones. Finally, the administration of that drug in advance of placement in E2 did not serve to increase paw-lick latencies nor to decrease rearing ones.

These observations were confirmed by the statistical analysis. The critical value of F (df=1,36, α =0.025) using Hays is 5.5. There were significant differences between paw-lick as well as rearing latencies in the groups tested in E1 versus E2, F's =36.8 and 13.7, respectively. However, there were no significant differences between paw-lick or rearing latencies in the groups pre-treated with naloxone versus saline, F's <1, n or was there any significant interaction between the factors on paw-licking or rearing latencies, F's <1. The test results from the subsidiary experiment are shown in Figure 11.

It is evident that the administration of morphine in the subjects pre-treated with saline occasioned long latencies to paw-lick and to rear. In contrast, subjects who received naloxone in advance of the morphine paw-licked and reared with relatively short latencies. These observations were confirmed by the analysis which revealed that there were statistically reliable differences between the groups in the latencies to paw-lick and to rear, F's=20.9 and 209.4, respectively (Fc=6.30, with 1,14 df and α =0.025).

The present results have shown that the place

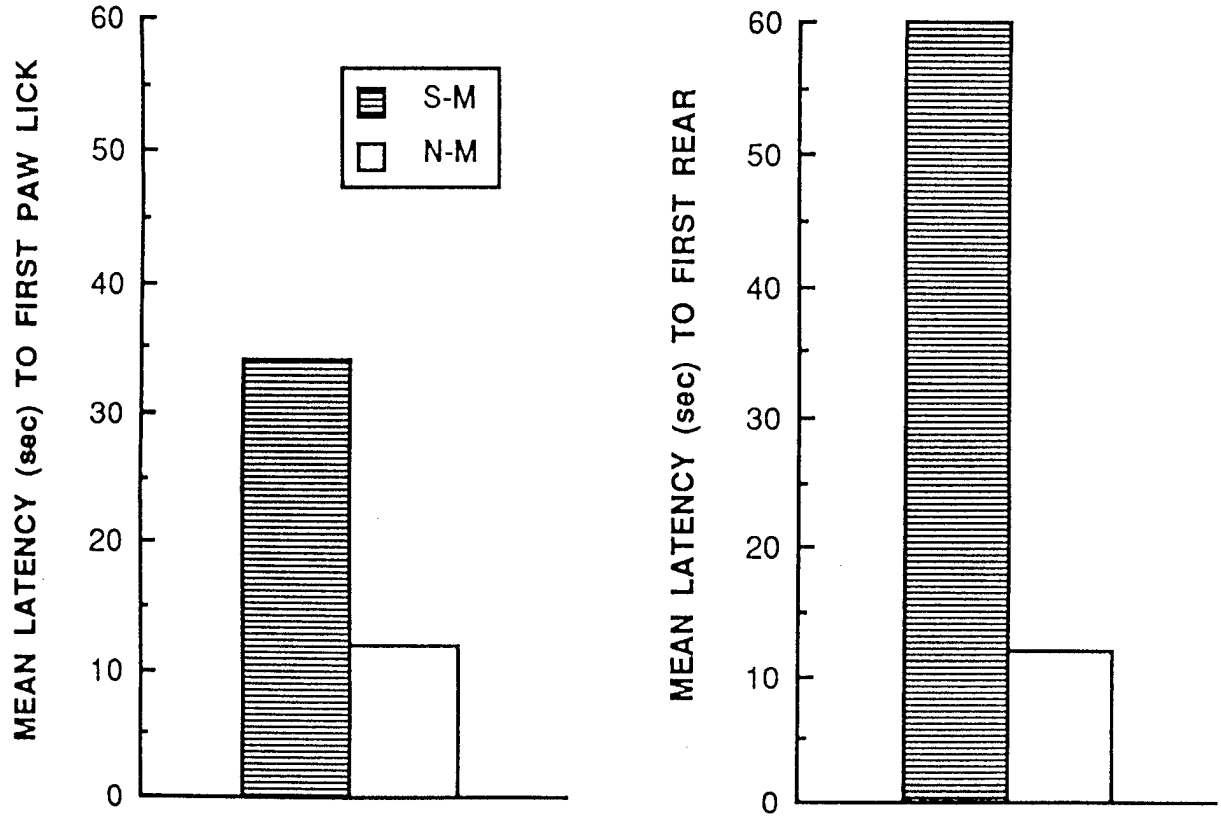

Figure 11. Mean latencies to paw-lick (left panel) and to rear (right panel) for the groups treated with saline before morphine (S-M) or with naloxone before the morphine (N-M).

