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

1e shows that pulses delivered at the rate of 2 per second to the distal end of the cut optic nerve caused nearly a tenfold increase in the ERG amplitude. When the pulses were turned off, the ERG amplitude decayed to the normal daytime level with a time constant of about 1 hour. When the current pulses were turned on several hours later, the ERG amplitude again increased and remained elevated throughout a 13-hour shocking period. Shocking the cut optic nerve trunk continuously at a rate of 2 per second was more effective than the endogenous efferent activity in increasing the amplitude of the ERG.

Spontaneous and driven discharges of single optic nerve fibers exhibit circadian rhythms which follow the periodicity of the endogenous efferent activity. The spike discharge of a single fiber in situ was recorded without cutting the optic nerve trunk. The animal was mounted in an aquarium as in the ERG experiments. However, in this experiment we exposed the optic nerve trunk through a hole in the shell, dissected free a single active nerve fiber, and recorded its activity with a glass suction electrode (*5*). The recorded ommatidium was optically isolated with a fiber-optic light pipe (*6*). Figure 1b gives the steady-state rate of discharge in response to a 6-second flash delivered to the ommatidium every hour for 56 hours while the animal remained in darkness. Figure 1c gives the rate of spontaneous activity recorded from the same optic nerve fiber. During the late evening hours, the spontaneous activity was minimum and the optic nerve responses maximum. In this preparation the daytime level of spontaneous activity was about equal to that recorded throughout the day and night when the optic nerve is cut (*5*).

We have shown thus far that the ERG and optic nerve responses exhibit circadian rhythms and that the rhythms follow the periodicity of the efferent optic nerve activity. Also, cutting the optic nerve abolishes the rhythms, and shocking the optic nerve reproduces the effects of the endogenous efferent activity. We therefore conclude that efferent fibers in the optic nerve trunk mediate circadian rhythms in the lateral eye.

The efferent input to the eye influences the intensity coding characteristics of the ommatidia. Figure 2 gives the daytime and nighttime intensity-response functions for the steady-state response from a single ommatidium in situ with the optic nerve trunk uncut. In this experiment we followed the procedure given above for the optic nerve recordings, except that here we measured the response of the recorded ommatidium over a large range of test intensities in the afternoon and in the evening. The animal remained in the dark during the entire recording period. The optically isolated ommatidium was allowed to adapt to the dark between test flashes.

Figure 2 reveals that during daylight hours the ommatidium exhibited (i) a mean spontaneous rate of 2 impulses per second, (ii) a threshold of about log $I = -9$ for steady-state response, (iii) a graded response over at least a 9-log-unit range of light intensity, and (iv) a distinct plateau in response at moderate intensities. At night the same ommatidium exhibited (i) a mean spontaneous rate of 0.2 impulse per second, (ii) a threshold of less than log $I = -11$, (iii) a graded response over at least a 9-log-unit range, and (iv) no clear plateau at moderate intensities. The fact that only the daytime characteristics match those recorded from ommatidia in situ with the optic nerve cut (*5*) supports the notion that ef-

