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Opioid and Nonopioid Interactions in Two Forms of Stress-Induced Analgesia

JUDY E. GRISEL,[1] MONIKA FLESHNER, LINDA R. WATKINS AND STEVEN F. MAIER

Department of Psychology, Campus Box 345, University of Colorado, Boulder, CO 80309

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GRISEL, J. E., M. FLESHNER, L. R. WATKINS AND S. F. MAIER. *Opioid and nonopioid interactions in two forms of stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV **45**(1) 161–172, 1993.—Stressful environmental events activate endogenous mechanisms of pain inhibition. Under some circumstances the analgesia is blocked by naloxone/naltrexone ("opioid"), while under others it is not ("nonopioid"). The existence of these two categories of analgesia leads to the question of how they are related. In a collateral inhibition model proposed by Kirshgessner, Bodnar, and Pasternak (1982), opiate and nonopiate mechanisms were viewed as acting in a mutually inhibitory fashion. In the present experiments, rats were exposed to either of two environmental stressors that produce a nonopioid stress-induced analgesia (SIA) following injections of the opiate antagonist naltrexone or agonist morphine. In the presence of naltrexone, SIA produced by either cold water swim (CWS) or social defeat was enhanced. These same SIAs were found to attenuate the analgesic effect of morphine, demonstrating that an activation of opioid systems can inhibit nonopioid analgesias. These results support an inhibitory interaction of opioid and nonopioid mechanisms in some forms of stress-induced analgesia.

Analgesia Opioid–nonopioid interactions Morphine Naltrexone Stress Cold water swim Social defeat

THE brain contains circuitry that is capable of inhibiting pain (5). A variety of research has indicated that there is not a single pain inhibition or analgesia system, but rather multiple systems with overlapping components and pathways [see (22,42,44) for reviews]. A key distinction has been made between analgesia systems that do and do not involve an opioid link. In practice, "opioid" analgesias have been defined as those that are reversible by administration of an opiate antagonist such as naloxone or naltrexone and cross-tolerant with morphine, while "nonopioid" delineates the general class of analgesias that are not affected by these manipulations. This classification was first made before the multiplicity of opioid receptors was well understood, and recent studies indicate that both analgesias may in fact be mediated through opioid mechanisms involving different receptor types. Thus, it may be more accurate to label these analgesias as "μ" and "non-μ" (47,49). However, the opioid/nonopioid terminology is still currently used, and that convention will be followed here.

The endogenous analgesic circuitry can be activated by environmental events that can be characterized as "stressful" [see (4) for a review], as well as by electrical or chemical stimulation (9,27,30,31). The phenomenon of stress-induced analgesia (SIA) has been investigated extensively, and exposure to a wide variety of stressful situations has been shown to diminish reactivity to nociceptive stimulation (29,42). Some stressors produce opioid analgesia as defined by the above criteria, while others lead to a nonopioid analgesia (3,6,12,25,26, 41,42,45). Similarly, stimulation of some brain regions results in opioid analgesia, while stimulation of others leads to the nonopioid variety (9,28).

The existence of these two categories of analgesia leads to the obvious question of how they are related. The most straightforward possibility is that they are independent—a triggering agent activates one, the other, or both. However, experiments by Kirchgessner, Bodnar, and Pasternak (23) first suggested that this might not be the case. They induced analgesia in rats either with morphine or by placing subjects in cold water [called a cold water swim (CWS)]. The morphine or CWS was preceded by ICV administration of the irreversible opiate antagonist naloxazone. Naloxazone blocked morphine analgesia, as would be expected. The surprising result was that naloxazone potentiated CWS analgesia rather than blocking it or having no effect. Kirchgessner et al. (23) interpreted this result to mean that there exists "collateral inhibition between opioid and nonopioid pain-inhibitory systems." Congruent results have been reported by Greeley et al. (17), Rochford and Stewart (32), Westbrook and Greeley (49), and Yoburn et al. (52).

In a collateral inhibition model, opiate and nonopiate mechanisms are viewed as acting in a mutually inhibitory fashion. Existing data show that naloxazone, naloxone, and naltrexone can potentiate nonopioid analgesia under some circumstances and that a manipulation that produces nonopioid analgesia can interfere with morphine analgesia (38). The pres-

[1] To whom requests for reprints should be addressed.

ent studies were undertaken to further explore the potential collateral model of opioid and nonopioid pain inhibitory systems. The first goal was to determine whether the opiate antagonist-produced potentiation of nonopioid analgesia is generalizable to other forms of stress-induced analgesia than those previously tested. We chose to explore the impact of naltrexone on nociception following a nonopioid analgesia produced by social defeat. A second goal was to test an as yet unstudied prediction of the collateral inhibition model. If opiate antagonists interfere with the activity of opiate systems, thereby enhancing the nonopioid analgesia, then opiate agonists might be expected to activate opiate systems and interfere with nonopioid analgesia. Here, we examined the effects of prior morphine on the analgesia following CWS or defeat. In addition, in each experiment animals were exposed to the stressor combined with the drug for 5 consecutive days to examine the temporal nature of the changes observed. Previous experiments have examined only a single combination of opiate antagonist and stressor, and the stability of the resulting interaction is unknown.

GENERAL METHOD

Subjects

Adult, male albino Sprague-Dawley (Holtzman) rats were used in all experiments. They were housed three to a cage with free access to food and water. Animals were acclimated to the colony room for at least 10 days prior to handling and 14 days prior to experimental manipulation. Handling days consisted of habituating animals to weighing, SC saline injections, and exposure to the restraint and tail-flick apparatus without thermal stimulation. Experiments were performed between 0900 and 1400 h during the light part of a 12 D : 12 L cycle in which lights were switched on at 0700. Room temperature was maintained at 22–23°C.

General Experimental Protocol

All experiments were 5 days long and successive test days consisted of identical behavioral procedures. The stressor was either 4 min of continuous CWS or 10 min of social defeat.

Drug Administation

Animals were given SC injections in their home colony rooms either 20 min (naltrexone HCl) or 10 min (morphine sulfate) before baseline analgesia measures were taken in the test room. Both drugs were prepared in physiological saline and delivered in a volume of 1 ml/kg. Naltrexone was administered at a dose of 14 mg/kg and morphine at 1.5 mg/kg. This dose of naltrexone was chosen because it had been used in prior (unpublished) studies in our laboratory and comparisons between studies were of interest. The dose of morphine was determined in pilot studies to be high enough to elicit a substantial analgesia in our naive rats but not so high that it prevented any observable interactions in our test paradigms. All three animals in the same cage were given the same injection.