associated with naloxone-stressor pairings was again able to maintain an analgesic reaction, when the rats were given saline and exposed to the hot plate. They also confirmed that the drug was unable to promote an analgesia, when given in advance of the animals being placed in E2, although the temporal interval between the drug and the stressor was capable of supporting analgesia (cf. Experiment 2). Such results thus confirm those observed in Experiment 3 and document further the role conditioning processes play in recruiting the naloxone analgesic effect. However, the present results have failed to provide any support for the hypothesis (Watkins & Mayer, 1982b) that such processes mediate analgesia by activating an opioid system: subjects given naloxone in advance of exposure to E1 were just as analgesic as those pre-treated with saline and placed in that environment. Combined with naloxone's reversal of morphine's analgesic properties, the present results converge on the conclusion that naloxone-stressor pairings enable the place in which these occurred to provoke a non-opioid form of analgesia.

Experiment 7

The results of Experiments 5 and 6 failed to provide evidence for an opioid involvement in the contextually controlled analgesia induced by naloxone-stressor pairings. Since the conditioned analgesia was maintained in rats who had been presumably rendered tolerant to morphine and was not reversed by a prior administration of naloxone, it seems reasonable to conclude that the analgesia was non-opioid. Such a conclusion then raises the question as to the relation between this analgesia and that provoked by an exogenous opiate, morphine. Suppose that rats were repeatedly exposed to pairings of naloxone and a stressor in a distinctive environment and were then tested with saline in that place. The results of the previous experiments suggest that those animals would be analgesic in comparison to those who had been given saline-stressor pairings. The question of interest, however, concerns the amount of analgesia which the naloxone-treated rats would display, if they were injected with morphine rather than with naloxone or saline. The collateral inhibition model of the endogenous pain control system (Akil & Watson, 1980) proposes that the opioid and non-opioid components are mutually inhibitory. Presumably, then, the administration of morphine in the context associated with naloxone-stressor pairings should reduce the amount of non-opioid analgesia provoked by that context. The present experiment used four groups of subjects to examine the relation between morphine analgesia and that occasioned by the place where rats were exposed to naloxone-stressor pairings. Two groups of subjects were repeatedly given naloxone-stressor pairings in a distinctive environment, while the other two received saline-stressor pairings. At the end of this training, one of the groups that had been trained with naloxone was tested with morphine, while the other one was given saline. Similarly, one of the groups trained with saline was tested with morphine, while the other group continued to receive saline.

Method

Subjects and apparatus. The subjects were 20 experimentally naive, male Wistar rats, with an average weight of 350 g. They were obtained from the colony maintained by the University and were housed, fed and watered in the manner described in Experiment 1. The apparatus was that used in Experiment 3.

Procedure. After 4 days of handling and weighing, the rats were assigned randomly to two groups (N=10 per group). Each day for four days, the animals were transported to the laboratory and placed in the plastic buckets for 15 min. They were then removed and injected sc with naloxone (5 mg/kg) or with saline. Fifteen minutes after the injection, subjects in each of the two groups were placed on a 52°C hot plate for 60 sec. The subjects that had been treated with naloxone were allocated randomly to a morphine or to a saline group (N=5 per group), as were those who had been trained with saline (N=5 per group). On four successive days, subjects were placed in the plastic buckets, injected sc with 5 mg/kg of morphine or with an equivalent volume of saline, replaced in the buckets for a further 15 min, and then exposed to the hot plate for 60 sec.