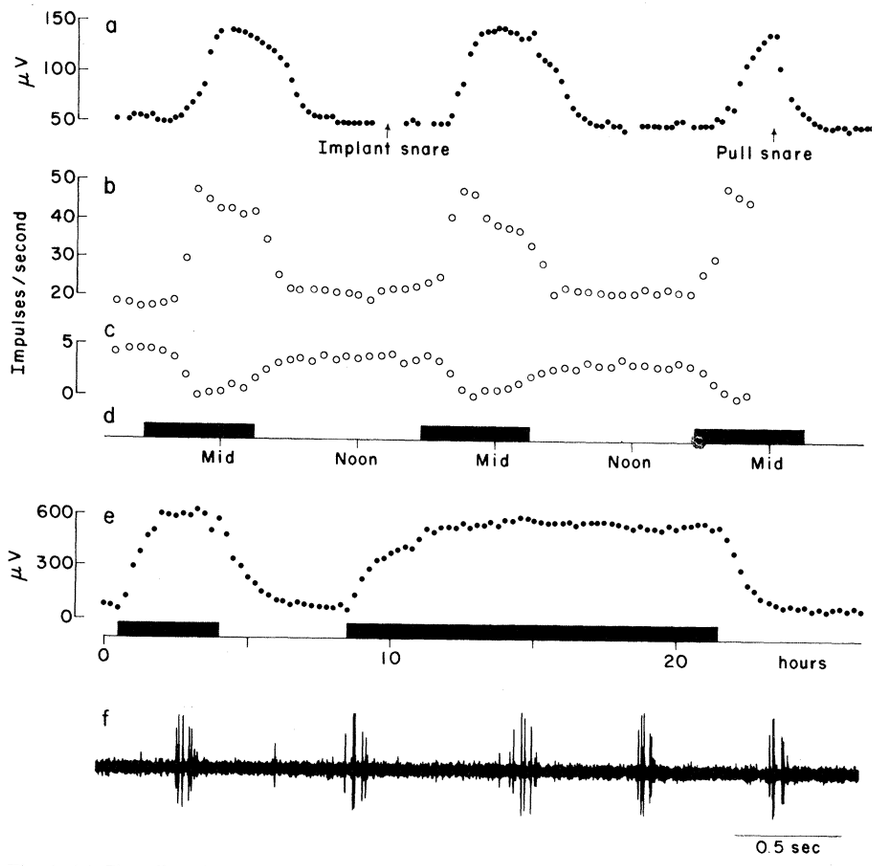

Fig. 1. (a) Circadian rhythm in the amplitude of the electroretinogram (ERG) of the lateral eye. Points give the peak amplitudes of the ERG in response to 50-msec test flashes of constant light intensity presented every 30 minutes while the animal remained in the dark. Local time is given in (d). Cutting the optic nerve ("pull snare") on the third day of the experiment abolished the free-running rhythm in the ERG responses. (b) Circadian rhythm in the response of a single dark-adapted ommatidium. Each point gives the mean firing rate of the ommatidium during the last 3 seconds of a 6-second flash presented every hour while the animal remained in the dark. The test flash delivered 10^5 quanta per second to the ommatidium at the cornea from 400 to 650 nm (corresponds to log $I = -7$ in Fig. 2). (c) Circadian rhythm in the spontaneous activity of a single dark-adapted ommatidium. Each point gives the mean rate of discharge of nerve impulses in a 25-second interval in the dark. The data in (b) and (c) were recorded from the same optic nerve fiber. (d) Periodicity of the endogenous efferent optic nerve activity recorded with a suction electrode from the proximal stump of the cut optic nerve. Dark bars give the periods of general efferent activity. The animal remained in the dark throughout the 3-day experiment. (e) Effects on the ERG amplitude caused by shocking the optic nerve in situ. Dark bars denote the periods during which constant-current pulses were delivered at the rate of 2 per second to the distal end of the cut optic nerve. Points give the peak amplitudes of the ERG elicited by 50-msec test flashes of constant intensity presented every 15 minutes while the animal remained in the dark. (f) Recording of endogenous efferent activity from the optic nerve trunk. The 3.5-second recording was made at 7:30 p.m. when efferent activity is about maximum. The synchronous bursts of impulses are characteristic of the efferent activity in *Limulus*. The data in (a) and (e) were obtained from crabs collected during the winter in Shark River, New Jersey, and stored in seawater aquariums in Syracuse. The data in (b), (c), (d), and (f) were recorded from freshly collected crabs at the Marine Biological Laboratory, Woods Hole, Massachusetts.

ferent inputs modulate the response characteristics of the lateral eye.

Changes in the position and shape of the intensity-response functions in Fig. 2 suggest changes in the quantum catch and gain of the photoreceptors (retinular cells). Circadian movement of screening pigment (*7*) into and out of the path of the incident light beam would change the number of photons absorbed by the retinular cells at each intensity (quantum catch) and thus would shift the intensity-response function along the intensity axis. However, subtracting the mean spontaneous rate from both functions indicates that the nighttime function is shifted both vertically and to the left, suggesting an increase in gain (response per absorbed photon) as well as in quantum catch. The migration of screening pigment does not provide a ready explanation for changes in gain, nor does it readily account for changes in spontaneous activity.

The physiological results reported here are consistent with the existing anatomical evidence for efferent fibers in the optic nerve trunk (*8*) and for efferent terminals on retinular and other pigment-containing cells of the ommatidium (*9*). The efferent terminals contain neurosecretory granules which appear to be transported from the brain (*8*) by several fibers in the optic nerve trunk. Cells believed to be neurosecretory are located in the central body of the brain near the ganglia of the median ocelli (*9, 10*). Preliminary results indicate that these ganglia connect directly to fibers in the optic nerve trunk (*11*); however, the neural pathways mediating the efferent and median ocellar influences have not yet been determined.

