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8. With the top of the incisor bar 3.0 mm below the ear bar center, the electrode tip was placed 6.5 mm anterior to the ear bar center, 0.5 mm lateral, and 8.1 or 8.3 mm below the dura. The conical exposed electrode tips were 0.5 mm long.
9. To determine the influence, if any, of lesioning current (as opposed to the total amount of millicoulombs delivered), half of these rats received the same 40 mcoulomb slowly as 100 μa for 400 seconds.
10. In order to produce stainless steel and platinum-iridium electrode anodal lesions of equivalent size, the number of coulombs is tripled when going from stainless steel to platinum-iridium.
11. The size of the lesions was determined without reference to weight gains, by projecting appropriately magnified cresyl violet–stained sections directly onto the frontal plates of the Konig and Klippel atlas and then tracing at 0.4-mm intervals the absent normal tissue. I used all of the even-numbered atlas plates through the extent of the lesion. Tracing the absent normal tissue instead of the periphery of the apparent lesion scar corrected for shrinkage into the lesion cavity. Shrinkage and scar tissue were greatest with the iron and steel lesions, especially the iron.
12. The superachiasmatic nucleus is at least partly included within the area damaged by all of the five most effective lesions, and it relays fibers from the eye that regulate circadian cycles [R. Y. Moore, *J. Comp. Neurol.* **146**, 1 (1972)]. Disruption of circadian cycles may in turn mediate hypothalamic obesity (R. M. Gold and G. Kapatos, manuscript in preparation).
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21. P. M. Quackenbush provided technical assistance, and G. Kapatos reviewed the manuscript. A preliminary report of this work was presented to the Eastern Psychological Association, Washington, D.C., May 1973.

13 August 1973

Morphine Action at Central Nervous System Sites in Rat: Analgesia or Hyperalgesia Depending on Site and Dose

Abstract. *Morphine was injected via fine-gauge cannulas permanently implanted in various subcortical sites in the rat brain. In this way the blood-brain barrier was avoided and precise quantities of the drug were delivered to the intended sites. Ten micrograms of morphine in the posterior hypothalamus resulted in significant analgesia, while the same dose injected into the medial septum, the caudate, or the periaqueductal gray matter yielded hyperalgesia. The morphine-produced hyperalgesia at the last-mentioned site was accompanied by stereotyped violent circular leaps, an effect of morphine not previously reported. Thus, intracerebral injections of morphine differ significantly from systemic injections and produce either analgesia or hyperalgesia, depending on site and dose.*

One of the major therapeutic uses of morphine has been for its analgesic action. Yet, little is known about sites in the central nervous system (CNS) which mediate the analgesic action of the drug. Attempts to relate a pattern of morphine distribution in the CNS with its analgesic effects in the intact animal after systemic injections have produced disappointing results (*1*).

A technique of potential value in the search for anatomic sites of morphine action is microinjection by means of permanently implanted intracerebral cannulas. This technique has been used in few morphine studies (*2*, *3*), although it has been applied extensively and fruitfully in neuropsychological studies of brain mechanisms controlling motivation (*4*). This method has the advantage of avoiding the blood-brain barrier [only about 0.1 percent of the systemically administered dose gains access to the CNS (*1*)] and permits precise quantities of the drug to be delivered into the intended site.

In the present studies, the microinjection method was used to administer morphine to discrete subcortical sites, and the analgesic action of morphine at these sites was evaluated. Injections of 10 μg of morphine into the posterior hypothalamus and into the third ventricle yielded dose-dependent analgesia, while injections at three other sites, the medial septal nucleus, the caudate nucleus, and the periaqueductal gray matter, yielded hyperalgesia (*5*).

