Stimulation-Produced Analgesia: Development of Tolerance and Cross-Tolerance to Morphine

Abstract. *Analgesia resulting from focal electrical stimulation of the brain of the rat shows tolerance with repeated exposures; this tolerance dissipates after a period of nonstimulation. Addiction to morphine reduces greatly the analgesia produced by electrical stimulation of the brain, which demonstrates cross-tolerance between morphine analgesia and stimulation-produced analgesia. Recovery of the stimulation-produced analgesia is seen after discontinuing administration of morphine. These results suggest that morphine and electrical stimulation produce analgesia by common mechanisms. The fact that tolerance occurs to the analgesic effect of electrical stimulation indicates that tolerance may be an alteration of an endogenous neuronal process.*

Focal electrical stimulation of specific sites in the brain produces powerful analgesia in rat (*1–3*), cat (*4*), and man (*5*). Several observations suggest that this stimulation-produced analgesia (SPA) affects a neural substrate similar to that acted upon by morphine. The analgesic action of both morphine and SPA appears to be concentrated in sites surrounding the third ventricle, cerebral aqueduct, and fourth ventricle (*3, 6*). Both effects can be antagonized by depletion of monoamines (*7*) and by the narcotic antagonist naloxone (*8*). Evidence is also available suggesting that both morphine and SPA exert their antinociceptive effects by activation of a neural system in the brain which inhibits transmission of nociceptive information through the spinal cord (*4, 9*). An invariable concomitant of repeated morphine administration is the development of tolerance to its analgesic effect. We now report that tolerance also develops to the analgesic effect of brain stimulation, and this tolerance shows cross-tolerance with morphine.

We have shown in three separate experiments that tolerance develops to SPA. In these experiments, 90-day-old male Holtzman rats were implanted with a bipolar electrode constructed of twisted stainless steel wires (0.2 mm in diameter) insulated with Teflon except for the bared cross section at the tips. All electrodes were stereotaxically aimed at the mesencephalic central gray matter, an area previously shown to produce analgesia (*10*). Brain stimulation was delivered by a Nuclear-Chicago constant-current stimulator and consisted of trains of biphasic, rectangular wave pulse-pairs. The pulse-pair consisted of a 50-μsec pulse followed 100 μsec later by a 50-μsec pulse of opposite polarity. Stimulation frequency was 20 per second unless otherwise stated. Electrode impedance was monitored continuously during all stimulation periods.

The test of analgesia was a modified version of the D'Amour-Smith tail-flick test (*11*). A baseline latency was established for each animal for each test session by four measurements of the tail-flick latency to radiant heat applied to the most distal centimeter of the tail. Trials were separated by 2 minutes. Baseline latency consisted of the average latency of the last three measurements. The effect of brain stimulation on tail-flick latency was measured by applying brain stimulation for 20 seconds before each of the three succeeding trials. Brain stimulation was never on during the actual test. The test latency was the mean latency of these three trials. Radiant heat was never applied to the tail for more than 6 seconds in order to prevent tissue damage. The degree of analgesia produced by brain stimulation is expressed as the percentage of maximum possible effect according to the formula

$$DA = [(TL - BL)/(6 - BL)] \times 100$$

where DA is the degree of analgesia; TL, the test latency; and BL, the baseline latency.

After a minimum of 2 weeks recovery from surgery, animals were screened for SPA. They were tested every 2 weeks at various stimulation intensities (1 to 7 ma) (*12*) until a level, which resulted in just submaximum analgesia, was found for each animal. The consistency of this analgesic level was measured in three additional verification sessions, again separated by 2 weeks. In experiment 1, 12 rats were then stimulated continuously for three 24-hour stimulation sessions separated by 48-hour rest periods. Current intensity was the same as that producing analgesia in the screening sessions, but the frequency was lowered to ten pulses per second. A test session, identical to the verification sessions, was run 24 hours after terminating each stimulation session and 2, 4, and 6 weeks after the last stimulation session. The following results of this experiment are shown in Fig. 1A. Significant tolerance, as compared to any control point, was present 24 hours after the first 24-hour stimulation session ($P < .05$; t-test), but reliably greater tolerance was observed 24 hours after the second stimulation session (S_1 versus S_2; $P < .005$; t-test). No further increase in tolerance was seen after the third stimulation session. Significant recovery from tolerance was observed 2, 4, and 6 weeks after the last stimulation session (S_3 versus E_1, E_2, or E_3; $P < .01$; t-test). Baseline latencies were unaltered throughout the experiment.