Analgesia Testing

A modification (1) of the tail-flick test (10) was used to measure pain sensitivity. Voltage to the bulb was adjusted to attain baseline latencies to radiant heat of 2–4 s in naive animals. If no flick occurred by 10 s, the trial was automatically terminated to avoid tissue damage. All baseline measures were the mean of three consecutive trials at 15-s intervals. A 1-cm diameter heat beam was sequentially applied at approximately 11, 8, and 5 cm from the proximal end of the tail. Rats were loosely restrained for tail-flick testing by being wrapped in cotton flannel cloth with their tails protruding and then secured to a Plexiglas tray by means of a velcro belt. Disturbance due to this handling was minimized by habituating animals to the restraint procedure for at least 3 days prior to the beginning of the experiment. On day 1 of experiments 2 and 4, baseline tail-flick measures were taken in the colony room as well as in the test room. Baseline measures in experiments 1 and 3 as well as all other tail-flick measures were taken in the test/stress rooms.

Data Analysis

Data were analyzed with repeated-measures analysis of variance (ANOVA) or factorial ANOVA. Neuman-Keuls comparisons were performed to test specific group differences with the required level of significance at 0.05.

EXPERIMENT 1

The purpose of the first experiment was to ensure that the opiate antagonist potentiation of CWS analgesia observed by Kirchgessner et al. (23) could be replicated under our laboratory conditions. Naltrexone rather than naloxone or naloxazone was used to increase generality and allow comparison with other experiments in our laboratory.

METHOD

Cold Water Swim

The CWS procedure took place in a 40 × 27 × 65-cm Plexiglas chamber that sat inside a 65 × 36 × 36-cm insulated cooler filled with water kept at 14°C. The Plexiglas chamber was perforated at the bottom so that the water level and temperature were constant throughout both chambers, and ice was added to the water in the cooler as necessary to maintain temperature. Animals were lifted in and out of the Plexiglas chamber by hand. Subjects were forced to swim in this water for 4 min. Immediately after their inescapable swim, rats were placed in individual holding bins, with wooden sides, Plexiglas tops, and wire mesh floors (40 × 25 × 28 cm), where they stayed except for brief removal during tail-flick testing.

Procedure

Fifteen adult, male Sprague-Dawley Holtzman rats, weighing between 400 and 500 g at the start of the experiment, were divided into two groups. Animals were injected in their home colony rooms with either 14 mg/kg SC naltrexone (n = 9) or equivolume vehicle (saline) (n = 6) for 5 consecutive days. Rats remained in their home cages between injections and testing. Nineteen minutes after injections, rats were brought to the testing room, tested for baseline pain sensitivity using the tail-flick procedure, and placed in the cold water swim apparatus. Each rat was given a 4-min exposure to 14°C water. Following CWS, rats were placed in individual holding bins, where they remained except for analgesia testing at 30

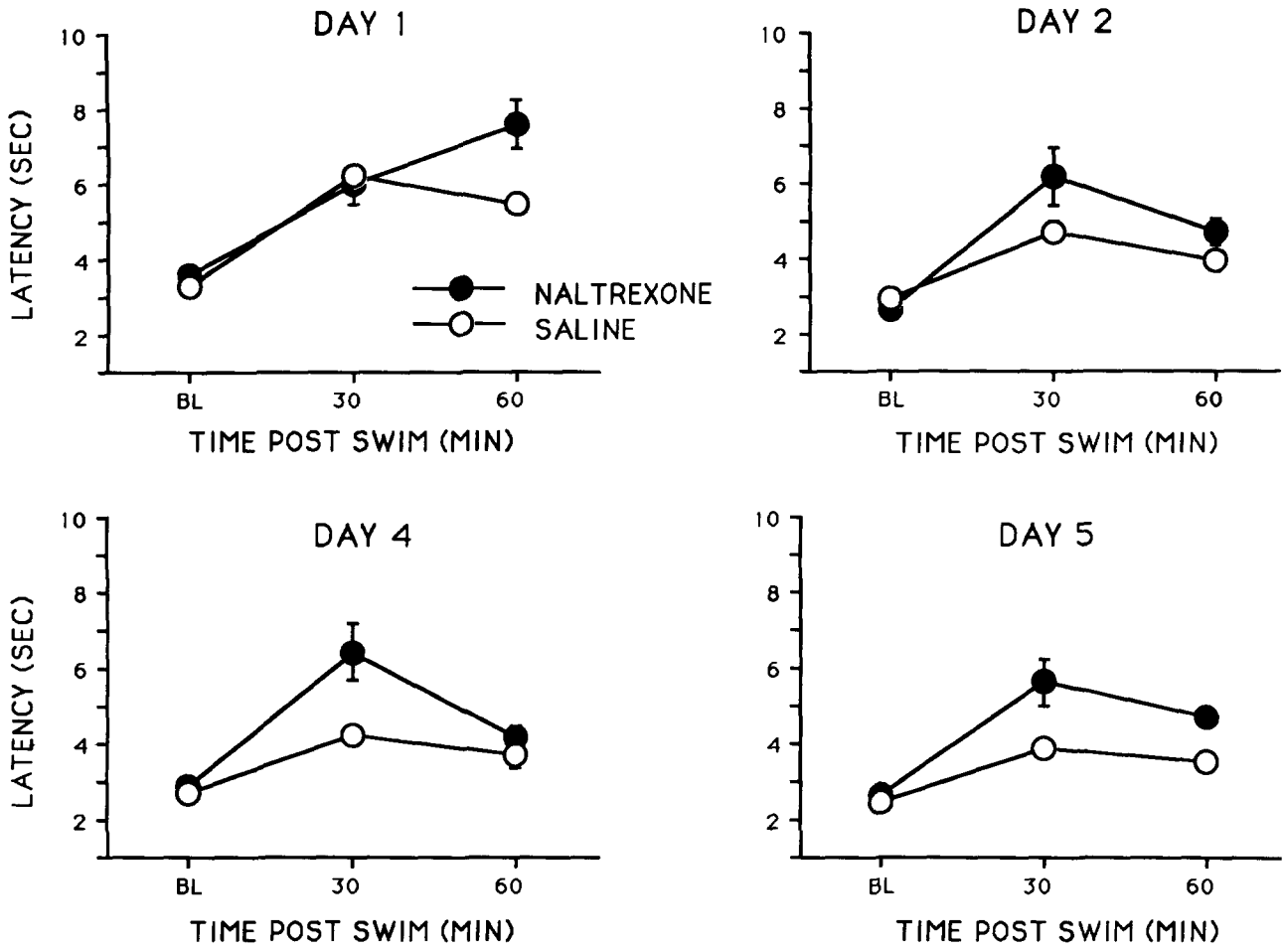

FIG. 1. Naltrexone + cold water swim (CWS). Mean tail-flick latencies (±SEM) for naltrexone and saline groups over 5 days of CWS. (Day 3 was omitted due to a procedural error.) Naltrexone (●) significantly potentiates the SIA resulting from CWS compared to saline controls (○).

and 60 min after the swim. Two measures were taken at each time point (10 and 5 cm from the proximal end of the tail). After the 60-min tail-flick test, they were returned to their home cages.

