irradiated or nonirradiated eyes given distilled water. In two eyes treated with pitch volatiles, injection of the lids, tearing, and slight mucous discharge were observed at 5 and 24 hours, but the eyes were normal thereafter. No corneal changes were observed. All six eyes treated with pitch volatiles and subsequently irradiated developed marked injection and edema of the lids, tearing, mucous discharge, and photophobia. These changes were pronounced at 5 and 24 hours after treatment, less pronounced at 48 hours, and had disappeared at 96 or 120 hours. Five hours after treatment the cornea appeared hazy and swollen with an opalescent ground-glass appearance and frequently surface pitting. A large central corneal ulcer, which stained with fluorescein, was present at 24 hours, smaller at 48 hours, present in only three eyes at 72 hours, and had disappeared by 96 hours. A granular ground-glass appearance and some swelling of the cornea remained for 24 to 48 hours after the ulceration had disappeared. All lesions appeared to heal completely and deeper structures such as the lens were not visibly damaged.

Two rabbits were killed 24 hours after treatment with pitch and subsequent irradiation. Histological examination of their eyes, stained with hematoxylin and eosin, showed marked congestion of the palpebral conjunctiva and extensive sloughing of the corneal epithelium with beginning superficial keratitis. No changes were observed in the iris, lens, or other ocular structures. Eyes treated with pitch alone showed edema and early cell necrosis of the corneal epithelium without other changes. Eyes treated with radiation alone were histologically normal.

These results indicate that exogenous photosensitizers such as coal-tar pitch components can cause phototoxic damage to the cornea and conjunctivae which can be assessed in animal models. The possibility of phototoxic eye damage should be borne in mind in evaluating the potential or actual effects of drugs or environmental contaminants.

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References and Notes

1. P. Leper, H. D. Dozovini, J. Doignon, in *Encyclopaedia of Occupational Health and Safety* (International Labor Office, Geneva, 1972), p. 1386.
2. K. D. Crow, E. Alexander, W. H. Z. Buck, A. Dupre, *Arch. Mal. Prof. Med. Trav. Secur. Soc.* **15**, 132 (1956); M. A. Everett and J. V. Miller, *Arch. Dermatol.* **84**, 99 (1961); Y. Izawa, R. Hayakowa, S. Hoshiono, K. Maeda, *Jpn. J. Dermatol. Ser. B* **79**, 324 (1969).
3. R. L. Hervin and E. A. Emmett, *Health Hazard Evaluation Determination Report No. 75-194-324* (National Institute for Occupational Safety and Health, Washington, D.C., 1976); *Health Hazard Evaluation Determination Report No. 75-102-304* (National Institute for Occupational Safety and Health, Washington, D.C., 1976); E. A. Emmett, R. L. Hervin, B. R. Belinky, in preparation.
4. G. Hodgson and H. Whitley, *Br. J. Ind. Med.* **27**, 160 (1970); C. Moret, *Opthalmology* **8**, 381 (1912); B. Bischler, *Opthalmologica* **114**, 56 (1947); S. Duke-Elder and P. A. MacFaul, *System of Opthalmology* (Mosby, St. Louis, 1972), vol. 14, part 2, pp. 1160–1162.
5. N. A. Susorov, *Voen. Med. Zh.* **3**, 75 (1970).
6. G. R. Romovacek, *Fuel* **55**, 287 (1976).
7. Supported in part by NIEHS grant ES-00159-11 and by the National Institute for Occupational Safety and Health. We thank K. Rowe for helpful advice, J. Ginsberg for interpretations of eye histology, and the American Coke and Coal Chemicals Institute for providing the coal-tar pitch distillate.

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Stereospecific and Nonstereospecific Effects of (+)- and (−)-Morphine: Evidence for a New Class of Receptors?

