and sexual behavior to be the result of sexual selection, generated by (i) small male parental investment, (ii) long-lived sperm, (iii) males being capable of multiple inseminations, and (iv) females being contagiously located at a predictable site and time in the host's intestines. Since the reproductive morphology of males and at least some of the population characteristics of *M. dubius* are characteristics of the entire phylum Acanthocephala, we suggest that our interpretation is a general situation in the phylum.

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22. It benefits a female to avoid copulation at the time she begins releasing eggs. If the sperm becomes depleted following the release of eggs, it would be advantageous to copulate again at such a time so as to maximize egg production. The report of O. M. Amin (*18*) of capped females with mature embryos suggests that more than one copulation can occur. In fact it should occur, for, if the cap was 100 percent effective, selection would eventually not favor the male capacity for multiple inseminations.
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Morphine and Enkephalin: Analgesic and Epileptic Properties

Abstract. *Systemic and intracerebroventricular administration of analgesic doses of morphine resulted in large increments of spontaneous multiple unit activity in the periaqueductal gray matter of the awake rat. Intracerebroventricular injection of methionine enkephalin gave analgesia in only 8 of 19 rats, but in all 8, and in no others, increased periaqueductal multiple unit firing was also seen. These findings support the view that the periaqueductal gray matter is actively involved in endogenous mechanisms of analgesia. A striking observation was that enkephalin caused electrographic and behavioral epileptic phenomena in most animals. This observation together with other recent findings suggests that endogenous enkephalin may play some role in epileptogenesis.*

Considerable attention has been paid to disclosing the site and mechanism of action of narcotic analgesic drugs. Stereospecific opiate binding sites have been discovered and mapped in the mammalian brain (*1–3*). A substance (enkephalin) has been identified in brain tissue that appears to be an endogenous ligand for the opiate receptor, and its peptidic structure has been described (*4*). The midbrain periaqueductal gray matter (PAG) seems to be a major site of opiate analgesic action. Significant opiate binding as well as enkephalin-containing fibers and fiber terminals are found here (*2, 3, 5*). Microinjections of morphine into PAG and electrical stimulation of this structure cause particularly potent analgesia (*6, 7*), and microinjections of enkephalin and other, more recently discovered, opioid peptides into the ventricular system or into the PAG likewise yield analgesic effects (*8*). We have reported that analgesic doses of either systemically administered morphine or PAG electrical stimulation augment spontaneous multiple unit firing in the PAG (*9*). These and other findings suggest, as we have previously concluded (*9, 10*), that activation of the PAG might normally be associated with pain inhibition.

In our study we sought to examine further the effects of systemically injected morphine on PAG multiple unit firing and to determine whether intracerebroventricular (ICV) administration of analgesic doses of morphine and enkephalin would also influence neural activity in this brain region. We found that analgesic doses of morphine, whether administered via the systemic or ICV route, reliably augmented PAG multiple unit activity. The ICV injections of enkephalin provided comparable analgesia in only some animals, but only those showing analgesia exhibited a significant increase in PAG multiple unit firing. An unexpected but striking observation was that enkephalin caused potent and long-lasting electrographic seizures in most animals.

Male Sprague-Dawley rats (300 to 400 g) were prepared with permanently implanted cannula guides, multiple unit recording electrodes, and skull electroencephalogram (EEG) leads according to standard procedures. Guides were made of 23-gauge stainless steel tubing and aimed for the lateral ventricle on one side. Multiple unit electrodes consisted of bundles of three to five 70-μm Nichrome wires cemented together and insulated except at their cross-sectioned tips. Each rat received one such bundle of electrodes aimed at caudal PAG; several rats were implanted with a second bundle in locus coeruleus. Stainless steel screws were threaded into the skull over frontal and occipital cortex for EEG recording. The ICV injections were administered via 27-gauge cannulas inserted into the guides. Testing began more than 1 week after surgery.