In the first experiment, 78 adult male albino Wistar rats, 200 to 250 g at the time of surgery, were randomly assigned to one of nine groups. All animals except controls were stereotaxically implanted with bilateral 30-gauge stainless steel cannulas at least 1 week before testing. The cannula tip was implanted 1 mm above the intended site. For the injection, a 35-gauge stainless steel needle affixed to a 10-μl Hamilton syringe was inserted into the cannula, with the needle tip extending 1 mm beyond the cannula tip. The volume injected into each site was 0.5 μl. The needle was withdrawn after 45 seconds to allow adequate absorption by the surrounding tissue and thus lessen the likelihood of the fluid being drawn back into the cannula or over the outer wall of the cannula and thus diffusing to other sites. Analgesia testing followed the injection either immediately or after a 20-minute delay; the time factor made no difference in the results. All animals were given habituation sessions (including mock injections and a 10-minute confinement in the experimental chamber) for 3 days to acclimatize them to the procedure before testing.

Analgesia was evaluated by a modified flinch-jump test. Ten sets of shocks were presented to the animal in alternating ascending and descending series. Shock intensity ranged from 0 to 2.2 ma in ten approximately equal steps. Shocks, delivered via a noiseless shock scrambler to the grid floor of the experimental chamber, lasted 1 second, and occurred at 30-second intervals. The animal was viewed through a one-way observation window by the experi-

menter (who was kept unaware as to the dose and group assignment of the subject). The animal's responses were classified in one of three categories: (i) no response; (ii) flinch, any observable slight movement of the animal after shock delivery; and (iii) jump, any simultaneous lifting of two paws from the grid floor after shock delivery. In the first series, the shock level was raised one step at a time until the first jump response was observed. Then the descending series was begun, with shock level being lowered stepwise until the first instance of no response. Then the ascending series was again begun and was terminated with the first jump response. Ascending and descending series continued until ten series were completed. Thus, the shock level was never raised beyond that eliciting the first jump response of the animal during the ascending series, and never lowered beyond the first causing no response in the descending series. The flinch threshold was calculated by taking the mean of the lowest intensity of shock eliciting the flinch response. Similarly, the jump threshold was calculated as the mean of the shock level eliciting the jump response. Total testing time for each animal was approximately 10 minutes.

Each animal was tested with two drug doses (1 and 10 μg, dissolved in 0.5 μl of Ringer solution), the lower dose first and the higher a day later. Control animals were also tested on two consecutive days to detect any effects of sequential testing; there was little difference between results for controls on the first and second days.

Immediately after the second testing, rats were injected bilaterally with 0.5 μl of trypan blue dye to stain the site of injection. The animal was then killed, and the brain was preserved in 10 percent formalin. Histological analysis ascertained the accuracy of cannula placement (*6*) and the extent of spread of the injected dye, which remained confined to an area less than 1 mm in diameter.

An analysis of variance showed that with the lower morphine dose (1 μg), there were no significant differences between the groups on the flinch and jump measures. However, when the morphine dose was raised to 10 μg, both the flinch (Fig. 1A) and jump (Fig. 1B), thresholds yielded significant differences ($P < .05$). On both measures, animals injected in the posterior hypothalamus or the third ventricle differed significantly from the control group. The animals injected in the medial septal nucleus or caudate nucleus showed significantly different responses to the two drug doses, with a reversal of the analgesia to hyperalgesia when the dose was raised from 1 to 10 μg (*7*). Animals injected in the posterior hypothalamus also responded differently to the two doses, with analgesia increased significantly at the higher dose.

In experiment 2, 11 additional animals were injected with either vehicle alone or 10 μg of morphine in the posterior hypothalamus. Half of the rats received the vehicle on the first day and drug on the second, while for the others the order was reversed. Again, testing was conducted with observers kept unaware of animals' drug treatment. Both flinch and jump thresholds were significantly elevated ($P < .01$) by morphine. Further testing of these animals indicated rapid development of tolerance to the analgesic effects of morphine; within two to three sessions, a doubled dose was required to obtain this analgesic effect. To test for isomer specificity, dextrorphan (10 μg) and levorphanol (10 μg) were injected into this site. The former, the analgesically inactive isomer, had no effect, while the latter, the active isomer, was analgesic.

