4. The surgery always involved the recession of one of the horizontal rectus muscles; the muscle (at the tendon) was moved backward on the globe and reattached. In about half of the cases, a resection of the other horizontal muscle was also performed. Resection involves the removal of most or all of the tendon and the reattachment of the muscle to its point of original insertion [H. M. Burian and G. K. Von Noorden, *Binocular Vision and Ocular Motility* (Mosby, St. Louis, 1974), pp. 434–450].
5. A conservative estimate of the amount of rotation of the eye produced by the surgery was assessed by alternate cover tests after 1 week. At the time of the pointing test, the amount of eye turn from surgery was usually more than that found 1 week later, as the eye "settles in" to its resting position.
6. A model predicting these opposite effects requires the concept of a cyclopean projection center for the origin of egocentric localization. The position of the covered eye will influence localization responses made with the uncovered eye (H. Ono and E. Weber, *J. Exp. Psychol. Hum. Percept. Perform.*, in press). If we assume that our strabismic patients also have some binocular interaction when pointing monocularly, we can account for the directions of the shifts. Assume that the covered or nonseeing eye, irrespective of whether it has been operated on or not, is registered as being too far nasal, that is, not converged to the same point in space as the seeing eye, but overconverged. The direction of the target would then appear to be shifted toward the uncovered eye.
7. The surgery can be considered anatomically symmetrical in the recess-resect procedure. It is as if the muscles are detached from the globe, the globe turned, and the muscles then reattached. In a mechanical sense the length-tension characteristics, and possibly any spindle afference, could also be unaffected by this form of surgery. But, much, if not all, of the tendon is removed in the resected muscle, so any potential afference from the tendon organs would be lost. These tendon receptors are found in human extraocular muscle [(*1*), p. 154]. In the recession-only procedure, there is mechanical, and presumably proprioceptive, asymmetry. No consistent pattern emerges between the recess-resect or recess-only procedures (Table 1).
8. P. B. C. Matthews, *Mammalian Muscle Receptors and their Central Actions* (Arnold, London, 1972), pp. 121–126. Because the surgery is almost entirely done on the tendons, we have assumed that only tendon organs are affected. It is possible, however, that the spindle organs in the distal third of the muscle may be affected as well (this is particularly so when there is a large resection of the medial rectus). We are currently looking at autopsy eye muscle specimens in an attempt to verify the receptor destruction that results from strabismus surgery.
9. E. Ludvigh, *Arch. Ophthalmol.* **48**, 442 (1952); see also (*1*), pp. 244–246.
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Long-Term Stress-Induced Analgesia and Activation of the Opiate System

Abstract. *Exposure of rats to a series of inescapable shocks produced in sequence both an early naltrexone-insensitive and a late naltrexone-reversible analgesic reaction. Activation of the opiate system was necessary and sufficient to produce an analgesic reaction 24 hours later on exposure to a small amount of shock. The amount of inescapable shock which induced naltrexone-reversible analgesia also produced hyperreactivity to morphine 24 hours later.*

Exposure to a variety of painful or stressful events produces an analgesic reaction (*1, 2*). This phenomenon, called stress-induced analgesia (SIA), has received considerable attention because it has been thought to provide insight into the psychological and physiological factors that activate endogenous pain control and opiate systems. Both electrical stimulation and opiate peptide microinjection into portions of the medial brainstem elicit analgesia (*3*). It has been speculated (*4*) that endogenous opiates are released in response to stress and inhibit pain by activating this midbrain system. However, the results of manipulations designed to assess the involvement of opiate systems in SIA, such as reversal by opiate antagonists, establishment of cross-tolerance to morphine, and pituitary and adrenal manipulations to prevent the release of β-endorphin, have been inconsistent (*2, 3*). Consequently a number of investigators have proposed that both opiate and nonopiate forms of SIA exist (*2, 3, 5*).

If there are opiate and nonopiate forms of SIA, it is important to learn what determines which form occurs. Recently, Lewis *et al.* (*5, 6*) suggested that the temporal characteristics of the aversive stimulation may be critical in determining which type of analgesia is elicited. They found that naloxone, dexamethasone, and long-term treatment with morphine blocked the analgesia elicited by 20 minutes of intermittent foot shock. However, 3 minutes of continuous foot shock resulted in an analgesia that was unaffected by these manipulations. Thus either the pattern, amount, or duration of shock is critical.