Abstract. *The unnatural (+) enantiomer of morphine had minimal activity in three opiate assays in vitro: the rat brain homogenate binding assay, the electrically stimulated guinea pig ileum assay, and the inhibition of adenylate cyclase in neuroblastoma × glioma hybrid cell homogenates. When (+)-morphine was microinjected into the periaqueductal gray (a site known to mediate morphine analgesia) of drug-naive rats, there was only minimal analgesia, but the hyperresponsivity usually observed after microinjection of (−)-morphine occurred. Also, when (+)-morphine was microinjected into the midbrain reticular formation of drug-naive rats, rotation similar to that following microinjection of (−)-morphine occurred. These behaviors were not blocked by naloxone. Significantly, they typically occur in precipitated abstinence in morphine-dependent rats. These observations suggest that there are at least two classes of receptors, one stereospecific and blocked by naloxone and the other only weakly stereospecific and not blocked by naloxone, and that precipitated abstinence may be due, in part, to a selective blockade of receptors of the former class but not of the latter.*

The recent exciting discovery of the endorphins (*1*), whose potential neuromodulatory role in the central nervous system (CNS) (*2, 3*) has attracted widespread interest, was made possible by the earlier discovery of a class of CNS receptors which possessed stereospecific affinity for opiates and which were further characterized as being blocked by naloxone (*4*). In the studies reported here, we compared the (+) and (−) enantiomers of morphine in several parallel opiate assays in vitro and in vivo, and we demonstrated that there are two distinct classes of receptors that mediate morphine effects. Receptors of the first class, possessing a high degree of stereospecificity and being blocked by naloxone, mediate morphine analgesia. The endogenous ligands for these receptors are apparently the endorphins (*5*). Receptors of the second class, possessing a low degree of stereospecificity and not being blocked by naloxone, mediate the syndrome of hyperexcitability and explosive motor behavior seen after direct microinjection of morphine into certain CNS sites (*6*). This behavior is strikingly similar to some components of the precipitated abstinence syndrome, suggesting that these receptors may play a significant role in opiate dependence.

Unnatural (+)-morphine was synthesized from natural (−)-sinomenine as outlined in Fig. 1. Briefly, (−)-sinomenine was converted to the key intermediate (+)-dihydrocodeinone, and then to (+)-codeine. *O*-Demethylation of (+)-codeine gave 88 percent yield of pure (+)-morphine. The overall yield from the starting material was 25 to 27 percent. In earlier work Goto and Yamamoto (*7*) effected the conversion of (−)-sinomenine to (+)-morphine with a 3 percent overall yield. The (+)-morphine was chromatographically and spectroscopically indistinguishable from an authentic sample of the (−) enantiomer except for the sign of optical rotation.

The unnatural (+)-morphine, assayed in three opiate assay systems in vitro, had the following effects: it was 10,000-fold weaker than its natural (−) enantiomer in its ability to displace [^{3}H]dihydromorphine from binding sites in rat brain homogenates (Fig. 2A). In electrically stimulated guinea pig ileum, (+)-morphine did not inhibit contractions at a dose 100 times greater than the dose of (−)-morphine or of (−)-normorphine that is normally effective in inhibiting contractions. Furthermore, (+)-morphine did not antagonize the action of (−)-morphine or of (−)-normorphine in this assay (Fig. 2B). Finally, in the assay of adenylate cyclase activity in neuroblastoma × glioma hybrid cell homogenates, (+)-morphine had less than 1/1000

of the inhibitory potency of (−)-morphine (Fig. 2C). Furthermore, (+)-morphine did not antagonize the inhibitory action of (−)-morphine in the adenylate cyclase assay (data not shown). The data thus show that (+)-morphine does not act as an antagonist of (−)-morphine in these assays in contrast to the antagonism of (−)-morphine by (+)-morphine on systemic administration in vivo reported by Takagi *et al.* (*8*).