Two supporting experiments were conducted. In the first, 12 rats of the same description as above were assigned to either naltrexone or saline groups. They were given these injections for 5 days and handled identically to those in the previous experiment except that they were not exposed to the swim procedure. Twenty minutes after injection, they were given baseline tail-flicks and then placed directly in the holding bins, where they remained until given additional tail-flick tests 34 and 64 min after baseline assays. The purpose of these groups was to determine any nonspecific effects of the drug administration.

In a separate experiment, 12 rats were given either naltrexone or saline and 20 min later exposed to cold water (14°C for 4 min). In this instance, body temperature changes were measured, as differences between groups could confound the interpretations of the effects of CWS on analgesia. Core body and skin temperature were determined at baseline, 30 min postswim, and 60 min postswim using a telethermometer with both rectal and skin probes.

RESULTS

Figure 1 shows tail-flick latencies for each group on each experimental test day. Data from day 3 are not included due to difficulties on this test day resulting from building construction that significantly disrupted experimental procedures. As can be seen, baseline latencies were comparable for both groups, $F(1, 58) > 1.0$, $p > 0.05$, and so were not included in the subsequent data analysis. The CWS produced increased tail-flick latencies in both saline and naltrexone groups, but animals receiving naltrexone prior to CWS showed even higher latencies than those that were given saline in combination with CWS, $F(1, 13) = 18.046$, $p < 0.001$. All animals were analgesic following exposure to the CWS relative to their baseline measures, and this analgesia decreased between the 30- and 60-min measures, $F(1, 13) = 11.446$, $p < 0.01$. The groups differed from each other at both the 30- and 60-min tests, and this difference did not depend upon the day of

testing. There was a significant effect of day, $F(3, 39) = 10.850$, $p < 0.001$, but no significant group × day interaction, indicative of the relative consistency across days of the naltrexone-potentiated analgesia despite overall changes in both groups.

Recall that an additional experiment examined the effect of naltrexone in the absence of CWS. In this instance, naltrexone did not result in analgesic responses different from either baselines or saline controls as there was no significant group effect, $F(1, 10) = 1.251$, $p > 0.05$.

Exposure to this swim procedure decreased core and skin body temperatures in both groups 30 min after swim. Core body temperature had returned to baseline levels by 60 min after the swim. There were no differences between naltrexone- and saline-treated animals in temperature levels at any time point. Temperatures for both groups are presented in Table 1. No effect of drug was observed on core temperatures, $F(1, 10) < 1.0$, $p > 0.05$, but the decrease in core temperature evident at 30 min following swim was reflected in a significant difference between 30- and 60-min measures, $F(1, 10) = 17.73$, $p < 0.001$. There was no drug × measure interaction, $F(1, 10) = 1.035$, $p > 0.05$. Again, there was no effect of drug on skin temperature $F(1, 10) > 1.0$, $p > 0.05$, there was a difference between the measures, $F(1, 10) = 63.897$, $p < 0.001$, but no group × measure interaction, $F(1, 10) = 2.428$, $p > 0.05$. Skin temperatures remained reduced from baseline values at both the 30- and 60-min measures, but in no instance was there a difference in temperature between the two groups, indicating that the potentiated analgesia when naltrexone is combined with CWS is not due to differences in body temperature.

EXPERIMENT 2

The results of Experiment 1 replicated previous findings of opiate antagonist potentiation of CWS analgesia and added the finding that the phenomenon is not transient but rather persists across repeated exposures. The purpose of Experiment 2 was to determine whether this result is particular to CWS or whether it extends to other stressors that produce nonopioid analgesia. A nonopioid form of social defeat was used for this purpose.

Social Defeat

Social defeat involved exposure to an established colony of two male Holtzman retired breeders that were housed in a separate room and maintained on a reverse 12 D : 12 L schedule, with the exception that low-intensity red lights were left on at all times in the colony room so that the experimenter could observe behavior during the "dark" cycle. These rats were housed in Plexiglas chambers 50 × 50 × 40 cm and received enough food to maintain their body weights (average about 650 g), but they generally did not have any food left when they were fed each day. This slight restriction tended to increase activity over an ad lib diet. Aggressors were evaluated for aggression toward an intruder following at least a 45-day period in which they were left undisturbed and allowed to form stable colonies. Those colonies that did not show aggression toward sample intruders were culled from the experiment. Testing occurred shortly after the lights went out at 0900 h as this is when the colonies tended to be most aggressive.

TABLE 1

SKIN AND CORE BODY TEMPERATURES (±SEM) OF RATS EXPOSED TO CWS FOLLOWING INJECTIONS OF EITHER NALTREXONE (NAL) OR SALINE (SAL)

	Baseline	30 min	60 min
Skin body temp.			
NAL	89.283/0.729	84.467/0.305	83.117/1.21
SAL	90.667/0.846	83.663/0.396	81.467/1.294
Core body temp.			
NAL	96.35/1.016	93.7/0.514	97.283/0.482
SAL	97.467/0.516	92.867/1.517	96.833/0.852

Procedure

Test subjects were male Holtzman rats weighing between 250 and 375 g. Animals received either naltrexone (14 mg/kg, SC; $n = 5$) or equivolume saline ($n = 5$) 20 min before exposure to social defeat. Nineteen minutes after injection, intruders were brought into the room housing the aggressive colonies and baseline tail-flick measures were determined. Intruders were then placed in the home cages of the retired breeder colonies for 10 min. During this time, four measures were recorded: time to first bite, time to the first observation of defensive behavior in the intruder (see below), total number of bites, and total time spent freezing. Bites were recorded upon vocalization of intruders unless there was clearly no contact between intruder and aggressor. Defensive behavior was characterized by several species-specific behaviors that included: rolling over on one's back or side and freezing, standing on two hindpaws with head and ears back and freezing, or freezing while on all four paws in the immediate presence of one of the aggressor rats. Freezing behavior was quantified when there was no movement present in the intruder, including a lack of vibrissae movement. Intruders were removed from the aggressor's cage for immediate tail-flick assay after 10 min. The analgesia data obtained following defeat consisted of four single trials, taken between 6 and 9 cm from the tip of the rat's tail and given immediately, 120 s, 360 s, and 600 s following the defeat experience.