A modification of the tail-flick method (*7, 11*) was used for analgesia testing. Animals were restrained in plexiglass tubes from which their tails extended. An opening in the top of the tube gave access to the animal's electrode connector for simultaneous electrophysiological recording. Baseline latencies to tail-flick withdrawal from the radiant heat source were averages of the first four (before treatment) trials in any test session and ranged between 3.0 and 3.5

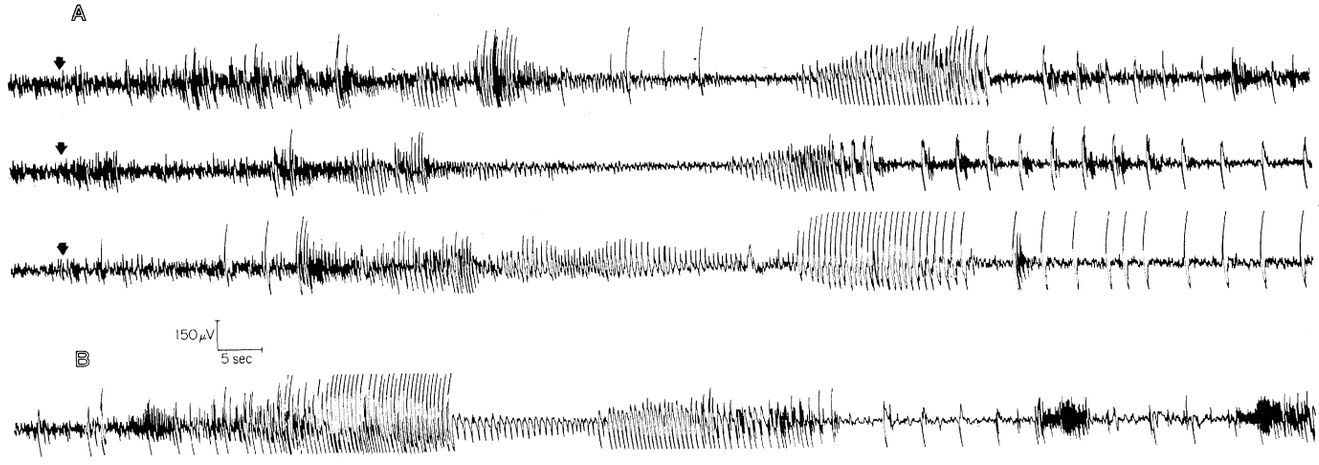

Fig. 1. Electroencephalogram recorded between occipital and frontal epidural electrodes in four different rats. (A) Records from three rats immediately after injections of 200 μg of methionine enkephalin into the lateral ventricle. Arrows indicate the end of the injection period. (B) Record from one rat 5 minutes after injection of 100 μg of morphine sulfate into the lateral ventricle.

seconds. A 2-minute interval separated tail-flick trials. To prevent tissue damage to the tail, the heat source was automatically shut off at 7.3 seconds if the animal had not already responded. Analgesia was defined by latencies attaining this predetermined cutoff.

Multiple unit and EEG recording began after determination of baseline tail-flick latency. Multiple unit activity (MUA) was recorded differentially between the two wires of the electrode bundle giving the highest amplitude signal. The MUA was amplified, sent through a high-pass filter and level detector and then integrated. Raw records were monitored on an oscilloscope, level detector output was monitored on a digital counter, and integrator output was monitored on a polygraph. Average MUA frequency (spikes per second) was determined over 10-second epochs with the aid of the counter. To establish a baseline MUA frequency, time was given to permit the animal to become drowsy as indicated by the appearance of intermittent periods of synchronous, high-amplitude (6 to 10 per second) waves in the cortical record. The level detector was then adjusted for that animal to allow passage of approximately 30 spikes per second during these periods of synchronous EEG. Changes in MUA frequency after drug administration were evaluated in reference to this baseline by maintaining the level detector at this setting for the remainder of the recording session.

Physiological saline was injected intraperitoneally 10 minutes prior to morphine administration; and MUA, EEG, and tail-flick latencies were measured in order to assess possible effects of the injection procedure alone. After morphine sulfate (10 mg/kg) was administered intraperitoneally, MUA and EEG were continuously monitored. Tail-flick testing began 1 minute after injection and continued at 2-minute intervals. After morphine analgesia was well established (10 to 20 minutes after injection), naloxone (1 mg/kg) was administered intraperitoneally, and recording and behavioral testing were continued as before.