Experiment 2 (with five rats) demonstrated that considerably less stimulation than employed in experiment 1 can result in tolerance to SPA. The first five points of Fig. 1B show the results of the second experiment. The first four points represent successive test sessions, each separated by 2 days. Since animals were stimulated only during the test sessions, they received a total of 60 seconds of stimulation on each of these days. Even with this minimum amount of stimulation, reliable tolerance rapidly develops (C_1 versus C_4; $P < .025$; t-test). Point C_5 is the degree of analgesia present 1 week after point C_4. As in experiment 1, significant recovery of analgesia occurs (C_4 versus C_5; $P < .05$; t-test), although this is not complete. Experiment 3 was identical to experiment 2 except that nine animals were stimulated and tested daily for 6 days. Stimulation-produced analgesia again showed tolerance, the degree of analgesia being reduced from an average initial value of 85 percent to a minimum value of 38 percent after six consecutive days ($P < .001$; t-test) (*13*). As in experiment 1, baseline latencies were unaltered in experiments 2 and 3.

A possible alternative explanation for this effect is that brain stimulation in these experiments results in tissue destruction at the site of stimulation. However, a number of observations and experiments make this explanation untenable. (i) Detailed study of the stimulation pulse configuration employed in these experiments has shown it to be noninjurious (*12*). (ii) It seems highly unlikely that the stimulation durations employed in experiments 2 and 3 (60 sec/day) could result in significant tissue damage. In fact, 60 seconds of stimulation separated by 2 weeks produced stable levels of SPA over several replications and even allowed recovery from tolerance (see Fig. 1A). Also, if tissue damage were responsible for the effect, the greater than 700-fold difference in duration of stimulation employed in experiment 1 versus experiments 2 and 3 should have resulted in a considerable difference in the reduction of the degree of analgesia observed; but this did not occur. (iii) The intensity of stimulation received by each animal was not correlated with observed tolerance either in any single experiment or in all experiments combined. (iv) In a direct test of the tissue damage hypothesis, we examined the effect on SPA of producing a small anodal electrolytic le-

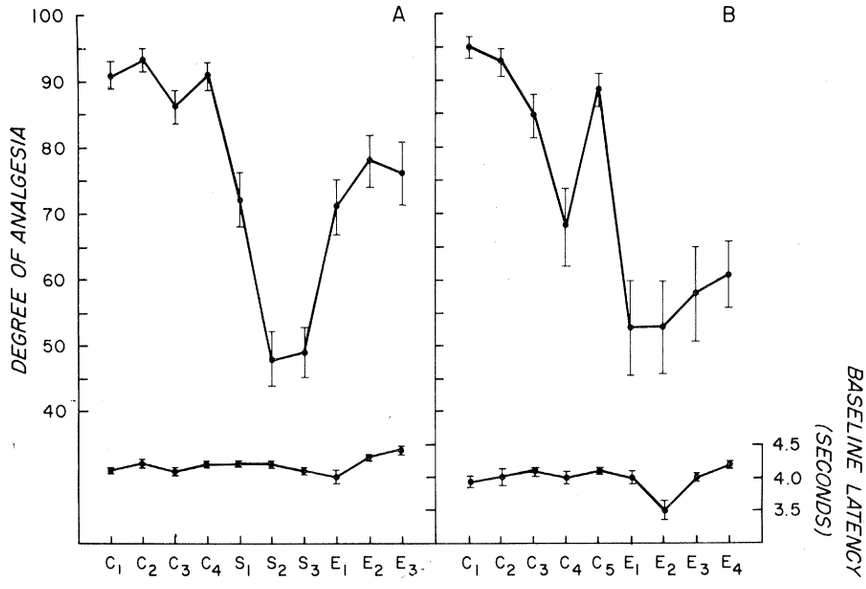

Fig. 1. (A) Effect of 24-hour stimulation periods on stimulation-produced analgesia (SPA). C_1 to C_4, control points separated by 2 weeks; S_1 to S_3, test points collected 24 hours after a 24-hour stimulation period; and E_1 to E_3, the degree of analgesia present 2, 4, and 6 weeks after S_3. (B) Effects of 60 seconds of repeated stimulation periods and electrolytic lesions on SPA. C_1, a control point; C_2 to C_4, the degree of analgesia present 2, 4, and 6 days after C_1; C_5, the degree of analgesia 1 week after C_4; and E_1 to E_4, test points 1 day and 2, 4, and 6 weeks after electrolytic lesioning at the stimulating electrode tips. Lower curves give baseline tail-flick latencies at each test point. The vertical bars represent standard error of the mean.