Nine additional animals with medial septal cannulas were tested in experiment 3 with either vehicle or 10 μg of morphine, again in counterbalanced order. The drug significantly lowered the flinch threshold ($P < .05$, one-tailed) but had no significant effect on the jump threshold. The hyperalgesic effect here was not as pronounced as the analgesic effect in the posterior hypothalamus.

In experiment 4, morphine (10 μg) was injected into the periaqueductal gray matter of six rats. Electrical stimulation of this area has been reported to result in profound analgesia, which was likened to narcotic analgesia and hypothesized to be due to facilitatory firing of pain-inhibitory pathways (*8*). However, when we injected morphine into this area the result was extreme hyperalgesia, not analgesia. This hyperalgesia was so pronounced, with rats unable to tolerate a low intensity of shock which normal animals tolerate without even flinching, that it was not possible to complete analgesia testing. This hyper-

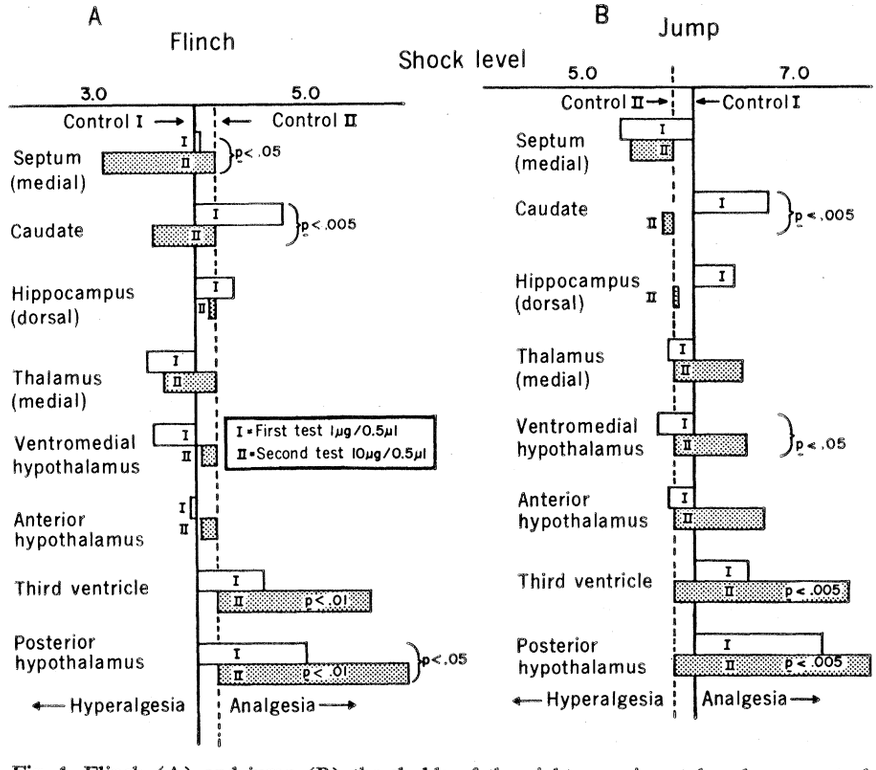

Fig. 1. Flinch (A) and jump (B) thresholds of the eight experimental and one control groups. The baselines for the control group are shown by the center vertical lines, the solid line for the first day (I) and the broken line for the second day (II). The open bars are data representing the first day (1 μg of morphine), the dotted bars are data for the second day (10 μg). The bars to the left of the baseline represent hyperalgesia, while those to the right represent analgesia. Probability (P) values within bars represent significant differences from the control; those outside of bars represent significant differences between doses (that is, between tests I and II).

algesic effect of morphine in the periaqueductal gray matter (later confirmed in an additional group of ten rats) was accompanied by violent uncontrolled jumping characterized by stereotyped circular leaps; these were set off by any slight stimulus, either auditory or visual (*9*). Some animals suffered fatal self-inflicted concussions by jumping and striking their heads repeatedly against the top of the cages. Morphine blocks the release of acetylcholine in peripheral (*10*) and central (*11*) tissues. Thus, its hyperalgesic action here may be the opposite of electrical stimulation; that is, morphine may block cholinergic neural transmission in these pain-inhibitory pathways. The stereotyped circular leaps may be caused by morphine acting in addition on a dopaminergic system.

