# Opioid-Like Analgesia in Defeated Mice

Abstract. *Mice exposed to repeated attacks by other mice showed decreased nociception in response to radiant heat focused on their tails. This form of analgesia was blocked by centrally acting opiate antagonists and was not observed in morphine-tolerant mice; furthermore, mice repeatedly subjected to defeat showed much less analgesia after receiving morphine than mice that were not subjected to defeat. Mice of the CXBK strain, which respond weakly to morphine, displayed only moderate analgesia following defeat. These findings suggest that endogenous opioid–mediated analgesic mechanisms are readily activated by situations involving biologically significant forms of stress, such as defeat.*

The possible role of endogenous opioids, or endorphins, in the modulation of pain has been studied intensively since the discovery of these peptides and their receptors (*1*). Endorphins appear to be directly involved in the suppression of pain as a result of electrical stimulation of discrete neural structures and acupuncture (*2*). Yet a physiological function for endorphins in the types of analgesia that are engendered by certain life experiences has not been established. Anecdotes are often told about individuals who failed to react to pain in affect-evoking situations. This stress-induced analgesia has been confirmed experimentally (*3*). That endogenously modulated nociception is dependent on endorphin activity has been questioned because moderate doses of the opiate antagonist naloxone often fail to reduce stress-induced analgesia (*4*) and because full cross-tolerance between morphine analgesia and stress-induced analgesia has not been demonstrated (*5*). However, under certain conditions such analgesia can be attenuated by a low dose of naloxone and by the induction of tolerance to morphine (*6*, *7*); moreover, this analgesia may be correlated with indices of opioid activity in the brain and in blood plasma (*8*). Many researchers now differentiate between opioid and nonopioid mechanisms of analgesia (*6*).

We report that defeat in a social confrontation between two mice leads to pain suppression that appears to be mediated by endogenous opioids. Initially, the number of attacks sustained by a mouse was related to the magnitude and time course of the analgesia. We determined the baseline response of nine adult $B6AF_1$ mice (*9*) to radiant heat focused on the tail (*10*) and then placed these mice individually into the home cages of adult male CFW mice. After the intruder mouse had been attacked 20 times, each attack including a bite, it was removed from the resident's cage. The degree of nociception induced by the attack was immediately assessed by the tail-flick assay. The intruder mouse was then placed in the home cage of another resident. This procedure was repeated five times and lasted 5 to 10 minutes. The intruder mouse was returned to its home cage after the tail-flick test following the 100th bite. Five additional tail-flick tests were then administered at 10-minute intervals.

As the total number of bites sustained by the intruders increased, more and more intruders became submissive and tolerated the heat stimulus for longer periods (Fig. 1A). Tail-flick latencies returned to normal 40 to 50 minutes after the last attack. Resident mice did not show altered levels of nociception (Fig. 1A).

In another experiment, 15 intruders were exposed to residents behind a wire screen and thus not attacked. These mice showed significantly less analgesia than the defeated mice after the fifth tail-flick test (mean tail-flick latency, 3.63 ± 0.33 seconds) [$F(1, 23) = 29.72$, $P < .01$]. Another group ($N = 15$) that was not exposed to residents also showed significantly less analgesia than the defeated mice on the fifth tail-flick test (mean latency, 2.82 ± 0.35 seconds) [$F(1, 23) = 42.27$, $P < .01$]. Finally, mice were placed one at a time into an empty cage, and the animal's back and tail (areas bitten most frequently by attacking residents) were pinched 100 times with mouse tooth forceps at a rate approximating that of an attacking mouse. Tail-flick latencies were assessed after every 20 pinches. Pinching failed to produce the analgesia observed in the defeated mice (mean latency after 100 pinches, 1.87 ± 0.12 seconds) [$F(1, 13) = 45.9$, $P < .01$].