In the in vivo assays, (+)-morphine was microinjected into two brain sites previously shown to mediate morphine-specific effects. A series of intracerebral microinjection studies by Jacquet and Lajtha (*6, 9*) had established that the periaqueductal gray (PAG) mediates the analgesic action of morphine. That is, microinjection of 10 μg of (−)-morphine into the PAG resulted in pronounced analgesia. Paradoxically, morphine microinjection into the PAG also resulted in a concurrent hyperresponsivity syndrome characterized by explosive motor behavior (with the animal leaping as high as 60 cm vertically) which could be set off by any slight auditory or visual stimulus (*10*). This behavior was found to be specific for morphine (and heroin) and did not occur after microinjection of other opiates such as levorphanol, dextrorphan, methadone, and etorphine. The time course and duration of the two behaviors, analgesia and hyperresponsivity, differed. Significantly, microinjection into the PAG of the recently discovered opioid peptide, β-endorphin, was found to result in analgesia but not hyperresponsivity (*3*). These observations suggested that two separate systems of receptors may be involved.

When (+)-morphine was microinjected into the PAG of unanesthetized rats through bilateral cannulas that had been implanted 1 week previously (*11*) at a dose of 5 μg per 0.5 μl at each bilateral site [corresponding to an effective dose of (−)-morphine in the PAG], no discernible effects occurred. When the dose of (+)-morphine was increased eight times to 40 μg per 0.5 μl per site in a new group of animals, only a minimal degree of analgesia accompanied by a pronounced hyperresponsivity was observed. Even at this high dose, the resulting minimal analgesia was significantly poorer ($P < .02$) than that following microinjection of (−)-morphine (5 μg per 0.5 μl per site) into the same animals 2 days later (Fig. 2D). The hyperresponsivity observed at this dose, characterized by lowered auditory and visual thresholds and exaggerated startle responses in the form of violent, repetitive vertical leaps accompanied by shrill

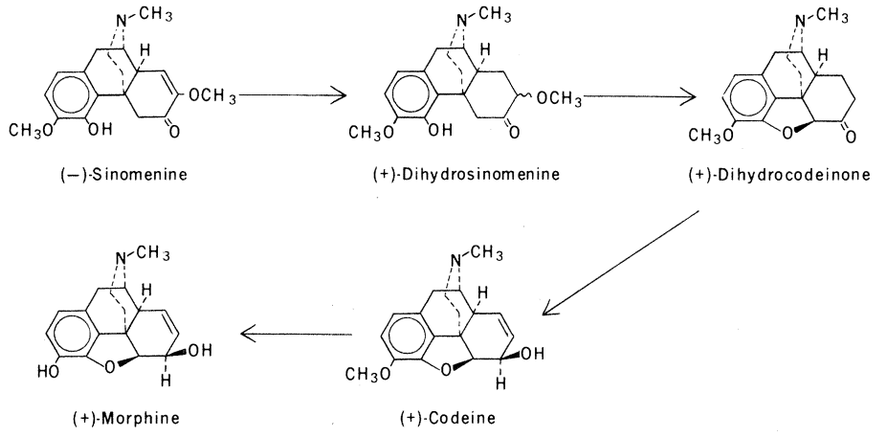

Fig. 1. Improved procedure for the conversion of (−)-sinomenine (*24*) to (+)-morphine. Catalytic hydrogenation of (−)-sinomenine over 10 percent palladium on charcoal quantitatively afforded dihydrosinomenine (*25*). Stirring this material with ten times its weight of polyphosphoric acid (60° to 70°C, 1.25 hours) followed by quenching with ice-ammonium hydroxide provided 75 percent of pure (+)-dihydrocodeinone (after extraction with chloroform and crystallization from chloroform-ether) which was identical with material prepared by the original method of Goto and Yamamoto (*7*) (50 to 65 percent yield). Dihydrocodeinone was converted to (+)-codeine as previously described by Iijima *et al.* (*26*). *O*-Demethylation of (+)-codeine to (+)-morphine was accomplished in 88 percent yield as previously described for the (−) enantiomer (*27*). The (+)-morphine hydrate prepared in this manner was chromatographically and spectroscopically indistinguishable from an authentic sample of the (−) enantiomer, except for opposite optical rotation, and had the following properties: melting point, 253° to 255°C (decomposes); and specific optical rotation, $[\alpha]_D^{23}$, + 132.1° (concentration = 1, methanol) [the literature values (*7*) are: melting point, 247° to 248°C (decomposes), and $[\alpha]_D^{25}$, + 132° (concentration = 1, methanol].