An additional control experiment was conducted in the same manner except intruder animals were not directly exposed to the aggressive colonies. All manipulations were the same except an air-permeable Plexiglas barrier (50 × 40 cm) was inserted in the aggressor's cage, separating the aggressors from the intruder so that no contact could occur.

RESULTS

Figure 2 illustrates results of Experiment 2. Defeat did not produce a clear analgesic response. Postdrug baseline measures did not differ between groups, $F(1, 48) > 1.0$, $p < 0.05$, and were not included in the ANOVA. Repeated-measures ANOVA applied to the postdefeat tail-flick latencies revealed a reliable effect of days, $F(4, 32) = 7.443$, $p < 0.001$. The group effect collapsed across days was not reliable, $F(1, 8) = 3.184$, $p > 0.05$. However, rats that received naltrexone before defeat were analgesic on the first 3 days of testing as revealed by the significant group × day interaction, $F(4, 32) = 4.656$, $p < 0.01$. There was a difference between groups that was dependent upon the time of tail-flick, $F(3, 24) = 5.093$, $p < 0.007$. Neither the measure × day or the triple interaction of group, measure, and day were reliable. Analysis of simple effects determined that the groups differed

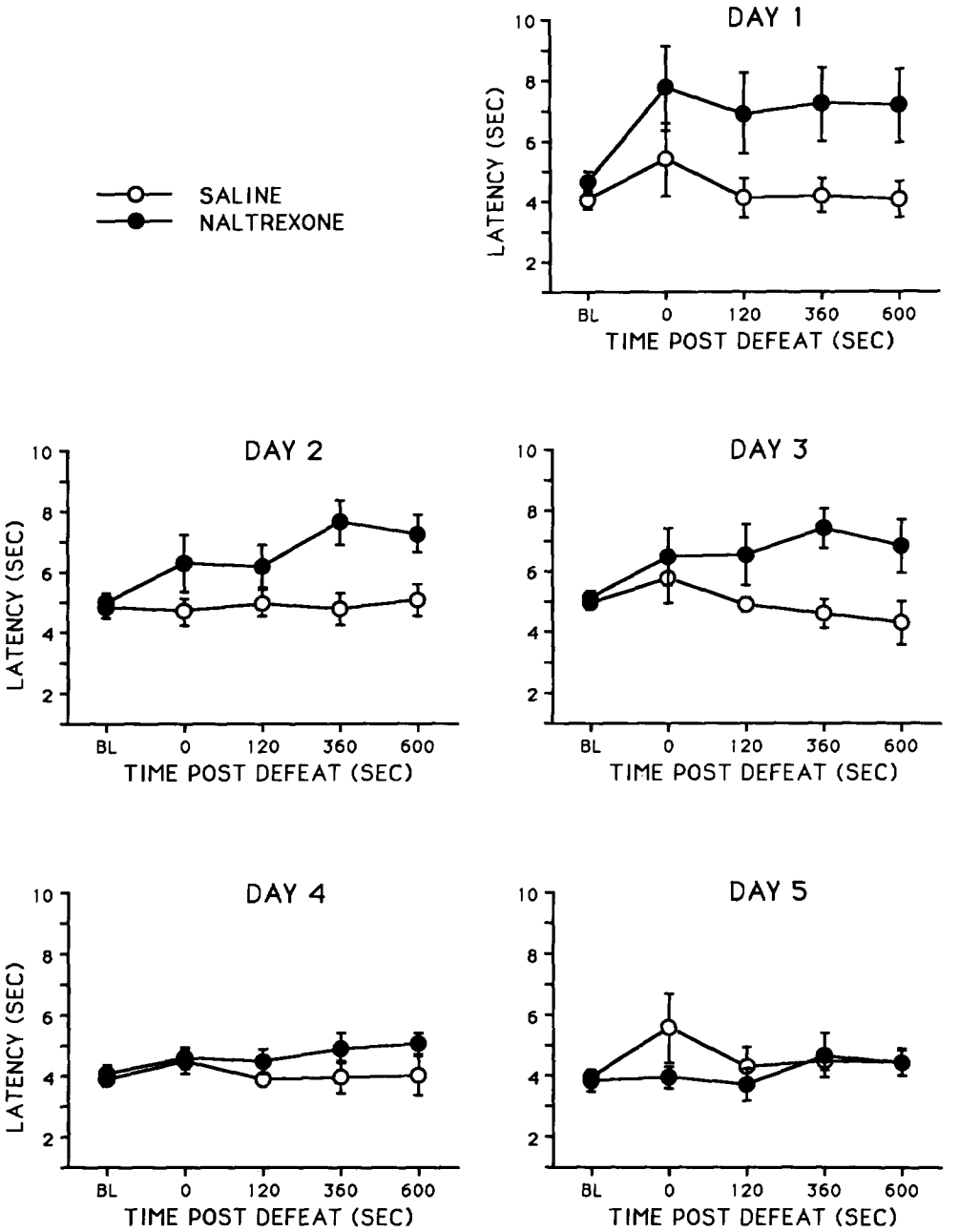

FIG. 2. Naltrexone + social defeat. Mean tail-flick latencies (±SEM) for naltrexone and saline animals in Experiment 2. Naltrexone (●) combined with social defeat produces analgesia over 5 days of chronic treatment compared to animals who receive saline and defeat (○).

reliably on days 1, 2, and 3 ($p < 0.05$) but that these differences were no longer present on the last 2 days of testing.

Naltrexone did not alter any of the defeat measures. Table 2 demonstrates the results of the factorial analysis: There were no differences in the number of bites, time to first bite, time to first instance of observable defensive behavior, or total time spent freezing between the two groups over all test days as all $F(1, 8) < 1.0$ and all $p > 0.05$. There were no reliable interactions involving either day of testing, $F(1, 8) = 1.897$, $p > 0.05$, or behavior measured $F(1, 8) < 1.0$, $p > 0.05$. When the experiment was repeated while preventing aggressive con tact between the colonies and intruders, naltrexone had no effect on pain sensitivity $F(1, 8) < 1.0, p > 0.05$.