sion (100 μa for 15 seconds) at the stimulating electrode tips. The results of this experiment are shown in the last five points of Fig. 1B. After a test at point C_5, animals from experiment 2 were lesioned and tested 1 day and 2, 4, and 6 weeks later. As would be expected, the lesions reduced SPA (C_5 versus E_1; $P < .05$; t-test); but in this case no significant recovery occurred (E_1 versus E_2, E_3, or E_4; $P > .25$; t-test). In this same figure, after induction of tolerance by stimulation significant recovery is shown to occur in the same animals after only 1 week. In addition, while electrolytic lesions resulted in reliable decreases in tissue impedance, no changes in impedance systematically related to tolerance were found during induction of tolerance to SPA. (v) Finally, it should be pointed out that when other workers examined the effect of repeated brain stimulation on a variety of stimulation-elicited behaviors, it was found that this results in no change or in an increase in sensitivity to the stimulation (*12, 14*).

We also examined the possibility that repeated exposure to radiant heat on the tail might result in a sensitization of responsiveness, thus accounting for the tolerance effect. Animals in experiment 3, after complete recovery from tolerance, were stimulated as before but were not tested until the sixth stimulation day. Tolerance approximately equal to that found during the repeated testing procedure resulted ($P > .05$; t-test).

In order to examine the possibility of cross-tolerance with morphine analgesia, we prepared 14 animals as described above. Animals were tested three times at 2-week intervals in order to demonstrate stable analgesia. They were then injected (subcutaneously) three times daily over a 21-day period with increasing doses of morphine sulfate until a dosage of 600 mg kg^{-1} day^{-1} was reached. Animals were tested for SPA 1, 2, 16, 30, and 44 days after the last dose of morphine. In Fig. 2, average SPA was reduced from 85 to 53 percent ($P < .005$; t-test) and 44 percent ($P < .005$; t-test) 1 and 2 days, respectively, after the last morphine injection. During the 2 days following morphine withdrawal rats lost 19 percent of their morphine maintenance weight. Thereafter weight gradually returned to preaddiction levels. The absence of a change in baseline latencies during this period, in contrast to a study in which a different behavior was used (*15*), indicates that our measure was unconfounded by withdrawal. The similarity in time courses of recovery from cross-tolerance and stimulation-produced tolerance also suggests a common mechanism probably not influenced by morphine withdrawal (see Fig. 1A).

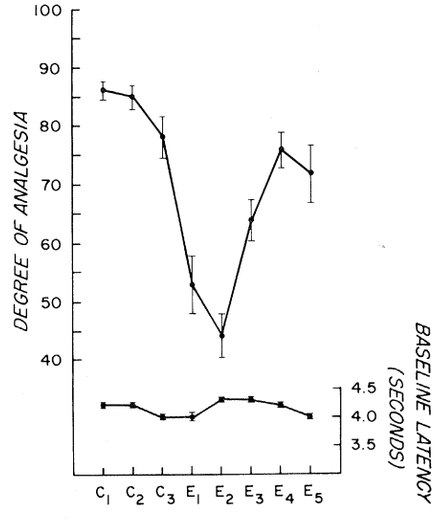

Fig. 2. Effect of morphine addiction on stimulation-produced analgesia. C_1 to C_3, control points separated by 2 weeks; and E_1 to E_5, the degree of analgesia present 1, 2, 16, 30, and 44 days after the last dose of morphine. The lower curve gives baseline latencies at each test point. The vertical bars represent standard error of the mean.

We also examined the effect of making animals tolerant to SPA on the effectiveness of an AD_{80} (analgesic dose effective in 80 percent of the animals) dose of morphine (4 mg/kg, subcutaneously). No difference was found between the stimulation-tolerant animals and unstimulated controls. While it could be argued that our test procedure was too insensitive to disclose cross-tolerance, we feel this result is explicable in terms of the quantity of neural substrate acted upon by morphine and electrical stimulation. Morphine appears to exert its analgesic effect over a diffuse substrate of neural tissue extending from the ventral medial diencephalon caudally through the periaqueductal gray matter and structures in the floor of the fourth ventricle (*3, 6*). Thus, morphine should render this entire substrate tolerant, and stimulation anywhere within it should show cross-tolerance. On the other hand, SPA should result in tolerance of only a small percentage of this neural tissue (*16*). Thus, when morphine is injected, a large proportion of substrate is nontolerant and relatively normal analgesia results.