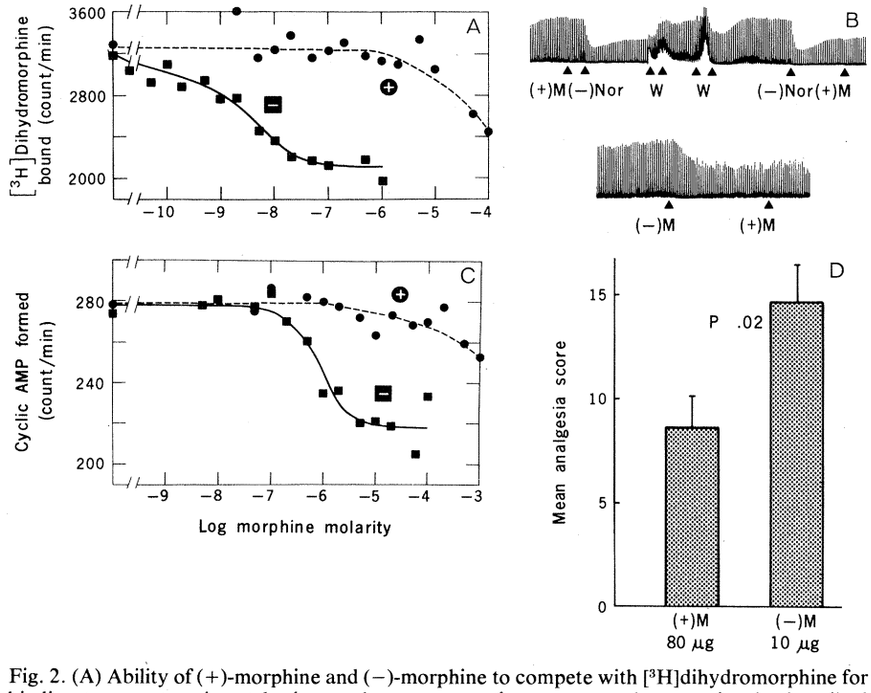

Fig. 2. (A) Ability of (+)-morphine and (−)-morphine to compete with [^{3}H]dihydromorphine for binding to receptors in rat brain membrane preparations, measured as previously described (*28*). (B) Effects of (+)- and (−)-morphine and (−)-normorphine on the electrically stimulated guinea pig ileum. Note the lack of inhibition following additions of (+)-morphine to the bath, and its lack of antagonism of the inhibitory effects of (−)-morphine and (−)-normorphine. The doses were (upper tracing) $10^{-5}M$ (+)-morphine and $4 \times 10^{-7}M$ (−)-normorphine; (lower tracing) $2 \times 10^{-7}M$ (−)-morphine and $2 \times 10^{-5}M$ (+)-morphine. (C) Adenylate cyclase activity of neuroblastoma × glioma hybrid cell homogenates measured in the presence of the indicated concentrations of (+)-morphine or (−)-morphine. Enzyme activity was measured by the procedure of Salomon *et al.* (*29*) as modified by Sharma *et al.* (*30*). (D) Mean analgesia scores and standard errors of rats ($N = 8$) microinjected with 80 μg (40 μg per 0.5 μl per site) of (+)-morphine into the PAG and, 2 days later, with 10 μg (5 μg per 0.5 μl per site) of (−)-morphine into the same site. Analgesia was measured by a battery of tests, including pinches, pinpricks, and thermal stimulation [described in detail in (*6*)]. A *t*-test of the difference between paired scores showed significance at $P < .02$.