EXPERIMENT 3

The foregoing results support the contention that a blockade of opiate systems can potentiate nonopioid SIA. This is

TABLE 2

BEHAVIORAL MEASURES TAKEN DURING DEFEAT (±SEM) OF RATS GIVEN EITHER NALTREXONE OR SALINE

	Time to 1st Bite (seconds)	Total Number of Bites	Time to 1st Submission (seconds)	Total Time in Submission (seconds)
Saline and defeat	350.48/51.63	9.0/2.47	242.36/39.95	582.0/101.08
Naltrexone and defeat	434.52/51.58	9.4/4.05	318.64/70.23	484.8/162.79

consistent with the collateral inhibition model proposed by Kirchgessner et al. (23). Experiment 3 was designed to explore an untested prediction of the model, that activation of opiate systems should inhibit nonopioid analgesia.

Animals again received five daily exposures to CWS but each was preceded by administration of morphine. Eighteen rats, with the same characteristics as in Experiment 1, were divided into three groups. Twelve animals were injected with morphine (1.5 mg/kg, SC) and 6 were injected with equivolume saline. Nine minutes after injections, they were brought to the test room, where baseline tail-flick latencies were determined. Ten minutes after the injection, half the morphine rats ($n = 6$) and all six saline rats were exposed to 4 min of 14°C water. Following swim, these animals were placed in the holding bins until removal at 30 and 60 min for tail-flick measures. The remaining six morphine rats were placed directly into the holding bins, without swim, to await tail-flick assay.

RESULTS

The results of Experiment 3 are depicted in Figure 3. A comparison of baseline measures before and after morphine on day 1 indicated that the small 1.5-mg/kg dose of morphine exerted a strong analgesic effect, but neither predrug (all three groups) or postdrug (morphine groups) baseline measures differed between groups on day 1, $F(2, 15) < 1.0$, and $F(1, 10) < 1.0$, respectively. Importantly, animals that received both morphine and CWS were less analgesic than those that were given only CWS, as shown in the overall group differences, $F(2, 14) = 6.918$, $p < 0.01$. There were differences between the tail-flick measures $F(1, 14) = 51.040$, $p < 0.0001$, reflecting the decrease in analgesia between the 30- and 60-min assays, and between the analgesia levels across days $F(4, 56) = 16.833$, $p < 0.0001$, especially due to the development of tolerance in the morphine groups. Interestingly, even though the analgesic effect of morphine decreased across days and was completely absent by day 5 morphine still reduced the analgesia produced by CWS. There was a significant group × day interaction $F(8, 56) = 2.438$, $p < 0.05$, and differences between groups at both 30 min, $F(2, 15) = 10.695$, $p < 0.001$, and 60 min, $F(2, 15) = 4.811$, $p < 0.05$, postswim. CWS and morphine produced equal levels of analgesia on day 1 but CWS was more potent than morphine thereafter as analysis of simple effects revealed group differences to be significant on days 2–5 ($p < 0.05$). Neuman-Keuls comparisons indicate a reliable difference ($p < 0.05$) between the saline-swim and the other two groups, which did not differ from each other.

EXPERIMENT 4

The previous experiment provided a clear demonstration that morphine and a nonopioid stressor interact in an antagonistic manner. Experiment 4 explored whether this result would generalize to social defeat.

METHOD

As in Experiment 2, 18 male Sprague-Dawley rats were divided into three groups. Twelve were injected with morphine (1.5 mg/kg, SC) and the remaining 6 received saline. The apparatus was the same as in Experiment 2. Nine minutes after injection, animals were moved down the hall and baseline tail-flick measures were taken in the experimental room. Half the morphine and all saline animals were then immediately exposed to 10 min of social defeat as described above. The remaining rats were placed in the aggressors' cages with a Plexiglas barrier preventing contact between aggressors and the intruder for 10 min. For both groups experiencing defeat, behavioral measures, as in Experiment 2, were taken throughout the defeat period. Immediately following this 10-min period, all rats were placed in gentle restraint and subjected to 10 min of tail-flick assay, taken at four time points: immediately, 2 min, 6 min, and 10 min postdefeat.

RESULTS

This experiment used a new colony of retired breeders that were more aggressive than the previous colony (see Table 3). Again, there were no differences in predrug measures of analgesia, $F(2, 15) < 1.0$, $p > 0.05$, or postdrug measures on day 1 between the two morphine groups, $F(1, 10) = 1.804$, $p > 0.05$. Here, defeat alone did result in analgesia, as did morphine administration. Groups differed significantly: Morphine analgesia was greater than that produced by defeat and was attenuated by defeat $F(2, 15) = 17.032$, $p < 0.0001$ (see Fig. 4). There was a change in analgesia over days, $F(4, 60) = 26.881$, $p < 0.0001$, and the overall group differences were dependent upon day of testing, $F(8, 60) = 3.921$, $p < 0.001$, as they were only significant on days 1–4. By day 2, animals that were exposed to defeat in addition to an injection of morphine were less analgesic than those that received morphine without direct exposure to the aggressive colonies. Differences between measures, indicating the decrease in analgesia levels over the 10-min measurement period, were significant, $F(3, 45) = 14.871$, $p < 0.0001$. A group × tail-flick measure interaction was also evident, $F(6, 45) = 2.458$, $p < 0.05$, demonstrating that these changes depended upon the defeat and drug status of the group. There were differences across days, $F(6, 90) = 61.491$, $p < 0.0001$, which depended upon group, $F(12, 90) = 9.330$, $p < 0.0001$. There was no measure × day interaction, but the three-way relationship involving group was significant, $F(24, 180) = 1.633$, $p < 0.05$. As Fig. 4 reveals, and Neuman-Keuls comparison confirmed, the group that received only morphine was more analgesic