Intracerebroventricular microinjections of all substances were delivered automatically by an infusion pump over a period of 10 to 20 seconds. Morphine sulfate was dissolved in normal saline (30 μg in 10 μl), and methionine enkephalin was dissolved in Ringer solution (200 μg in 10 μl). Ringer solution at *p*H 4.0 (10 μl) was administered as a control for the acidity of the enkephalin used here.

Cannula and electrode placements were verified histologically. Only data from animals with accurate placements are reported. The tips of all PAG electrodes lay within 0.5 mm of the midline, below the aqueduct, in or just adjacent to the dorsal raphe nucleus.

Prior to treatment, as the EEG of the relaxed animal shifted spontaneously from synchrony to desynchrony, there was usually a small increase in PAG multiple unit activity (about 35 versus 30 spikes per second). Intraperitoneal injections of saline inevitably aroused the animal, desynchronizing the EEG, but they never significantly altered MUA frequency or tail-flick latency. By contrast, all seven rats given morphine (10 mg/kg) intraperitoneally showed complete analgesia and large increments in PAG multiple unit firing (median increase, 167 percent; range, 95 to 790 percent). The median latencies to onset of analgesia and onset of MUA increase were 15.0 minutes (range, 3 to 36 minutes) and 10.0 minutes (range, 3 to 21 minutes), respectively. Interestingly, these two latencies were highly correlated across animals (Spearman rho, .99; $P < .01$). The effect of this dose of morphine on cortical EEG was assessed in six of the seven rats. After several minutes of desynchrony due to the injection procedure, uninterrupted cortical synchrony was seen for the remainder of the observation period.

Naloxone administration restored tail-flick latencies and EEG patterns to the levels observed before morphine was given. It completely reversed the MUA increase in three of the seven rats, and partially reversed MUA in the remaining four rats.

Three of the seven rats had a second set of multiple unit recording electrodes in the locus coeruleus. At the same time that these animals showed increases of 167, 332, and 790 percent in PAG, two showed no MUA changes at the locus coeruleus placement, and one showed a moderate decrease.

Intracerebroventricular injection of 30 μg of morphine caused complete analgesia in all ten rats tested and a rise in PAG multiple unit firing in nine of these (median increase, 100 percent; range, 33 to 350 percent). The median latencies to the onset of analgesia and the MUA increase were 4.5 minutes (range, 2 to 40 minutes) and 5.0 minutes (range, 2 to 35 minutes), respectively. Once again, the two latencies were positively correlated (Spearman rho, .91; $P < .01$). Five of the ten animals were naive; and in all of these, morphine caused sustained EEG synchrony. The others had received an ICV injection of enkephalin 5 days earlier; and, in these, ICV morphine caused electrical seizure activity in three animals and did not alter EEG in two. No conclusions can be drawn from this small

sample about possible effects of prior enkephalin administration. Naive and nonnaive rats did not differ in their behavioral or MUA responses to ICV morphine.

A single intraperitoneal injection of naloxone (3 mg/kg) reversed the behavioral and electrophysiological effects of ICV morphine in four of eight animals tested. A second naloxone injection at least partly reversed these effects in three of the remaining four animals.

Nineteen animals received ICV injections of methionine enkephalin (200 μg). Six had received an ICV injection of morphine or Ringer solution (pH 4.0) 5 days earlier; the remaining 13 were naive. Eight of the 13 naive rats manifested complete or near complete analgesia after enkephalin, lasting between 1 and 40 minutes (median, 4 minutes). No other animal showed clear alterations in tail-flick latency. All eight rats manifesting analgesia also showed increases in PAG multiple unit firing after enkephalin lasting 15 seconds to 26 minutes, and no other animal did. For these eight, the median MUA increase was 225 percent (range, 50 to 550 percent). The latencies to onset of analgesia and MUA changes were within 1 minute for most animals, precluding computation of a meaningful correlation. However, the MUA increase was always apparent during the time in which analgesia was observed. Because these effects were of such brief duration, and because they were not seen at all in many rats, no attempt was made either to reverse or prevent their occurrence by naloxone administration.