distress vocalizations, was identical to that observed after microinjection of (−)-morphine (5 μg per 0.5 μl per site) in the PAG. Naloxone given intraperitoneally at 10 mg/kg did not block the weak (and probably nonspecific) analgesia or the hyperreactivity. On the contrary, systemically administered naloxone appeared to potentiate the toxic effects of (+)-morphine. Two of five animals pretreated with systemically administered naloxone (10 mg/kg) died with symptoms of narcotic overdose (desanguination of the eyes, ears, and paws; flaccidity of muscle tone; and labored respiration) after microinjection of (+)-morphine. Thus, naloxone did not confer protection against the toxic effects of (+)-morphine in the PAG.

These results suggest the existence of at least two classes of opioid receptors in the PAG: one which is highly stereospecific and blocked by naloxone and which mediates the analgesic effect of morphine, and another which is less stereospecific and not blocked by naloxone and which mediates the hyperreactive effects of morphine.

Another CNS site which mediates morphine-specific effects was recently found in the midbrain reticular formation (MRF) (*12*). Microinjection of 20 μg of (−)-morphine into this site resulted in explosive bursts of violent ipsilateral rotation, which occurred at up to two to three turns per second. This pronounced rotation behavior was also found to be specific for morphine, and did not occur after microinjection of other opiates (with the single exception of heroin) or other CNS drugs. Moreover, this morphine-specific behavior was neither blocked nor reversed by naloxone given either intracerebrally (into the same CNS site) or systemically. When (+)-morphine was microinjected unilaterally into the MRF at a dose of 20 to 40 μg/μl, bursts of ipsilateral rotation behavior occurred in one-fourth to three-fourths of the animals in a dose-dependent fashion. However, when (−)-morphine at a dose of 20 μg/μl was microinjected into the opposite side approximately 2.5 hours later, the animals reversed their direction and increased the vigor and duration of rotation, rotating to the side ipsilateral to the (−)-morphine microinjection. Naloxone given intraperitoneally at a dose of 10 mg/kg did not block or reverse this rotation behavior. No analgesia was ever observed after microinjection of either (+)- or (−)-morphine into this site.

The MRF is a region reported to be low in opiate binding (*13*), which suggests that the receptor observed in our in vivo assay is of a different kind than that measured by radioreceptor assay. The MRF receptor appears to be of the same class as that in the PAG mediating hyperresponsivity, since morphine microinjection into both the PAG and the MRF resulted in lowered auditory and visual thresholds, heightened emotionality, and explosive motor behavior, and these behaviors were not reversed by naloxone. This receptor exhibits only a low degree of stereospecificity for morphine.

Interestingly, these behaviors have never been observed after systemic administration of morphine. Even at very high systemic doses, animals typically remain immobile in a stuporous state, interspersed with fits of abrupt running but never violent jumping or rotating. In contrast, both rotation (*14*) and vigorous jumping ["flying" (*15*)] have been observed after systemic administration of naloxone in morphine-dependent rats. Hitherto, these behaviors have been regarded as symptoms of opiate dependence, occurring only during precipitated abstinence [but see (*16*)]. However, in our assay, these behaviors were observed in drug-naive rats after the first morphine microinjection into the PAG or MRF. These apparently discrepant observations may be explicable in terms of differing morphine distributions in the brain. After intracerebral microinjection, morphine is delivered to a single discrete brain site, whereas after systemic administration, it is distributed throughout the CNS, activating multiple neuronal systems, some of which may exert an inhibitory influence on its hyperexcitatory effects in the PAG and MRF. Significantly, other CNS areas, such as the caudate and amygdala, have been reported to be extremely rich in stereospecific opiate receptors (*13, 17*), although the physiological significance of the naloxone-sensitive opioid receptors in these sites has never been made clear. The evidence indicates that they do not have a significant role in morphine analgesia (*18*). The caudate is part of the extrapyramidal system, and the high opiate binding there may reflect its role in morphine-induced body rigidity and immobility—the opposite syndrome to that observed after morphine stimulation of the PAG or MRF. The simultaneous activation of these CNS sites by systemic administrations of morphine may serve to inhibit the excitatory effects of morphine in the PAG and MRF. After naloxone administration in chronically or acutely (*19*) morphine-treated animals, there is a selective blockade of the naloxone-sensitive but not of the naloxone-insensitive receptor, leading to removal of the inhibition, and resulting in the violent behavioral expression of the latter receptors (*20, 21*).