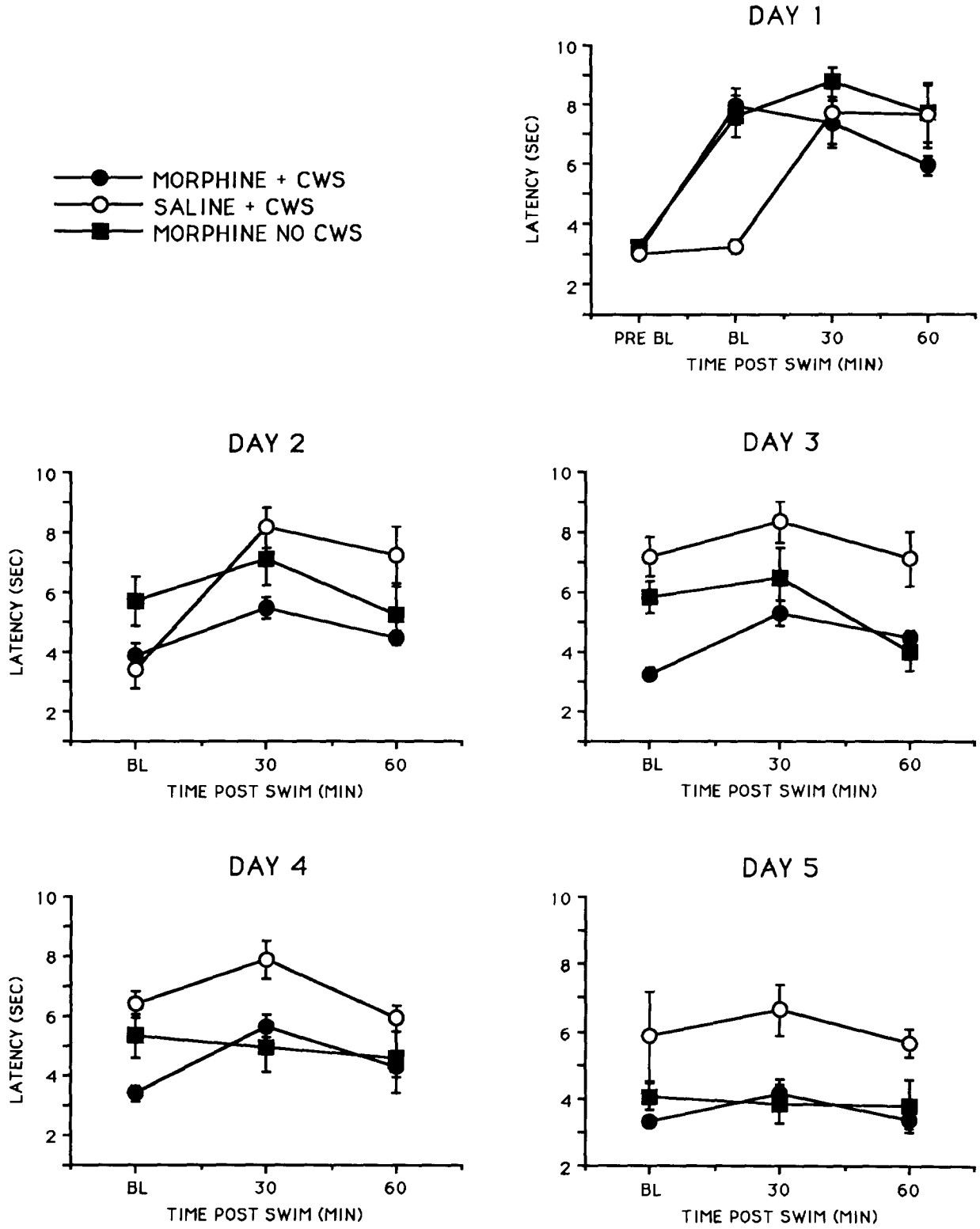

FIG. 3. Morphine + cold water swim (CWS). Mean tail-flick latencies (±SEM) for groups experiencing morphine (■), morphine + CWS (●), or saline + CWS (○) over 5 days of testing. Animals receiving morphine + CWS were less analgesic than those just receiving CWS.

TABLE 3
BEHAVIORAL MEASURES TAKEN DURING DEFEAT (±SEM) OF RATS GIVEN EITHER MORPHINE OR SALINE

	Time to 1st Bite (seconds)	Total Number of Bites	Time to 1st Submission (seconds)	Total Time in Submission (seconds)
Saline and defeat	232.95/36.63	45.17/7.27	188.17/29.57	1476.0/59.335
Morphine and defeat	277.19/38.32	34.5/4.72	188.91/30.89	1482.67/144.77

than either of the other two groups, which did not differ from each other.

There were no observable differences in the defeat experience between morphine- and saline-defeated animals, as factorial analysis revealed all $F(1, 10)$ to be less than 1.52 and all $p > 0.05$, (see Table 3).

GENERAL DISCUSSION

Two forms of nonopiate analgesia were affected by manipulations of opiate systems in these experiments. These results indicate an interaction of opioid and nonopioid mechanisms in some forms of stress-induced analgesia. Opioid and nonopioid analgesias were demonstrated to be mutually inhibitory, supporting the model of collateral inhibition as put forth by Kirshgessner, Bodnar, and Pasternak (23). Nonopioid SIA produced by either CWS or social defeat was enhanced in the presence of the opiate antagonist naltrexone. These same SIAs were found to attenuate the analgesic effect of morphine.

Animals administered naltrexone for 5 consecutive days in combination with CWS demonstrated a potentiation of the SIA. Experiment 1 replicated the results reported by Kirchgessner et al. (23) and Yoburn et al. (52) that opiate antagonists potentiate analgesia produced by CWS and added the new finding that this potentiation continues to occur with repeated exposures to naltrexone and CWS. By classic criteria, this analgesia would be presumed to be mediated by nonopiate mechanisms because it is not attenuated by the opiate antagonist naltrexone. Unlike traditional characterizations of nonopiate analgesia, however, naltrexone did have an effect in that it potentiated this SIA.

CWS has also been reported to produce SIAs that are either unaffected by opiate antagonism or reduced by antagonist administration (16,19,42). The effects of opiate antagonism appear to depend upon the specific parameters of the CWS procedure, including manipulations of both time and temperature. For example, Girardot and Holloway (16) found three different results: that analgesia produced from CWS in 2°C water was significantly antagonized by 14 mg/kg systemic naltrexone following intermittent swim (18 10-s exposures, 3/min), was unaffected by continuous swim (3.5 min), and potentiated after prolonged intermittent swim (60 1-s exposures 12/min). Bodnar and Sikorszky (8) found that naloxone (10 mg/kg, SC) antagonized the analgesia resulting from 3.5 min of CWS in a 15°C bath but did not effect analgesia after baths of the same duration of 8 or 2°C. Although there are some patterns evident in these and other data relating to SIA, as yet there are no conclusive or complete explanations at either the behavioral or physiological levels.