A prominent and very characteristic abnormal EEG pattern was seen in 15 of the 19 animals tested with enkephalin (Fig. 1A). This activity began within the first minute after injection and endured for at least 3 minutes and, in some animals, for as long as 1 hour. The abnormality started with the appearance of rapidly repetitive spikes that lasted for 20 to 30 seconds and typically evolved into slower high-amplitude sharp waves (4 to 6 per second). This pattern was often followed by approximately 30 seconds of unusually low amplitude, desynchronized EEG and then a second buildup of regularly repetitive sharp waves or spike and wave complexes (2 to 3 per second) lasting about 20 seconds. Finally, a long-duration period of recurrent spike and polyspike and wave complexes appeared at a frequency of 1 per 5 to 7 seconds. Typically animals remained motionless throughout the duration of these cortical events, except for periodic sudden twitches and aperiodic "wet-dog shakes." Twitches often occurred in temporal association with the EEG spike and spike wave discharges. The discharges could occur alone, however, indicating that they were not movement artifacts. This sequence of events appeared to be a form of epileptic activity.

Four additional rats whose cannulas were misplaced dorsal to the lateral ventricle also showed these seizures after enkephalin treatment, without manifesting changes in either tail-flick latency or MUA. In another small group of rats, ICV injection of large doses of morphine (100 μg) caused seizures quite comparable to those caused by enkephalin (Fig. 1B), but at considerably longer latency.

Four of the 15 rats in which enkephalin caused seizures were on a later occasion given naloxone 15 minutes before or 15 minutes after enkephalin administration. In intraperitoneal doses up to 2 mg/kg, naloxone neither prevented nor reversed enkephalin-induced seizures.

A total of nine rats (five with well-placed cannulas; four with cannulas misplaced dorsal to the ventricle) were injected ICV with 10 μl of Ringer solution at pH 4.0 to control for the acidity of the enkephalin used in this study. No changes in tail-flick latency, MUA, or EEG were observed.

Our results confirm and extend our earlier finding (*9*) that systemically administered morphine substantially increases spontaneous multiple unit firing in PAG. With half the dose of morphine used in the previous study, a robust increment in PAG activity was still observed. Intracerebroventricular injection of morphine also increased PAG multiple unit activity, and a significant positive correlation was found between the latency to analgesia and the latency to the rise in MUA after both intraperitoneal and ICV morphine administration. Although enkephalin caused analgesia in fewer than half the animals tested, PAG activity increased only in those animals showing the behavioral effect. These observed associations between PAG multiple unit activity and inhibition of a spinal nociceptive reflex are consistent with our previously expressed view (*9, 10*) that the PAG participates actively in an endogenous, centrifugal mechanism of pain inhibition.

Seemingly at variance with our observation of increased PAG multiple unit firing are the results of studies in which systemic or iontophoretic injections of morphine and enkephalin fail to affect or primarily inhibit single neuron activity in this brain area (*12*). However, in these studies either decerebrate cats or anesthetized rats were used, and such preparations might significantly alter PAG activity, affecting responsiveness to opioid drugs. In this regard, we have observed (*13*) that systemic injections of morphine do not alter PAG multiple unit firing in rats anesthetized with urethane. It might also be that single and multiple unit techniques preferentially sample different cell populations in PAG, and that these populations respond differently to opiates. It is, in fact, likely that the majority of PAG cells comprising the MUA signal are small, and perhaps many are relatively inactive under baseline conditions. Such cells are only rarely sampled by the microelectrode technique (*14*).

A striking new finding was the observation that enkephalin caused seizures in most animals. Because seizures and analgesia did not always occur in the same animal (*15*), and because even when these effects were seen together their time courses differed greatly (seizures generally persisting well beyond the short-lived analgesia and increased MUA), it seems unlikely that seizures caused these other changes or that all three effects depend directly on a common underlying mechanism. However, this possibility cannot entirely be excluded and merits more intensive investigation.

The potency and reliability of enkephalin-induced seizures call into question the physiological role of this endogenous peptide. Several recent findings may shed some light on this issue (*16–20*). We now find (*16*) that ICV injections in the rat of either methionine or leucine enkephalin at doses as low as 25 μg produce no analgesia but reliably cause clear abnormalities in the cortical EEG, for example, paroxysmal activity such as that shown in Fig. 1A or abnormal hypersynchrony with occasional spiking. Our preliminary evidence indicates that at these lower doses such abnormalities are always greatly attenuated by naloxone and often completely antagonized. These findings suggest that, when normal regulatory processes are disrupted, endogenous enkephalin may play a significant role in the etiology or elaboration of epileptic phenomena. Others have shown that various fragments of the pituitary hormone β-lipotropin, including methionine enkephalin, produce wet-dog shakes such as we observed during seizures (*17–19*), rigidity (*17, 18*), sedation (*20*), and catalepsy (*17, 20*) when injected into the rat lateral ventricle (*17–19*) or PAG (*20*). These effects were interpreted as suggesting a possible role for opioid peptides in the etiology (*17*) or control (*20*) of psychopathology. However, our results make it imperative to