Maier and his colleagues have shown that the inescapable shock used to induce learned helplessness (80 5-second shocks at 1-minute intervals) (*7*) produces both the usual short-term SIA measured within 30 minutes of the end of the session and a long-term form that can be reinstated 24 hours later by a brief exposure to shock (*8*). Both of these analgesic reactions were blocked by naltrexone (*9*) and were completely cross-tolerant with morphine (*10*). Moreover, this opiate-mediated SIA is only activated if the subject was previously exposed to inescapable shock (*8*). Rats that were allowed to escape shock did not experience analgesia, whereas subjects administered an equivalent amount of uncontrollable shock did. This was true for both short-term and long-term SIA. The organism's learning that there is no escape from an aversive stimulus may, therefore, be another variable determining whether opiate systems are activated.

It should be noted that the stressor in studies of SIA has typically been inescapable. Further, if learned helplessness is important in triggering opiate-mediated SIA, then the shock parameters should be critical. Many shocks over an extended period may be required for such learning to occur. A brief exposure—whether intermittent or continuous—should be insufficient for such learning and should lead only to nonopiate-mediated changes in pain reactivity. It follows that both nonopiate and opiate forms of SIA should occur sequentially during a long series of inescapable shocks.

To test this idea, we injected 20 rats with naltrexone (14 mg/kg) and 20 rats with saline. This dose was used because it blocks both short- and long-term analgesia (*9*). Twenty minutes later the rats were placed in restraining tubes and tested for baseline pain sensitivity by measuring the latency period before they flicked their tails at least 0.5 cm laterally away from radiant heat. Half of the rats in each group were given 80 5-second, 1-mA inescapable shocks through tail elec-

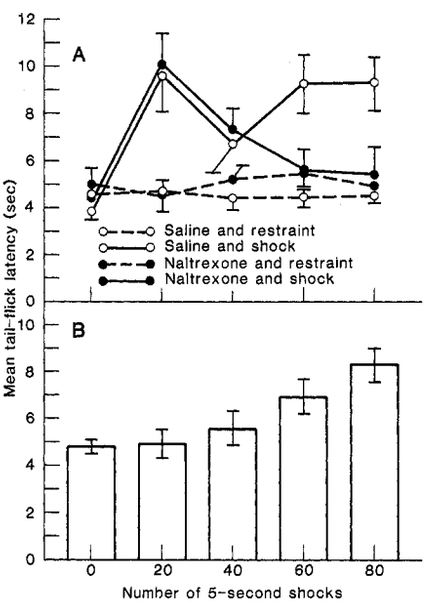

Fig. 1. (A) Mean tail-flick latencies during inescapable shock or restraint following administration of naltrexone or saline. (B) Mean tail-flick latencies for subjects given 0, 20, 40, 60, or 80 inescapable shocks and then, 24 hours later, exposed to a few additional shocks.

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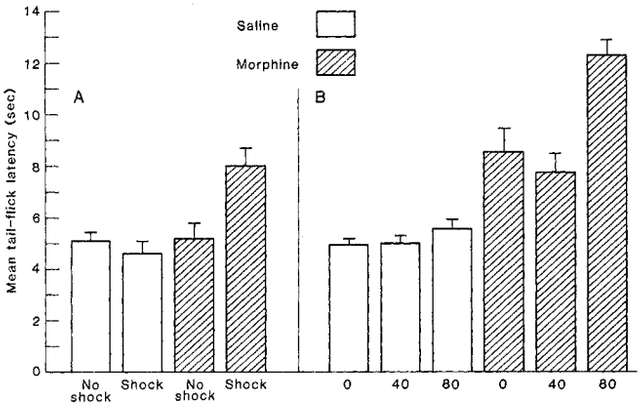

Fig. 2. (A) Mean tail-flick latencies for subjects administered morphine or saline and then, 24 hours later, given shock or no shock. (B) Mean tail-flick latencies for subjects given 0, 40, or 80 inescapable shocks and then, 24 hours later, administered morphine or saline.

trodes on a variable interval (mean, 1 minute) schedule; the other half received only restraint. The apparatus and procedure are fully described elsewhere (*9*). After every 20 trials of shock or an equivalent period of restraint the rats were given tail-flick latency tests. The electrodes were removed and testing proceeded without the rats being handled or removed from the apparatus. Care was taken to avoid shocking and testing the subjects on the same portion of the tail.