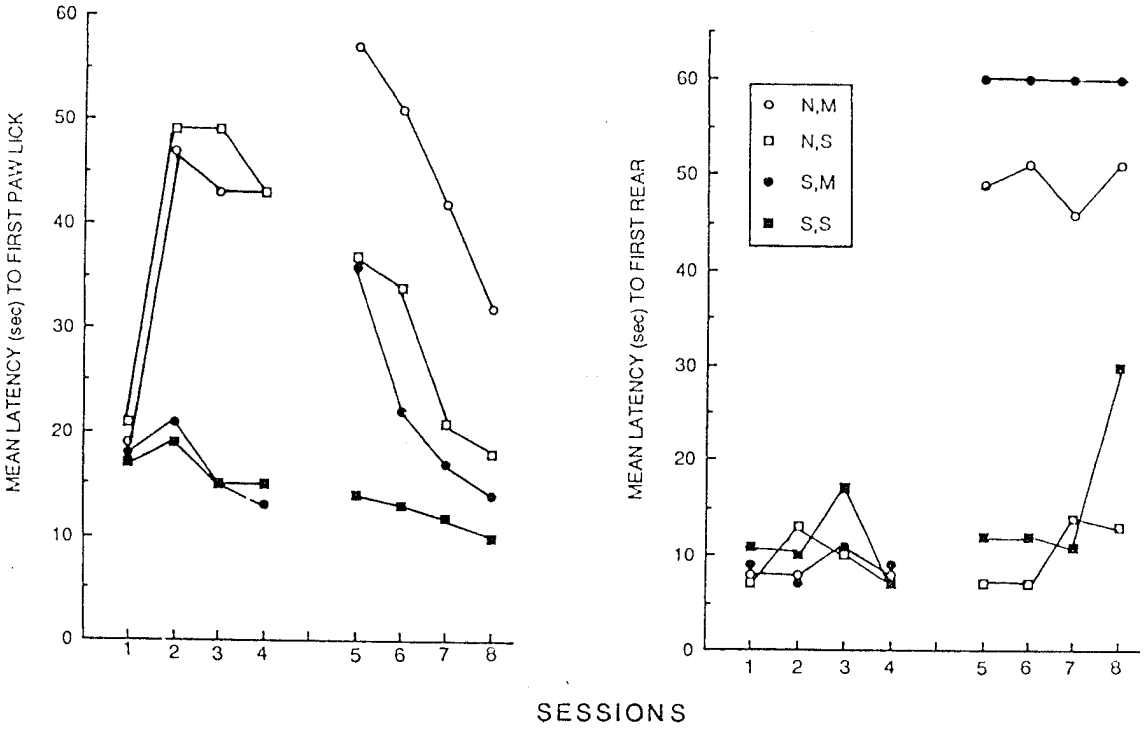

Figure 12. Mean latencies to paw-lick (left panel) for subjects exposed to naloxone (N) and then to either morphine (M) or saline (S) and for those exposed to saline (S) and to either morphine (M) or saline (S) in Experiment 7. The right panel shows the rearing latencies for these four groups of subjects.

Results and discussion

The mean latencies to paw-lick and to rear across the two stages of the experiment are shown for each of the groups in the left and right panels, respectively, of Figure 12.

Subjects exposed to naloxone-stressor pairings in the distinctive environments rapidly acquired long latencies to paw-lick, while maintaining short latencies to rear. In contrast, subjects exposed to saline-stressor pairings paw-licked shortly after placement on the hot plate. On the final day of the initial training, the mean latency to paw-lick in the naloxone-treated subjects was 44.9 sec, with a range of 23.3 to 60 sec, while the mean latency in the subjects given saline was 11.9 sec, with a range of 7.8 to 20.3 sec. The latencies to rear overlapped considerably in the two treatments, with the mean of the naloxone subjects being 11.8 (range:5.1 to 22.5) sec and the mean latency of the saline animals being 11.6 (range: 2.5 to 35.8) sec. However, the data of major interest were the latencies to paw-lick and to rear, when the subjects from these two conditions were given morphine or saline. An inspection of the paw-lick latencies in stage 2 of the experiment shows that subjects who continued to receive saline paw-licked shortly after exposure to the hot plate in comparison to the paw-lick latencies displayed by the subjects in the other three groups. In these groups, the administration of saline in the place associated with naloxone-stressor pairings, provoked a conditioned analgesia which appeared to be as potent as that induced by morphine in the subjects pre-trained with saline. Of most interest, however, the conditioned analgesia induced by a history of naloxone-stressor pairings summated with the analgesic properties of morphine to bring about a "superanalgesia" in the rats given morphine where they "expected" naloxone.