In simple systems such as the isolated guinea pig ileum (*22*) and neuroblastoma × glioma hybrid cells (*23*), an important mechanism of opiate dependence appears to be the development of a latent hyperresponsivity to compensate for the depressant action of opiates on the stereospecific naloxone-sensitive receptor. The observations reported here suggest that in the rat brain another important mechanism may be selective stimulation by opiates of the naloxone-insensitive receptors following blockade by naloxone of the naloxone-sensitive receptors, which normally act to inhibit the former. These observations further suggest that if it were possible to selectively antagonize only the excitatory effects of morphine at these naloxone-insensitive receptors, it might be possible to dissociate some of the undesirable and desirable effects of morphine—for instance, to block the development of some aspects of opiate dependence but not the occurrence of analgesia—and thus approach the goal of a potent analgesic having a minimal dependence liability.

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References and Notes

1. J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature (London)* **258**, 577 (1975); A. F. Bradbury, D. G. Smyth, C. R. Snell, N. J. M. Birdsall, E. C. Hulme, *ibid.* **260**, 793 (1976); R. Guillemin, N. Ling, R. Burgus, *C.R. Acad. Sci. Ser. D* **282**, 783 (1976); B. M. Cox, A. Goldstein, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1821 (1976).
2. L. Terenius, A. Wahlstrom, L. Lindstrom, E. Widerlov, *Neurosci. Lett.* **3**, 157 (1976); F. Bloom, D. Segal, N. Ling, R. Guillemin, *Science* **194**, 630 (1976).
3. Y. F. Jacquet and N. Marks, *Science* **194**, 632 (1976).
4. A. H. Beckett and A. F. Casy, *J. Pharm. Pharmacol.* **6**, 986 (1954); P. S. Portoghese, *J. Pharm. Sci.* **55**, 865 (1966); A Goldstein, L. I. Lowney, B. K. Pal, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1742 (1971); C. B. Pert and S. H. Snyder, *Science* **179**, 1011 (1973); E. J. Simon, J. M. Hiller, I. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1947 (1973); L. Terenius, *Acta Pharmacol. Toxicol.* **33**, 377 (1973).