The emergence of analgesia was associated with a specific defeat behavior. When attacked, threatened, and pursued by the resident, the intruder initially reacted with flight and defensive behavior (*11*). Eventually, however, the intruder displayed all elements of a specific posture of defeat, characterized by an upright position, limp forepaws, upwardly angled head, and retracted ears (Fig. 2). The defeated animal assumed this pos-

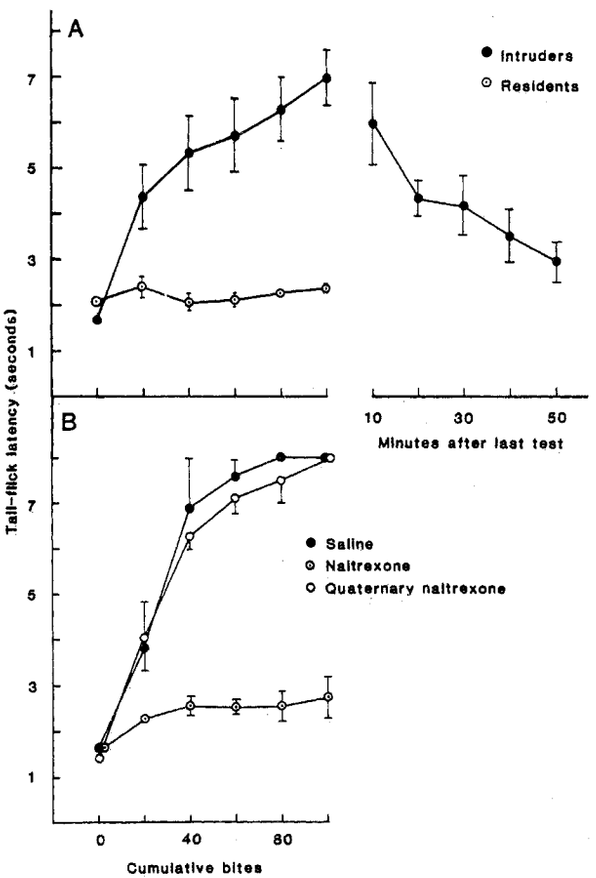


Fig. 1. (A) Tail-flick latencies in intruder mice ($N = 9$) as a function of being bitten and in resident mice ($N = 5$) as a function of attacking an intruder. Values are means ± standard errors. Latencies were determined before any fighting experience and after 20 bites. The heat stimulus turned off automatically after 8 seconds. (B) Tail-flick latencies in intruder mice that were injected with saline ($N = 5$), naltrexone ($N = 7$), or quaternary naltrexone ($N = 6$) 20 minutes before the first test, expressed as a function of exposure to bites by nontreated resident mice.


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Fig. 2. Defeated mouse in characteristic posture.

ture even before being attacked, squealed when approached, and failed to track the opponent with shifts in body position. In three series of experiments, every mouse showing the full posture of defeat concomitantly showed full analgesia in the tail-flick assay. Analgesia was not directly dependent on the number of bites—and presumably the amount of pain—but on whether or not the bites produced defeat. Although most mice assumed the posture of defeat after receiving 50 to 70 bites, some individuals did so after only 20 bites. A few failed to show defeat after as many as 100 bites (*12*). To eliminate observer bias in the determination of defeat, we subsequently defined the intruder's analgesic response after it received a standard number of bites exceeding the number necessary to produce defeat in most mice.

We next attempted to block the analgesia in defeated mice with opiate antagonists. Following determinations of baseline tail-flick latency, we injected separate groups of mice intraperitoneally with naloxone (0.3, 1.0, 3.0, or 10.0 mg/kg) or saline. Twenty minutes later the mice were subjected to 70 bites and then given the tail-flick test. Naloxone-treated mice showed significant dose-related decreases in the level of analgesia following defeat. Mean tail-flick latencies were as follows: saline ($N = 5$), 7.62 ± 0.38 seconds; naloxone at 0.3 mg/kg ($N = 6$), 3.57 ± 0.87 seconds [$t$ (10) = 3.52, $P < .01$]; naloxone at 1.0 mg/kg ($N = 16$), 2.90 ± 0.52 seconds [$t$ (20) = 5.02, $P < .01$]; naloxone at 3 mg/kg ($N = 7$), 3.41 ± 0.69 seconds [$t$ (11) = 5.19, $P < .01$]; and naloxone at 10 mg/kg ($N = 16$), 2.41 ± 0.40 seconds [$t$ (20) = 5.54, $P < .01$].