The neurochemical events underlying these diverse effects of morphine are unknown. Other investigators have implicated the cholinergic (*10*), serotonergic (*12*), and catecholaminergic (*13*) systems. A "narcotic receptor" that specifically binds naloxone was found to be concentrated in the striatum (*14*); however, Wei *et al.* (*15*) found that when naloxone was injected into brains of morphine-dependent rats, the greatest incidence of withdrawal symptoms followed injections in the medial thalamus, with few symptoms being elicited by injections in the basal ganglia. However, in both reports acetylcholine was suggested as the most likely neurotransmitter involved in the action of morphine. Further work is necessary before any firm conclusions can be made.

In conclusion, our results show that intracerebral injections of morphine have effects significantly different from those of systemic injections; this is similar to results with other centrally acting drugs (*16*). Depending on site and dose, intracerebral morphine injections result in either analgesia or hyperalgesia, the latter accompanied in some cases by violent motor activity.

In addition, these results indicate that microinjection, when used with care, is a fruitful method of investigating morphine action. Among the steps we took to eliminate the various sources of error inherent in this system were (i) use of fine-gauge cannulas—30-gauge (outer diameter 0.30 mm) for the guide cannula and 35-gauge (outer diameter 0.13 mm) for the injection cannula—to minimize damage to dorsal sites as well as to the intended site; (ii) injecting 1 mm beyond the cannula tip to minimize backwash and diffusion to other sites (via the outer walls of the cannula into ventricular spaces); (iii) slow injection of a small volume (0.5 μl) to allow fluid to be absorbed by the tissue and to avoid tissue damage that may result from greater volume; (iv) thorough habituation of the animals to the injection procedure and experimental chamber before testing; and (v) a reliable but sensitive analgesia test that also minimized stress to the subject. We believe that conflicting results obtained with the microinjection method are probably due to lack of care in any of these aspects.

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Electrophysiological Identification of a Visual Area in Shark Telencephalon

Abstract. *Optic nerve stimulation in the shark evokes short-latency telencephalic field potentials localized to the ipsilateral, posterior central nucleus. Such a well-defined visual area in elasmobranch telencephalon further challenges classical formulations of forebrain evolution. Moreover, its ipsilateral representation confirms recent evidence for a crossed thalamotelencephalic visual projection.*

The classical view of forebrain evolution (*1*) suggests that in more primitive nonmammalian vertebrates, such as cartilaginous fish, the telencephalon is largely dominated by olfactory input with less prominent representation of other sensory modalities. In the course of vertebrate phylogeny these other modalities presumably gain increasing telencephalic representation, and, with specialized exceptions, olfactory domination decreases. However, the resurgence of comparative neuroanatomy in the past decade has generated considerable data inconsistent with this view. As the sampling of nonmammalian vertebrate species increases, substantial evidence is accumulating that nonolfactory modalities have well-defined telencephalic representations. This has been most comprehensively investigated with respect to visual pathways ascending to the telencephalon, and specific thalamotelencephalic visual pathways have now been identified in amphibians, reptiles, and birds (*2*, *3*). Thus, there is little doubt that specialized visual receiving areas exist in the telencephala of nonmammalian terrestrial vertebrates, and an analogous story is developing with respect to other nonolfactory modalities (*4*).

The only vertebrates in which the classical formulation of forebrain evo-