These results strongly support our earlier contention that focal electrical stimulation of the brain and morphine activate common mechanisms to produce analgesia (*2, 3, 17*). Our data do not provide any direct evidence concerning the mechanism of the development of tolerance to SPA or narcotic analgesics. It is clear, however, that tolerance is not a process unique to the introduction of drugs into the central nervous system but can be a property of certain neural systems when activated in other ways. Our observation of tolerance to the analgesic effects of electrical stimulation leaves open the possibility that tolerance is a normal regulatory mechanism of some neural systems. Such a mechanism, when exacerbated, could mediate not only

the observed tolerance to some drugs but also responses to internal pathological conditions and environmental influences, thus providing a potential tool for understanding the well-known cyclic nature of various psychopathologies.

DAVID J. MAYER
RONALD L. HAYES
Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298

References and Notes

1. D. V. Reynolds, *Science* **164**, 444 (1969).
2. D. J. Mayer, T. L. Wolfle, H. Akil, B. Carder, J. C. Liebeskind, *ibid.* **174**, 1351 (1971).
3. D. J. Mayer and J. C. Liebeskind, *Brain Res.* **68**, 73 (1974).
4. J. C. Liebeskind, G. Guilbaud, J. M. Besson, J. L. Oliveras, *ibid.* **50**, 441 (1973).
5. D. E. Richardson and H. Akil, paper presented at annual meeting of American Association of Neurological Surgeons, Los Angeles, 11 April 1973.
6. K. Tsou and C. S. Jang, *Sci. Sin.* **13**, 1099 (1964); A. Herz, K. Albus, J. Metys, P. Schubert, Hj. Teschemacher, *Neuropharmacology* **9**, 539 (1970); Y. F. Jacquet and A. Lajtha, *Science* **182**, 490 (1973); M. J. Kuhar, C. B. Pert, S. H. Snyder, *Nature (Lond.)* **245**, 447 (197[illegible] L. G. Sharpe, J. E. Garnett, T. J. Cicero, *Behav. Biol.* **11**, 303 (1974).
7. S. S. Tenen, *Psychopharmacologia* **12**, 278 (1968); R. A. Verri, F. G. Graeff, A. P. Corrado, *Int. J. Neuropharmacol.* **7**, 283 (1968); H. Akil, thesis, University of California, Los Angeles (1972); — — and D. J. Mayer, *Brain Res.* **44**, 692 (1972).
8. H. Akil, D. J. Mayer, J. C. Liebeskind, *C. R. Acad. Sci. Ser. D* **274**, 3603 (1972).
9. M. Satoh and H. Takagi, *Eur. J. Pharmacol.* **14**, 60 (1971); *Jpn. J. Pharmacol.* **21**, 671 (1971).
10. Histological verification of electrode placement revealed that almost all electrodes were located in the mesencephalic central gray matter at coordinates: anterior-posterior, − 0.2 to 0.0; depth, − 0.5 to − 3.0 according to the atlas of L. J. Pellegrino and A. J. Cushman [*A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)].
11. F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.* **72**, 74 (1941).
12. Stimulation intensities of 1 to 7 ma may appear excessively high, but it is important to consider pulse duration in conjunction with intensity. Our short pulse duration (50 μsec) falls on a portion of the strength-duration function which necessitates high intensities for effectiveness. Stated differently, a critical variable when stimulating neural tissue is coulombs, not peak current alone [J. C. Lilly, in *Electrical Stimulation of the Brain*, D. E. Sheer, Ed. (Univ. of Texas Press, Austin, 1961), p. 60; see L. Stein and O. S. Ray, *Psychopharmacologia* **1**, 251 (1960)]. Lilly *et al.* [J. C. Lilly, J. R. Hughes, E. C. Alvord, Jr., T. W. Galkin, *Science* **121**, 468 (1955)], using intensities two to three times our highest value, showed that almost identical biphasic pulse configurations produce no observable tissue damage over stimulation periods considerably longer than those we used. Informal histological examinations of electrode loci revealed no evidence of tissue abnormality.
13. Approximately the same amount of tolerance results from widely different durations of stimulation. A publication by D. J. Mayer [*Neurosci. Res. Program Bull.* **13**, 94 (1975)] treats the important implications of this observation.
14. G. V. Goddard, *Nature (Lond.)* **214**, 1020 (1967); F. A. Jarvis and F. B. Colavita, *Physiol. Behav.* **7**, 671 (1971); F. Szeligo and F. B. Colavita, *T. I. T. J. Life Sci.* **2**, 141 (1972); G. G. Ball, *Science* **184**, 484 (1974).
15. H. A. Tilson, R. H. Rech, S. Stolman, *Psychopharmacologia* **28**, 287 (1973).
16. Effective current spread by use of a variety of pulses is commonly reported to extend from 1 to 2 mm from the electrode tip. A previous report on SPA is consistent with these observations [D. J. Mayer, T. L. Wolfle, H. Akil, B. Carder, J. C. Liebeskind (*2*)].
17. J. C. Liebeskind, D. J. Mayer, H. Akil, in *Advances in Neurology*, vol. 4, *Pain*, J. J. Bonica, Ed. (Raven, New York, 1974), p. 261.
18. Supported by PHS grants DA00576-01 and DA00551-01. We thank D. W. Richardson for assistance in portions of this study.