To determine whether there were statistically reliable differences among the groups across stage 2, a set of post-hoc, orthogonal contrasts was written and analysed with the technique described by Rodger (1967). With the significance level set at 0.025, to take into account the use of two dependent variables, this yields a critical value of 7.52. The analysis revealed that there were statis-

tically reliable differences between the subjects who always received saline and those in the three other groups, F=36.5. There were no statistically reliable differences between the conditioned analgesia induced by naloxone-stressor pairings, and that produced by morphine in the rats pretrained with saline, F<1. Finally, the two groups just described differed from the subjects given morphine in the place associated with naloxone-stressor pairings, F=48.95, confirming the presence of superanalgesia.

An examination of the rearing latencies, in stage 2 of the experiment, reveals that morphine-treated subjects took much longer to rear than those given saline. Subjects who were given this drug where they "expected" naloxone reared more rapidly than those who were given the drug after training with saline. In fact, these latter subjects were never observed to rear. Except for the final session, subjects who continued to receive saline reared as rapidly as those who were given saline where they had come to "expect" naloxone. The analysis confirmed that there were statistically reliable differences between the subjects who always received saline and those in the other groups, F=22.78. There were also differences between the subjects given morphine after saline training and those given saline after naloxone training, F=70.88. However, the contrast which tested for the differences between the two groups just described and the group who received morphine after naloxone training yielded an F=2.70.

The present results have confirmed that naloxone-stressor pairings imbue the place in which these occurred with the ability to sustain long latencies to paw-lick in saline-treated subjects. Further, these latencies were just as long as those which were observed in the rats who were given morphine after training with saline. Although equally analgesic on the latencies to paw-lick, the subjects who "expected" naloxone continued to rear as rapidly as they had always done, while those given morphine simply never reared. Finally, the present experiment has revealed that subjects who received morphine in the place associated with naloxone-stressor pairings showed enhanced analgesia as indexed by the latency to paw-lick. Taken together with the results of Experiments 6 and 7, this finding suggests that the contextually controlled, non-opioid analgesia summates with opiate analgesia, a suggestion which is inconsistent with the hypothesis that these components of the endogenous pain control system collaterally inhibit one another.

General Discussion

The present experiments have confirmed that pairings of the opioid antagonist, naloxone, and a heat stressor renders rats analgesic, as indexed by the latencies with which they paw-lick when exposed to a hot plate (Greeley et al., 1988; Rochford & Stewart, 1987). Although dose dependent (Experiment 1), the analgesia was not due to some non-specific action of the drug at the higher doses, since it depended upon the functional concentration in the blood-brain at the time of exposure to the stressor (Experiment 2).

Experiment 3 showed that conditioning processes play an important role in recruiting the analgesic effects which accrue from repeated naloxone injections. In the absence of the drug, its associated environment supported analgesia, but in the absence of that environment, the drug did not promote analgesia. Of course, the procedure used in this experiment was designed to bring the analgesic effect under discriminative control, differing from that used in say Experiments 1 and 2. However, the discrimination arrangement merely makes explicit what characterised these other experiments, namely, the provision of cues which signalled the administration of naloxone. Accordingly, the association between the sights and sounds of the laboratory and the naloxone-stressor pairings may have imbued these antecedent cues with the ability to trigger the endogenous pain control system, coming to render the animals analgesic when exposed to the heat stressor. Naloxone's analgesic effects, therefore, are contingent phenomena, governed by the cues which have signalled naloxone-stressor pairings in the past.

Experiment 4 provided evidence that a history of exposure to naloxone in a distinctive environment was not sufficient to make the rats analgesic, when challenged with the drug and the heat stressor in that environment. Rats given exposure to naloxone in that place were selectively analgesic, when the drug had been paired with the heat stressor, but not when the drug had been administered in the absence of pain. On exposure to naloxone and the stressor in that environment, the subjects who had received the drug there, in the absence of pain, paw-licked just as rapidly as animals who had been trained with saline in that environment. Naloxone's analgesic effects, therefore, appear to be doubly contingent, depending not only upon the presence of cues which have signalled naloxone-stressor pairings, but also upon the association between the drug and pain.One way to accommodate both of these contingencies is to suppose that the effect is mediated by the contextual triggering of the opioid component of the endogenous pain control system. For example, it could be argued that exposure to the heat stressor releases opioids whose analgesic effects were blocked by naloxone's occupation of receptor sites. Although ineffective, their release would constitute the neural basis for the brain's unconditioned reaction (UR) to the stressor and would come to be triggered as a conditioned response (CR) by antecedent cues. Suppose, further, that naloxone was unable to reverse the action of these opioids, once they had been released (Watkins & Mayer, 1982b). One could then argue that pain was necessary for the effect because it caused the release of opioids and so provided the basis for their conditional release by antecedent cues. Further, since the rats were exposed to these cues in advance of administration with naloxone, the drug might have been unable to reverse their action upon the opioid system. However, Experiments 5 and 6 did not provide any support for this hypothesis concerning an opioid-mediated analgesia. Subjects presumably rendered tolerant to morphine, after exposures to naloxone-stressor pairings in a distinctive environment, were just as analgesic, when tested with saline in that environment, as subjects who had received saline between the training and test. Rats were also trained in a discrimination between pairings of naloxone and the stressor in one environment and of saline and the stressor in another. On test, the animals treated with saline in the home cages and then