determine whether seizures were elicited by the compounds these investigators used. If so, such seizures may have caused ictal or postictal behaviors that manifested in their animals as wet-dog shakes, rigidity, sedation, and perhaps even catalepsy.

On the other hand, as many have suggested, enkephalin no doubt plays somewhat different roles in the different central nervous system sites where opiate receptors are found. The proximity to lateral ventricle of limbic structures known to be rich in opiate receptors and to be particularly sensitive to epileptogenic manipulations suggests that this injection site might favor the occurrence of seizures but be less than optimal for eliciting analgesia. This hypothesis would serve to explain the relative unreliability of the enkephalin-induced analgesic effects we observed.

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Efferent Optic Nerve Fibers Mediate Circadian Rhythms in the *Limulus* Eye

Abstract. *When the horseshoe crab is kept in constant darkness, the lateral eye produces larger electroretinographic and optic nerve responses at night than during the day. These circadian rhythms are mediated by synchronous bursts of efferent impulses in the optic nerve trunk. The endogenous efferent activity appears to increase both the gain and the quantum catch of the photoreceptors.*

The response characteristics of peripheral sensory organs determine the nature of information transmitted to the brain. Studies of a number of animals show that efferent nerve signals transmitted from the brain can, in turn, modulate the characteristics of some sensory organs (*1*). Efferent nerve activity may therefore play an important role in processing sensory information.

Here we report that efferent activity in the optic nerve of the horseshoe crab, *Limulus polyphemus*, modulates the response characteristics of the lateral eye. The endogenous efferent signals mediate circadian rhythms in the electroretinogram (ERG), in the optic nerve responses, and in the spontaneous discharge of the optic nerve. The efferent activity appears to increase both the gain and the quantum catch of the photoreceptors.

Circadian oscillations in the amplitude of the ERG are shown in Fig. 1a. In this experiment an animal which had been maintained in a natural light-dark cycle was clamped to a rigid platform in a seawater aquarium located in a light-proof, shielded cage. A corneal electrode (*2*) was positioned on one of the lateral eyes, a fiber-optic bundle was aligned in front of the eye, the cage was closed, and ERG responses to brief test flashes were recorded every 30 minutes while the animal remained in darkness. Figure 1a shows that the amplitude of the ERG was higher at night than during the day. On the afternoon of the second day (first arrow) we opened the cage and implanted a snare around the optic nerve trunk. Pulling the snare at midnight of the following day cut the optic nerve and caused a rapid decline of the ERG amplitude to the low daytime level (*3*). When the nerve was cut during the day (data not shown), the ERG amplitude remained at the daytime level and no further circadian changes in amplitude could be detected. These results suggest that at night tonic efferent activity in the optic nerve increases the ERG amplitude.

Efferent activity can indeed be recorded from the optic nerve at night. Regular bursts of impulses were discharged by fibers in the proximal stump of the cut optic nerve in the record in Fig. 1f. The different spike amplitudes in each burst indicate that several efferent fibers fired impulses in near synchrony. The frequency of bursting was maximum (2 per second) during the early evening hours. During the late evening and early morning hours, silent periods interrupted the periods of bursting. Little or no efferent activity was detected during the day. The periodicity of the efferent optic nerve activity (dark bars in Fig. 1d) was approximately the same as that of the ERG responses (Fig. 1a).

Illumination of the median ocelli modulates the ongoing efferent optic nerve activity and thereby influences the responses of the lateral eyes. No circadian rhythm could be detected in the ERG of the median ocelli, and excision of the median ocelli did not abolish the circadian oscillations in the lateral eye responses. The effects of median ocellar illumination will be described in a later report (*4*).

Pulses of current delivered to the optic nerve in situ produce the same effects as the endogenous efferent activity. Figure