13 December 1974; revised 11 February 1975

Thick and Thin Filaments in Postmitotic, Mononucleated Myoblasts

Abstract. *Addition of cytochalasin B to primary muscle cultures allows the physical separation of postmitotic myogenic cells from replicating presumptive myoblasts and replicating fibroblasts. Mononucleated, postmitotic myoblasts proceed without fusion to synthesize myosin and actin and to assemble these proteins into thick and thin filaments. Although sarcomeres oriented in tandem are not evident and Z, H, and I bands are atypical in these mononucleated myoblasts, the irregularly scattered clusters of myofilaments are assembled into remarkably normal interdigitating arrays. These scattered clusters of stacked thick and thin filaments permit the cell to contact spontaneously in the presence of cytochalasin B.*

It has been known for some time that multinucleated myotubes form by the fusion of mononucleated myogenic cells (*1–3*). Some investigators have argued that there is no obligatory coupling between fusion and the regulatory events associated with the initiation of contractile protein synthesis or the assembly of these proteins into striated myofibrils (*2–4*). They contend that the correlation in vitro between fusion and the initiation of contractile protein synthesis is more coincidental than causal and that, in the absence of fusion, mononucleated postmitotic myoblasts assemble thick and thin filaments (*3–7*).

This view has been challenged. Some workers (*8*) claim that neither transcription nor translation of myosin messenger RNA (mRNA) takes place in mononucleated myogenic cells, and that these events occur only in multinucleated myotubes. Others (*9*) argue that while myosin and creatine phosphokinase mRNA's are transcribed in mononucleated myogenic cells, these mRNA's are not translated until after the cells have fused to form myotubes [see also (*10*)].

In our experiments cytochalasin B (CB) was added to muscle cultures. This antibiotic, which does not block the synthesis of myosin or actin (*11–13*), blocks fusion and separates postmitotic myotubes and myoblasts from replicating myogenic and fibrogenic cells (*4, 14, 15*). Cultures were prepared from breast muscles of 10-day chick embryos and CB was added to a final concentration of 5 μg/ml (*12*). After 2 to 4 days in CB the cells were prepared for electron microscopy (EM) by fixation in glutaraldehyde.

Myogenic cultures and most "myogenic clones" consist of a heterogenous population of cells including myotubes, replicating "presumptive myoblasts" and their daughters, the postmitotic "myoblasts" (*2, 3, 6*), as well as replicating mononucleated cells which operationally are indistinguishable from fibroblasts (*16*). Contrary to some reports (*17*), neither we nor others (*18*) can reliably distinguish among replicating presumptive myoblasts, fibroblasts, and mesenchyme cells. In this report only postmitotic, mononucleated cells that can fuse and actually display thick and thin filaments are termed myoblasts.

Cytochalasin B has very different effects on postmitotic myotubes and myoblasts as compared to replicating cells. Forty-eight

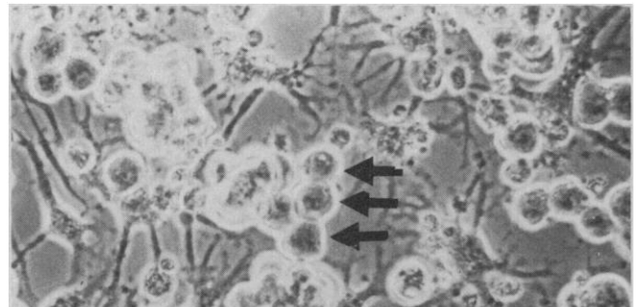

Fig. 1. Intermediate-power phase micrograph of a 5-day breast muscle culture exposed to CB for the last 48 hours. All of the myotubes have retracted from the substrate and will degenerate. The arborized cells adhere to the substrate and are characterized by their many spidery processes (single arrow). In another focal plane are clusters of spontaneously contracting, postmitotic, round, mononucleated myoblasts loosely attached to one another (three arrows). The arborized cells do not bind fluorescein-labeled antibody against myosin or tropomyosin, whereas the round cells bind both antibodies (*6, 14*). Scale bar, 20 μm.