exposed to either of the two environments were selectively analgesic in the place associated with naloxone-stressor pairings. Likewise, rats given naloxone in advance of exposure to these environments were just as selectively analgesic in the place associated with naloxone-stressor pairings. Since the naloxone was administered in advance of exposure to the place where they were selectively analgesic, the animals could not have been activating the opioid component of the endogenous pain control system.

These arguments also apply to an account of naloxone-induced analgesia which distinguishes between the analgesia observed in the presence of the drug and that which can be detected in the absence of the drug when the animals are tested in the place where naloxone-stressor pairings occurred. Rochford and Stewart (1987), for example, have drawn such a distinction, arguing that naloxone provokes a non-opioid form of analgesia and at the same time enables the associated context to trigger an opioid analgesia, when the animals are tested there in the absence of naloxone. Rather, the results of Experiments 5 and 6 are consistent with the notion that naloxone's blockade of opioid receptors eventuates in a non-opioid form of analgesia which can be activated by antecedent cues.

Viewed in this way, naloxone's analgesic effects can be understood in terms of the collateral inhibition model of the endogenous pain control system (Akil & Watson, 1980). According to this account, exposure to the heat stressor activated endogenous opioids whose analgesic effects are blocked by naloxone's occupation of receptor sites. This blockade in combination with pain frees the non-opioid component of the system from the inhibition usually exerted by the release of opioids. Presumably, this non-opioid component then comes to be activated by antecedent cues, rendering the animal analgesic when exposed to the heat stressor. This account of the organization of the endogenous pain control system appears to imply that the occupation of receptors by opiates might diminish the non-opioid form of analgesia hypothesised to be conditioned to contextual cues. However, Experiment 7 showed that animals who were treated with morphine in the place associated with naloxone-stressor pairings were superanalgesic, indicating that the conditioned non-opioid analgesia summated with the opiate analgesia produced by the morphine. Since the rats were exposed to the place associated with naloxone-stressor pairings and were then injected with morphine, it is possible that the superanalgesia is peculiar to the non-opioid analgesia being triggered in advance of morphine's occupation of opioid receptors and attendant opiate analgesia. Accordingly, it might be of interest to replicate the design of Experiment 7 but administer morphine to the rats before they were exposed to the place where they had received naloxone-stressor pairings. If such rats did not display the superanalgesia, then there might be grounds for supposing that there were asymmetries in the relations between the opioid and non-opioid components of the endogenous pain control system, such that opiates can inhibit the subsequent recruitment of the non-opioid component, while prior activation of the non-opioid component may summate with opiate analgesia.

Naloxone-treated subjects persistently reared with short latencies, when exposed to the hot plate. These latencies were not due to some non-specific action of the drug. Rather, they were governed by the interaction between the drug and the heat, since they were very long in the absence of any heat (Experiment 4). Viewed as an index of general activity, the rearing latencies observed in naloxone-treated subjects presumably means that they were affected by nociceptive stimulation. Further, the relation between the latencies to paw-lick and to rear differed in the naloxone- and the saline-treated subjects: the former reared rapidly but failed to paw-lick, while the latter, after extended training, paw-licked rapidly but took a long time to rear.