These differences in the impact of naltrexone on CWS analgesia following different stress parameters may reflect the relative activity of opiate and nonopiate analgesia mechanisms. SIAs are typically described categorically as either opiate or nonopiate, but stressors might instead activate a blend of processes, with pure opiate and nonopiate analgesia being ends of a continuum. Perhaps opiate antagonists block only relatively pure opiate analgesias and have no affect when the stressor results in a purely nonopiate activation. Although the specific mechanisms of a collateral inhibition model are not yet clear, one might expect that opiate antagonists can potentiate analgesia when activation is mixed. Here, removal of opiate inhibitory input on nonopiate systems may outweigh any reduction in opiate-induced analgesia.

The potentiation of analgesia resulting from opiate antagonism would be especially likely if there is an asymmetry between the ability of opioid activation to produce measurable analgesia and inhibit nonopioid systems. It might be reasonable to suppose that opiate activity can more potently inhibit nonopiate analgesia systems than it can produce behavioral analgesia. The opiate–nonopiate interaction might occur on the same neuron or in a small set of neurons, most likely in the spinal cord (47,48,50). The production of analgesia by endogenous opiates, on the other hand, would require activation of a larger neural circuit. Such an asymmetry might be especially apparent at low levels of opiate activation. Because neither the exact substrates of nonopiate analgesia nor the mechanisms for the interaction are known, this hypothesis is not readily testable. Nonetheless, a necessary assumption of the collateral inhibition model is that the inhibitory interaction between opioid and nonopioid substrates requires less activity than does the outcome of observable analgesia. If this were not the case, any potentiation of nonopiate analgesic mechanisms by naltrexone would be canceled by the attenuation of opiate analgesia. Without the asymmetry assumption, a collateral inhibition model cannot produce an observable net effect of an opiate antagonist. Unfortunately, collateral inhibition is difficult to assess under circumstances in which the measured output (analgesia) is identical for both systems.

Experiment 2 extended the findings of Experiment 1 to a different stressor, social defeat. The defeat did not produce a measurable analgesia by itself in this experiment, probably because the colonies were not aggressive. Behavioral measures taken throughout the defeat experience indicated fewer bites and total time spent in freezing as well as increases in the average time to first bite or first instance of defensive behavior, as compared to other studies in our laboratory in which defeat did result in analgesia (see Experiment 4). It is important to note here that in none of the studies were there any differences in the defeat experience between groups, that is, analgesia differences between experimental groups do not reflect differences in aggression or defeat within a study. In Experiment 2, analgesia did result from a relatively low level of aggressiveness if naltrexone had been administered. Like CWS, social defeat in rats has been shown to produce three

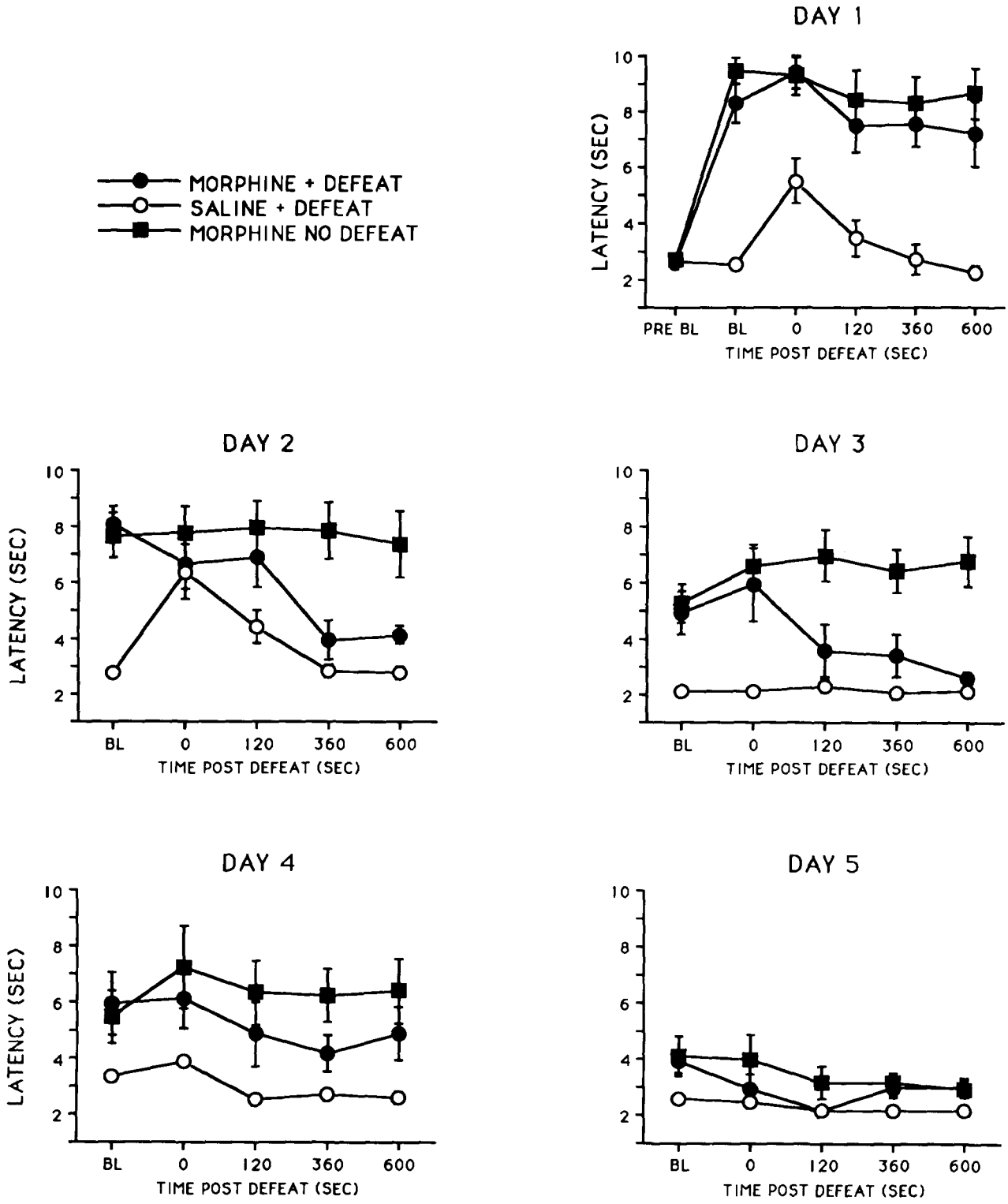

FIG. 4. Morphine + social defeat. Mean tail-flick latencies (±SEM) for groups experiencing morphine (■), morphine + defeat (●), or saline + defeat (○) over 5 days of testing. Animals receiving morphine + defeat were less analgesic than those just experiencing defeat.