Figure 1A shows the mean tail-flick latencies for each test. Subjects given saline and inescapable shock displayed analgesia after 20 trials. This analgesia dissipated after an additional 20 trials but increased again after a further 20 trials (a trend analysis revealed that this function differs significantly from linearity at $P < .05$). Thus there were indeed two peaks of analgesia, one early and one late. The subjects that received naltrexone and inescapable shock exhibited only the early peak. An analysis of variance revealed that the changes in pain reactivity depended on whether the subject received shock and on the number of shocks experienced (the responses of the shocked groups were significantly different from those of the restrained groups at $P < .001$) and that the effect of number of shocks depended on whether the subject had received naltrexone ($P < .05$). Thus, if reversal by opiate antagonists is taken as a criterion, nonopiate and opiate reactions occurred sequentially on continued exposure to the same inescapable stressor.

There are two potential difficulties with this conclusion. First, approximately 40 minutes separated the injection of naltrexone from the 20-trial test, whereas 80 and 100 minutes elapsed before the 60- and 80-trial tests. Perhaps 40 minutes is not long enough for the drug to exert its effect. To test this possibility, we repeated the experiment but injected naltrexone 80 minutes before the session. The results were unchanged. Second, since we both shocked and tested the tail, one might contend that the analgesia observed after 20 trials was a local change in pain reactivity, and masked the reversal of analgesia by naltrexone. We evaluated this possibility by measuring paw-lick latencies on a hot plate after 20 trials of shock, and again obtained a significant decrease in pain reactivity which was not blocked by naltrexone. Thus the early analgesia is not restricted to the tail and is not mediated by endogenous opiate systems.

We previously showed (*10, 11*) that long-term SIA also appears to be opiate-mediated. Particularly interesting is the finding that administration of naltrexone before inescapable shock blocks this SIA. This suggests that activation of the opiate system during inescapable shock sensitizes the system to reactivation 24 hours later on exposure to shock. The results of the first experiment suggest that 60 to 80 inescapable 5-second shocks are required to activate the opiate system. If the necessary sensitization occurs during such activation, 60 to 80 shocks should be required to elicit long-term SIA. To test this, we exposed five groups of ten rats each to 0, 20, 40, 60, or 80 inescapable shocks. All the rats were restrained for equal periods of time. Shock schedules were programmed so that the variable number of shocks occurred during the latter portion of restraint. Twenty-four hours later the subjects were exposed to a small amount of foot shock (five 5-second, 0.6-mA shocks) and then administered three tail-flick latency tests (*10*).

Figure 1B shows the mean tail-flick latencies for each group. Clearly, the subjects that received 80 shocks experienced a substantial increase in analgesia. Analysis of variance revealed a significant effect of group ($P < .005$), and Newman-Keuls post hoc comparisons revealed that the subjects receiving 80 shocks differed significantly from those receiving 0, 20, or 40 shocks. Thus it appears that the opiate system must be sufficiently activated during the initial inescapable shock treatment for long-term SIA to occur.

The results of the above experiment agree with our previous finding (*9*) that naltrexone administered before exposure to inescapable shock blocks the long-term SIA typically observed 24 hours later. Both experiments suggest that activation of an opiate system is necessary to the production of a sensitized system so that analgesia can be reinstated 24 hours later by exposure to a small amount of shock. Further, since naltrexone exerts its antagonistic effect by blocking opiate receptors (*11*), a postrelease alteration produced by endogenous opiates may be responsible for this sensitization. Thus one should be able to produce long-term SIA not only by exposing subjects to inescapable stress but also by activating the opiate system directly by injecting morphine. Inescapable shock per se may not be necessary; merely activating opiate processes should be sufficient and should mimic the sensitizing effects of inescapable shock.

To determine whether activation of opiate processes produces long-term SIA, we injected 20 rats with morphine (4 mg/kg) and 20 rats with saline. Twenty-four hours later half of the rats in each group were given five brief shocks followed by three tail-flick latency tests. The rest of the subjects were treated identically except that they were not shocked. These controls were necessary to determine whether there were any residual effects of morphine at the time of testing.

Only the animals that received morphine and shock displayed a significant degree of analgesia ($P < .005$, 2×2 analysis of variance) (Fig. 2A). Thus it seems that activation of the substrates responsive to opiates is sufficient to produce analgesia 24 hours later on exposure to brief shocks. Morphine appears to produce the same effect as does inescapable shock, perhaps by exerting a comparable sensitizing influence on opiate receptors, a secondary messenger system (*12*), or perhaps the pituitary-adrenal axis (*13*).