5. We include the enkephalins in the term "endorphins."
6. Y. F. Jacquet and A. Lajtha, *Science* **185**, 1055 (1974).
7. K. Goto and I. Yamamoto, *Proc. Jpn. Acad.* **34**, 60 (1958); *ibid.* **36**, 145 (1960); *ibid.* **30**, 769 (1954).
8. K. Takagi, H. Fukuda, M. Watanabe, M. Sato, *Yakugaku Zasshi* **80**, 1506 (1960).
9. Y. F. Jacquet and A. Lajtha, *Brain Res.* **103**, 501 (1976).
10. Failure to reliably observe this behavior in some laboratories may be due to use of larger-gauge cannulas, which create a larger area of damage in the PAG. Our histological results show that our use of fine-gauge cannulas (35 gauge, with an outer diameter of 0.13 mm) results in minimal damage. We have observed that a lesion in the PAG often results in a diminution of the occurrence of this behavior.
11. Y. F. Jacquet, in *Methods of Narcotics Research*, S. Ehrenpreis and A. Neidle, Eds. (Dekker, New York, 1975), pp. 34–57.
12. Y. F. Jacquet, M. Carol, I. S. Russell, *Science* **192**, 261 (1976).
13. J. M. Hiller, J. Pearson, E. J. Simon, *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 1052 (1973).
14. B. Hine, E. Friedman, M. Torrelio, S. Gershon, *Neuropharmacology* **14**, 607 (1975); E. T. Iwamoto, H. H. Loh, E. L. Way, *J. Pharmacol. Exp. Ther.* **197**, 503 (1976).
15. J. Bläsig, A. Herz, K. Reinhold, S. Zeiglgänsberger, *Psychopharmacologia* **33**, 19 (1973).
16. H. O. J. Collier and C. Schneider, *Nature (London)* **224**, 610 (1969).
17. M. J. Kuhar, C. B. Pert, S. H. Snyder, *ibid.* **245**, 447 (1973).
18. Y. F. Jacquet and A. Lajtha, *Science* **182**, 490 (1973).
19. It has been reported that jumping can be induced in mice given a single morphine dose 1 to 4 hours before naloxone challenge, although large doses of morphine and naloxone are required for this effect [E. L. Way, D. A. Brase, E. T. Iwamoto, J. Shen, H. H. Loh, in *Opiates and Endogenous Opioid Peptides*, H. W. Kosterlitz, Ed. (Elsevier, Amsterdam, 1976), pp. 311–318; J. J. Jacob, E. C. Tremblay, G. M. Michaud, in *ibid.* pp. 377–384]. This suggests that only high concentrations of morphine at the receptor sites can activate these receptors. Note that in our studies, relatively high concentrations of morphine are delivered to discrete CNS sites.
20. Microinjection of 2 μg of etorphine into the PAG of the rat resulted in analgesia but not hyperreactivity; yet etorphine has a high dependence liability. Significantly, only a relatively high dose of etorphine was effective in causing analgesia following microinjection in the PAG. Perhaps when the blood-brain barrier is bypassed by intracerebral injection, the lipophilicity of etorphine prevents its diffusion within the aqueous extracellular fluid and thus hinders access to receptors. The high intracerebral dose of etorphine, which is comparable to a systemically effective dose, is probably absorbed into the circulatory system and thus gains access to the other CNS sites which are hypothesized to exert an inhibitory action on the excitatory receptors in the PAG. Some previous results (Y. Jacquet, unpublished observations) showing that prior systemic administration of morphine prevented the occurrence of the hyperresponsivity but not of analgesia following morphine microinjection into the PAG are in accord with these suggestions.
21. It is not clear at present whether these two classes of opiate receptors correspond to any of the multiple opiate receptors described by others [W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler, P. E. Gilbert, *J. Pharmacol. Exp. Ther.* **197**, 517 (1976); P. E. Gilbert and W. R. Martin, *ibid.* **198**, 66 (1976); J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, in *Opiates and Endogenous Opioid Peptides*, H. W. Kosterlitz, Ed. (Elsevier, Amsterdam, 1976), pp. 275–280].
22. H. O. J. Collier and D. L. Francis, *Nature (London)* **255**, 159 (1975).
23. S. K. Sharma, W. A. Klee, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3092 (1975).
24. (−)-Sinomenine was a generous gift from M. Takeda (Tanabe Laboratories) and W. Nagata (Shionogi Research Laboratory).
25. K. Goto, *Sinomenine, an Optical Antipode of Morphine Alkaloids* (Kitasato Institute, Tokyo, 1964), p. 22.
26. I. Iijima, K. C. Rice, J. V. Silverton, *Heterocycles* **6**, 1157 (1977).
27. K. C. Rice, *J. Med. Chem.* **20**, 164 (1977).
28. W. A. Klee and R. A. Streaty, *Nature (London)* **248**, 61 (1974).
29. Y. Salomon, C. Londos, M. Rodbell, *Anal. Biochem.* **58**, 541 (1974).
30. S. K. Sharma, M. Nirenberg, W. A. Klee, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 590 (1975).
31. We thank M. Aceto and L. Harris for providing the impetus for this work and A. Brossi, A. E. Jacobson, E. L. May, and H. O. J. Collier for helpful discussions. This work was partially supported by NIDA grant 00367. This is the second report on studies of the (+)-morphinan series; the first report has been published (*26*).