How should one view this apparently inverse relation between paw-licking and rearing? One way to do so is to suppose that both paw-licking and rearing constitute attempts to adapt to environmental conditions; paw-licking transiently alleviates pain by its cooling action on the skin and rearing serves to explore and learn about the environment. The behaviour of saline-treated rats on both the hot and ambient plates will serve as

examples of 'normal' adaptive behaviour under these different environmental conditions. When placed repeatedly on an ambient plate nondrugged rats gradually reduce the latency with which they rear. Rearing here may reflect exploration and a reduction in fear of a novel enviornment. When placed on the hot plate, however, exploratory tendencies give way to attempts to reduce the pain. Thus, an increased latency to rear on the hot plate may not indicate greater fear but a reordering of appropriate behaviours to meet the exigencies of the situation.

Since naloxone decreases the tendency to rear during exposure to the ambient plate, it might be argued that naloxone maintains or increases fear of novelty (perhaps by preventing habituation). Yet, when placed on the hot plate naloxone-treated rats rear most readily. The introduction of pain to the novel environment of the hot plate apparatus overrides naloxone's tendency to delay rearing and, in fact, reverses the tendency to one of more rapid rearing. It seems unlikely that rapid rearing on the hot plate indicates a reduction in fear. Indeed, it may reflect the opposite, an enhancement of fear which interferes with the acquisition of a more appropriate adaptive response - paw-licking.

Another way of describing the relation between paw-licking and rearing is to suppose that these behaviours constitute the expressions of distinct, mutually inhibitory, central motivational states, along the lines proposed by Bolles and Fanselow (1980). Paw-licking might then be described as a recuperative behaviour that is engaged by hurt, while rearing might be thought of as a defensive behaviour that is provoked by the fear associated with increasing pain or with a signal for that pain. If the arousal of fear inhibited the experience of hurting and vice versa, then such an argument leads to the conclusion that naloxone-treated subjects inhibited paw-licking, because they remained frightened by the hot plate, while saline-treated subjects eventually paw-licked rapidly, because they were no longer afraid. An implication of this conclusion is that anxiolytic drugs might serve to remove the conditioned analgesia induced by naloxone-stressor pairings, since they would act to diminish the conditioned fear. However, these observations must remain speculative, since the present experiments did not provide any converging evidence for the notion that the naloxone- and saline-treated subjects were differentially afraid.

Finally, the results of the present experiments have implications for the use of naloxone as an assay for an opioid involvement in stress-induced analgesia. Such an assay involves a 2 x 2 factorial design, in which rats are either administered with naloxone or saline, and then exposed or not exposed to a stressor, such as shock. Studies employing this design then test all of the animals for their responsiveness to nociceptive stimulation, typically a heat stressor. The aim of such studies is to determine whether shock enhances the paw-lick latencies in saline-treated subjects compared to non-shocked controls, and whether this shock-induced enhancement is influenced by naloxone. The present experiments, of course, have used two of these groups, those given saline and naloxone and tests on the hot plate and shown that the latter become analgesic, as indexed by their latencies to paw-lick. Since the drug interacts with the test to render animals analgesic, the use of naloxone in combination with a heat stressor to draw inferences about an opioid involvement in the analgesia produced by a stressor, such as shock, would seem to be a more complicated matter than that believed previously, at least in those studies in which repeated testing on the heat stressor is employed.

Summary

Seven experiments examined the apparently paradoxical analgesia which accrues when rats are repeatedly injected with an opiate antagonist, naloxone, and exposed to a heat stressor. Experiments 1 and 2 showed that such pairings came to enhance in a dose-dependent manner the latencies with which rats paw-licked in response to the stressor. Experiment 3 documented a role for conditioning processes in recruiting the naloxone-induced analgesia. Experiment 4 showed that the analgesic effect was due to the pairings of the drug and the heat stressor, since a history of exposure to naloxone in a distinctive environment did not render the animals analgesic, when challenged with the drug and the stressor. Experiments 5 and 6 provided evidence that the conditioned analgesia which accrued from drug-stressor pairings was non-opioid in nature, since the analgesia was observed in morphine tolerant rats and was not reversed by an administration of naloxone in advance of exposure to the conditioning context. Experiment 7 demonstrated that the administration of morphine in the context previously associated with naloxone-stressor pairings provoked an enhanced analgesia. While rats increased their latencies to paw-lick under naloxone, their latencies to rear decreased. The results were discussed in terms of the collateral inhibition model of the endogenous pain control system and some speculations were offered concerning the relation between paw-licking and rearing.

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