"classes" of analgesia. It is sometimes unaffected by opiate antagonism (33), attenuated (34), or, as demonstrated here, enhanced. Defeat-induced analgesia is better characterized in mice than in rats. There, the presence of opiate or nonopiate analgesia has been shown to be dependent upon specific parameters of intensity (35), duration (36), and strain (24).

It also might be noted here that although the dose of naltrexone used in these studies was large (14 mg/kg) it had no effect on tail-flick latencies in the absence of either CWS or defeat. These results probably do not reflect a dose effect as we have found that a 7-mg/kg dose also resulted in potentiated tail-flick latencies following defeat in other (unpublished) studies. Other groups have also found potentiated analgesia resulting from such stressors as a hot-plate test after lower doses, such as 5 mg/kg (17,32,49).

The fact that analgesia resulted from a combination of naltrexone with a defeat regimen that was insufficient to produce any measurable analgesia by itself is consistent with the idea that opiate antagonist enhancement of analgesia occurs under conditions of low opiate system activation. Further, such effects might be more easily observed after the application of relatively mild stressors that only weakly activate both systems. Under such conditions, opiate systems might readily inhibit nonopiate output but be insufficient to produce measurable opiate analgesia. Indeed, opiate antagonist potentiation of analgesia has been most often reported after the application of weak stressors (as judged by the amount of analgesia) such as confinement to a hot plate for a brief period of time (32,49). In the present experiments, the CWS produced only weak analgesia with no animals at cutoff tail-flick latencies, and defeat produced either no analgesia (Experiment 2) or weak analgesia (Experiment 4).

Experiments 3 and 4 further support a mutually inhibitory relationship between opioid and nonopioid analgesia as morphine combined with SIA in a subtractive manner. In Experiment 3, CWS produced analgesia, as did morphine. When both the opiate agonist and the nonopiate SIA were combined, the resulting analgesia indicated that these manipulations detracted from each other's analgesic potency. Rats that received a low dose of morphine (1.5 mg/kg, SC) combined with CWS for 5 consecutive days became significantly less analgesic than did rats that received only the CWS. Before observable morphine tolerance had developed (days 1–3), animals that received both morphine and CWS also tended to be less analgesic than those that received only morphine. Thus, the analgesia resulting from combining morphine and CWS produced less analgesia than did either manipulation alone. Moreover, the low dose of morphine continued to interfere with CWS analgesia even after tolerance had developed to morphine's analgesic effects. This again suggests an asymmetry in the ability of low levels of opiate activity to produce analgesia output vs. inhibiting nonopioid analgesia systems.

In Experiment, 3 there was evidence of conditioning of analgesia to the experimental room. There were increased baseline tail-flick latencies in the saline + CWS group over the 5 days of the experiment, and morphine seemed to prevent this effect. The differences between the saline + CWS and the other groups are therefore potentially confounded. However, an additional study demonstrated this conditioned analgesia to be brief, lasting less than 21 min, and therefore not able to account for the higher tail-flick latencies at 30 and 60 min after swim in the saline group. The conditioned analgesia was also shown to involve opiate mechanisms because it was partially reversed by naltrexone. Therefore, it appears that the conditioned analgesia does not persist long enough to be responsible for the high levels of analgesia throughout the test sessions and is unlikely to contribute to the differences between CWS groups due to its opioid nature. Moreover, this argument cannot be applied to the day-2 data because the increased baseline in the saline + CWS group did not appear until day 3. However, the inhibitory interaction was still present on day 2. Finally, this argument cannot be applied to Experiment 4 because conditioned analgesia did not occur. Baselines in the saline + defeat group did not increase across days.

Experiment 4 also demonstrated an inhibitory interaction between morphine analgesia and social defeat. Animals that received both defeat and morphine were less analgesic than those that had received only morphine. This interaction was especially interesting because defeat itself produced little analgesia, far less than that produced by morphine. On the first and second treatment days, there was a small analgesia observable immediately following defeat that had dissipated by 2 min after defeat. By the third day, there was no analgesia observable even immediately after defeat. In contrast, morphine-induced analgesia was more potent and persisted for the 10-min measurement period. Nevertheless, defeat interfered with morphine analgesia on all days (except day 5, by which time morphine tolerance had developed and there was no analgesia to interfere with) across the entire measurement period. This suggests a similar asymmetry as that implied in opiate systems. Nonopiate systems involved in analgesia appear to inhibit opiate processes more readily than they produce analgesic output under conditions of low activity.

These results differ from those expected to occur when two independent manipulations that produce analgesia are combined. It could be expected that: a) the resulting analgesia would be greater than that produced by either factor alone, with either additive or synergistic effects being possible; or b) the measured analgesia would be equal to that produced by the more powerful of the two factors due to ceiling effects. Indeed, previous studies have found an additive or multiplicative relationship between the analgesic effects of morphine and other opioid-mediated analgesic manipulations. For example, immobilization (2), stimuli paired with shock (37), and 2-deoxy-glucose (7) each summates with morphine and produces naloxone-reversible analgesia. Instead, the present results along with those reported by Kirchgessner et al. (24) and Steinman et al. (38) support the existence of a mutually inhibitory, rather than independent, relation between opiate and nonopiate systems. Again, the rules that determine whether there is no effect of one type of analgesia on another, an enhanced effect, or a subtractive effect as found here are not clear at this time. What does seem to be evident is that all of these outcomes are possible and that a thorough understanding of analgesia systems must incorporate a consideration of these effects and their fundamental substrates.

The mechanism by which the mutual inhibition might occur is unknown (38). A number of neuropeptides are known to interfere with morphine-produced analgesia. Cholecystokinin (13), thyrotropin-releasing hormone (46), adrenocorticotropic hormone (40), melanocyte inhibitory factor-1 (15,20), α-melanocyte-stimulating hormone (39), and FMRF-amide (21) have all been reported to inhibit the analgesic effect of opiates. All of these can be released by stress and so are potential candidates as mediators of the interference of morphine analgesia produced by defeat and CWS. Opioids can, in turn,

inhibit the release of at least some of these peptides (18,51). In addition, the κ-opiate receptor ligand dynorphin has been shown to inhibit μ-receptor activity in a number of studies (11,14,43), suggesting that the mechanism for collateral inhibition may involve differential action of receptor subtypes within the opiate class. Any of these could be involved in the mediation of the effects reported here. What is clear is that analgesia may not result only from the presence of either opiate or nonopiate mechanisms but rather the complex relationship of these two components.

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