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Odor-Aversion Learning in Neonatal Rats

Abstract. *Two-day-old rats were exposed to a novel odor and injected with an illness-inducing drug, lithium chloride. When tested at 8 days of age, these pups avoided pine shavings scented with the odor, whereas control pups did not. These results imply that rat pups are capable of associative learning at a much earlier age than was thought possible.*

Unlike some rodents such as the guinea pig, the rat's central nervous system (CNS) at birth is markedly underdeveloped and its sensory systems and behaviors are correspondingly primitive (*1*). The rat, however, matures rapidly. In about 4 weeks, its CNS approximates that of the adult, and its behavioral repertoire is rich (*1*). Consequently, by studying this mammal at various stages of development, one might discover important changes in its learning capabilities and identify neurological and neurochemical changes of functional significance to the learning process. An important step is to develop behavioral procedures that not only reveal learning in neonatal pups but also allow a systematic investigation of the variables likely to influence the learning process at various stages in the transition to adulthood.

To study the learning capabilities of the neonatal rat, however, the researcher must overcome a number of problems that are a direct consequence of the immaturity of its CNS. On the one hand, the neonate's sensory limitations restrict the nature of the stimulus experience about which it can be expected to learn, and, on the other, its limited behavioral repertoire makes it difficult to obtain a performance measure to identify the operation of associative-learning processes.

Perhaps because of such problems, there is little evidence of associative learning in pups less than 6 days of age, and we know almost nothing about the principal variables influencing the learning processes of the neonate nor about how these processes change as the organism matures. The purpose of this report is to describe several studies that have overcome some of these difficulties and have revealed evidence of associative learning in neonatal rats only 2 days old.

Several investigations of the neonatal rat's associative learning capabilities have employed Pavlovian conditioning procedures (*2, 3*). In these studies pups experienced vibrotactile stimulation (the conditioned stimulus, CS) paired with electrical shock (the unconditioned stimulus, UCS) to their forelegs. Reliable evidence of conditioned leg flexion has been reported for pups trained at least 6 days of age (*3*); results were mixed when the pups were less than 4 days old (*2, 3*). The possibility of associative learning at this early age thus remains to be determined.

We followed Pavlovian procedures that take advantage of recent developments in the study of the rat's responsivity to olfactory stimulation. (i) Shortly after birth, the rat pup is capable of discriminating among various odors (*4*). (ii) Adult rats acquire specific aversions to odors paired with an illness-inducing UCS (*5*). With these facts in mind, we attempted to induce aversions to olfactory stimulation in neonatal rats by pairing an olfactory CS with an illness-inducing UCS.

On the day of the odor-illness pairing, male and female rat pups were taken from the maternity cage and placed in a polyurethane bag (55 by 36 by 24 cm) containing fresh pine shavings scented with the odor CS. Approximately 5 minutes after being placed in this environment, pups were removed, injected intraperitoneally with the illness-inducing UCS (2 percent of body weight of a 0.15 *M* solution of lithium chloride) and then returned to the odor environment for an additional 30 minutes. When they were 8 days old, the pups were tested for aversion to the odor that had been paired with the illness. On each test they were placed in the center of a 30 by 20 by 10 cm compartment with a wire mesh floor. Beneath the floor were two 15 by 9 by 3 cm containers. One container was always filled with CS-scented pine shavings. The other container was filled with pine shavings either naturally scented or