In an additional experiment, we used two forms of naltrexone in order to differentiate between the central and peripheral components of analgesia blockade by opiate antagonists. In vitro assays show that naltrexone is about 40 times more potent than its quaternary derivative, but some behavioral tests indicate that the two forms have similar potency (*13*). We administered naltrexone (1 mg/kg), quaternary naltrexone (40 mg/kg), or saline to separate groups of mice 20 to 30 minutes before subjecting them to defeat. Naltrexone blocked analgesia in the defeated mice and quaternary naltrexone did not. Apparently this form of analgesia is mediated in the central nervous system and not at peripheral sites.

An important test for the involvement of endogenous opioids in stress-induced analgesia is the demonstration of full cross-tolerance to and from morphine. Mice were given tail-flick tests to establish baseline response levels and then were assigned to experimental and control groups. Experimental mice were implanted subcutaneously with a 75-mg pellet of morphine base (*14*); control mice received a placebo pellet. One week later, when body weight and tail-flick latencies had returned to control levels in the mice with morphine pellets, both groups were subjected to 70 bites and then given the tail-flick test. Whereas response latencies were substantially increased in the control mice, latencies in the mice with morphine pellets showed only a slight increase (Table 1).

Table 1. Cross-tolerance between morphine analgesia and defeat-induced analgesia. Values are means ± standard errors.

| Treatment groups | $N$ | Tail-flick latency (seconds) |
|---|---|---|
| *Challenge with defeat* | | |
| Morphine pellet (7 days) | 11 | 0.90 ± 0.36 |
| Placebo pellet | 10 | 4.55 ± 0.65 |
| *Challenge with morphine sulfate* | | |
| Daily defeat (14 days) | 12 | 0.43 ± 0.11 |
| Control | 14 | 5.66 ± 0.33 |

We next examined the analgesic response to morphine in mice with a history of defeats. Mice were subjected to 70 bites daily for 14 days. Control mice were not subjected to any attacks. On day 15, all the mice were injected with morphine sulfate (5 mg/kg, intraperitoneally) and given tail-flick tests. The mice that had been defeated repeatedly showed almost no analgesic response to morphine, while the control mice developed strong analgesia (Table 1). These experiments demonstrate full cross-tolerance between morphine analgesia and defeat-induced analgesia to an extent not observed in studies of analgesia induced by other forms of stress (*5*).

It is striking that exposure to attacks from another mouse for 5 minutes per day can produce tolerance equal to that caused by a 75-mg morphine pellet. To learn more about the development of cross-tolerance, we repeated the preceding experiments with varied parameters. In six mice subjected to daily defeat, the

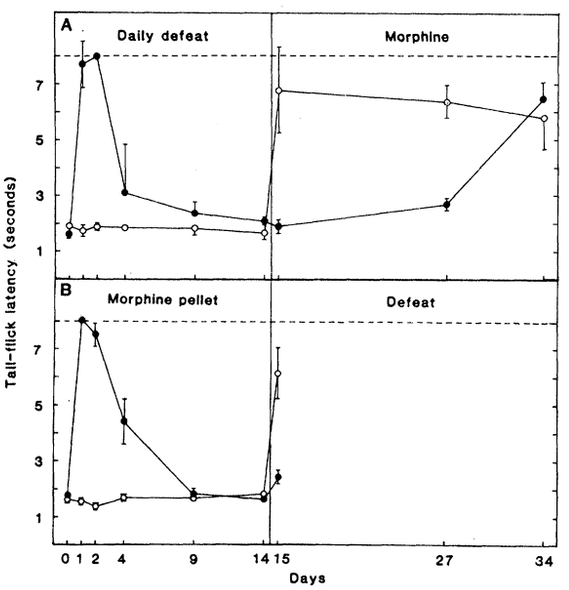
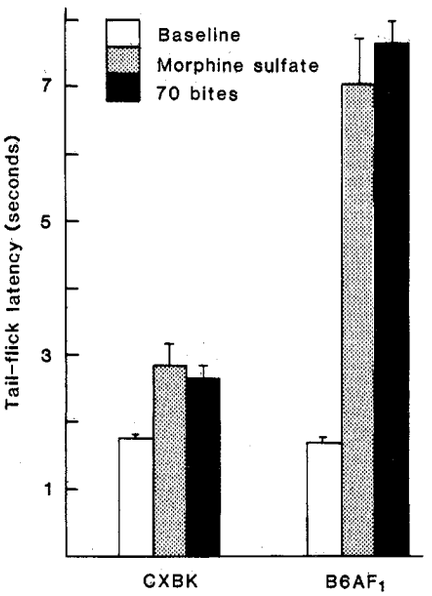