If the long-term analgesia produced by extended stress or morphine is mediated by sensitization of a common opiate-responsive process, then not only should brief shock 24 hours after morphine injection produce analgesia, a small dose of morphine 24 hours after extended stress should also have an exaggerated analgesic effect. Since 60 to 80 shocks

are necessary to activate opiate processes, 60 to 80 inescapable shocks should be required to produce sensitization to morphine. To test this hypothesis, we gave groups of 20 rats 0, 40, or 80 shocks and, 24 hours later, administered morphine (2 mg/kg) to half of the rats in each group and saline to the other half. Thirty minutes later the rats were given three tail-flick latency tests.

Only the rats that had been given 80 inescapable shocks were hyperreactive to morphine (Fig. 2B). A 2 × 3 analysis of variance revealed that the increase in analgesia observed depended on whether the animal had received morphine and on the number of shocks given ($P < .005$). Newman-Keuls post hoc comparisons revealed that the response of the group receiving 80 shocks and morphine differed significantly from that of all the other groups. Prolonged exposure to inescapable shock evidently elicits hyperreactivity in a system responsive to the opiates. Thus long-term SIA may occur because the system acted on by the endogenous opiates has been made hypersensitive, not because more of the ligand is released during reinstatement of analgesia.

We have demonstrated that both opiate and nonopiate forms of SIA exist and can be produced with the same stressor. This was suggested by Lewis *et al.* (*5*), but they compared continuous with intermittent shock. Our procedure entailed presentation of only one pattern of shock; therefore our findings suggest that a critical determinant of the form of SIA is the number of shocks or the duration of exposure. Prolonged exposure could be important because it allows the animal to learn that it is helpless, or perhaps because it simply provides more stress. Moreover, we have shown that the activation of opiate systems is necessary and sufficient to produce long-term SIA and that opiates and inescapable shock share some common action. This commonality appears to reside in a facilitation of the effectiveness of endogenous opiates rather than in facilitation of their release.

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Carcinogen-DNA Adducts in Mutagenesis Assays

Bigger *et al.* (*1*) state that the use of liver homogenates in conjunction with the *Salmonella* mutagenesis assay, developed by Ames and co-workers (*2*), as an in vitro screening test for carcinogens can yield misleading results. Bigger *et al.* studied a single carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) and found that metabolic activation and the formation of DNA adducts mediated by a rat liver homogenate (S9) system differed from results obtained in intact mammalian cell systems. However, they did not study DMBA adducts bound to the DNA of intact *S. typhimurium*, but instead substituted naked DNA. For the S9 and microsome assay system, a 2-hour incubation was used, but for the intact mammalian cells the exposure time was 24 hours. Thus it is possible that in the mammalian cells certain DMBA-DNA adducts were removed by DNA excision repair during the 24-hour period. In the studies with the S9 fraction the concentration of DMBA was many times higher than in those with the intact cell systems, and much higher than the concentration used in the Ames pour plate assay. Presumably, because of these differences, the amount of DMBA bound to DNA was three to five times higher in assays with the S9 fraction or microsomes than amounts obtained with intact cells. Furthermore, the precise structures of the adducts formed under the various conditions are not known with certainty, nor is it known which of these adducts plays a central role in the carcinogenic process.

Under appropriate conditions of incubation of the parent carcinogen benzo[*a*]pyrene with an S9 activation system, we detected a guanine adduct in the *S. typhimurium* DNA that is identical in chemical structure and stereochemistry to the major benzo[*a*]pyrene diol epoxide adduct found in intact rodent or human cells incubated with benzo[*a*]pyrene (*3*). Stark *et al.* (*4*) found that when *S. typhimurium* was incubated in an S9 assay system with aflatoxin B_1, the major DNA adduct formed was the same as that found in the liver DNA of rats exposed to this carcinogen. In addition, the *S. typhimurium* S9 assay system has proved useful in the screening of a wide range of potential carcinogens (*5*). We would agree that because of marked variations between assay systems, interspecies and intertissue variations, and the multistep nature of the carcinogenic process considerable caution must be exercised in making precise extrapolations from various in vitro systems to the intact organism. It seems likely that the *S. typhimurium* S9 assay system may give more reliable results with some carcinogens than with others. This assay system and assays that employ intact cells for metabolic activation require further study.

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We initiated comparative studies of carcinogen metabolism in different systems to examine the assumption that the subcellular Aroclor-induced rat liver metabolizing system, recommended by Ames *et al.* (*1*) for general mutagenesis

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