Fig. 3. (A) Tail-flick latencies in intruder mice ($N = 6$) subjected to 100 bites by resident mice every day for 14 days (●) and in control mice ($N = 6$) not subjected to attack (○). Tail-flick latencies were measured before the first attack (day 0) and periodically thereafter (days 1, 2, 4, 9, and 14). On days 15, 27, and 34 the defeated mice and the controls were administered morphine sulfate and given tail-flick tests 30 minutes later. (B) Tail-flick latencies in mice implanted with a morphine pellet ($N = 10$) (●) or with a placebo pellet ($N = 5$) (○). Tail-flick tests were performed before pellet implantation (day 0) and periodically thereafter (days 1, 2, 4, 9, and 14). On day 15, mice with morphine and placebo pellets were exposed to 100 bites and tested for tail-flick latency.

Fig. 4. Effects of morphine sulfate or exposure to 70 bites on tail-flick latencies in groups of CXBK mice (six received morphine and 14 were bitten) or B6AF$_1$ mice (six received morphine and five were bitten). Values are means ± standard errors.




time course of tolerance development was delineated more precisely by repeated tests for analgesia during the 2-week period. Also, groups of mice implanted with morphine pellets or placebo pellets underwent tests for cross-tolerance after 14 days.

Both the mice with morphine pellets and the defeated mice showed maximal analgesia on days 1 and 2 (Fig. 3, A and B). The time course of morphine tolerance development was comparable to that for mice subjected to repeated defeat (Fig. 3B). Tests for cross-tolerance on day 15 produced essentially the same results as those obtained in the preceding experiments; mice exposed to morphine pellets or to defeat failed to show analgesia when challenged with the opposite treatment. Tolerance to the effects of repeated defeat appears to be long-lasting. The response to morphine took about 3 weeks to return to control levels (Fig. 3A).

In a further experiment, we measured defeat-induced analgesia in CXBK mice. Compared to B6AF$_1$ mice, these mice have a reduced analgesic response to morphine, considerably fewer brain opiate receptors (*15*), and much less electroacupuncture analgesia (*16*). After receiving 70 bites, the CXBK mice showed much less analgesia than B6AF$_1$ mice (Fig. 4). In both strains the magnitude of analgesia following exposure to attack paralleled that following morphine administration to groups of mice that were not subjected to attack.

Most of the studies of stress-induced analgesia in which rats were used have implicated nonopioid-mediated pain pathways (*8*). In the few studies reporting naloxone-reversible analgesia, mice were used (*17*). The present results may reflect a species difference in the capacity of rats and mice to activate endogenous opioid systems in response to stress (*18*). However, analgesia from cold water, which is nonopioid-mediated, has been reported in mice (*19*). In addition, opioid-mediated pain-inhibition systems can be activated in rats when certain temporal and spatial requirements of the stimulus presentation are met (*6*). For example, analgesia produced by intermittent foot shock for prolonged periods (30 minutes) is naloxone-reversible, but analgesia produced by continuous foot shock for brief periods is not. Analgesia produced by shocking the forepaws of rats is naloxone-reversible, whereas analgesia produced by shock to the hindpaws is not. These results would suggest that defeat-induced, naloxone-reversible analgesia is probably not limited to mice.

Unlike foot shock analgesia, analgesia from defeat can be induced over a wide range of experimental parameters. Significant naloxone-reversible analgesia is evident in mice receiving only 20 bites in just 1 minute; analgesia produced by foot shock over a similar period is not reversible with naloxone. Furthermore, defeat-induced analgesia does not require that the stimulus be delivered to any one part of the body. We recently found that mice take about 300 percent longer to react to a hot plate after being bitten just 20 times.

Defeat in a social confrontation is stressful and engenders analgesia. Yet, attacking mice show no increase in tail-flick latencies, even though they receive occasional retaliatory bites by the intruders and experience substantial pituitary-adrenal activation while attacking (*20*). Apparently, the special biological significance of the defeat experience, and not simply the experience of being stressed, is critical to the occurrence of opioid-like analgesia.

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