The free-running oscillations in Fig. 1 appear to be generated by an endogenous clock mechanism which exhibits properties typical of circadian clocks in other organisms. For example, the period of the free-running oscillations generally differs from 24 hours and, consequently, under conditions of constant darkness the phase of the oscillation progressively shifts with respect to the local light-dark cycle. Also, the phase of the free-running oscillations can be shifted by exposing the animal to brief periods of illumination during the subjective night.

Circadian rhythms appear to be characteristic of some invertebrate visual systems (*12*). Daily oscillations in retinal responses are generally believed to result from rhythmic migration of screening pigment in the compound eye (*13*). In some animals, however, the circadian effects are more complex. For example, both neural and humoral processes appear to modulate the circadian rhythms of pigment migration and visual responses in the crayfish eye (*14*). In *Aplysia* the isolated eye itself exhibits a circadian rhythm but the neural activity of the eye in situ can be modulated by efferent optic nerve activity (*15*). The data in this report indicate that in *Limulus* the lateral compound eye receives a periodic efferent input which does more than influence the location of screening pigment.

Pronounced efferent activity has been recorded from the optic nerve of both squid and octopus; however, its function is not yet understood (*16*). Although efferent retinal nerve fibers have been described in a number of vertebrates, their function has been studied in detail only for the avian retina (*1*). Efferent activity appears to increase the sensitivity of the avian retina by decreasing inhibitory influences (*17*). A similar mechanism has been reported for the turtle retina (*18*). In both cases the efferent influences are exerted on amacrine cells and not on photoreceptor and pigment cells, as appears to be the case in *Limulus*.

What role, if any, circadian changes in the response characteristics play in *Limulus* behavior is not known. Since a steady-state response of 20 impulses per second requires 5 log units less light intensity at night than during the day (Fig. 2), the circadian changes in retinal sensitivity may partially compensate for the diurnal fluctuations in ambient illumination. The decrease in spontaneous activity at night further enhances retinal sensitivity and appears to adapt the eye for functioning at very low light intensities. In this regard it is interesting to note that the period of elevated retinal sensitivity corresponds reasonably well to the animal's normal period of locomotor activity (*19*) and also to the time the animals congregate in shallow water to mate (*20*). In view of the relative simplicity of the *Limulus* visual system, future research may well reveal the significance of these circadian rhythms in the lateral eye.

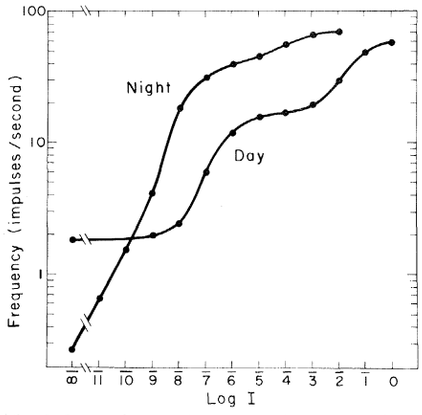

Fig. 2. Intensity (*I*) characteristics for daytime and nighttime responses from an ommatidium in situ with the optic nerve trunk uncut. The mean firing rate of the single optic nerve fiber during the last 3 seconds of a 6-second flash is plotted on the ordinate as a function of the logarithm of the relative light intensity plotted on the abscissa. "Day" responses were recorded from 3 to 4 p.m. and "night" responses from 9 to 10 p.m. The animal remained in the dark between the test flashes and between the daytime and nighttime recording periods. In darkness ($\log I = -\infty$) the spontaneous activity of the optic nerve fiber was about 2 impulses per second during the day and 0.2 impulse per second at night. At $\log I = 0$ approximately 10^{12} quanta per second are incident on the ommatidium at the cornea from 400 to 650 nm.

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Physiological Evidence for Habenula as Major Link Between Forebrain and Midbrain Raphe

Abstract. *The lateral habenula is one of the few forebrain areas that project to the midbrain raphe nuclei. Electrical stimulation of the habenula markedly suppressed serotonergic neurons in the midbrain raphe. The suppression was blocked by systemic or microiontophoretic administration of picrotoxin, which suggests that γ-aminobutyric acid is the inhibitory transmitter in the habenula-raphe pathway. These results support the concept that the habenula may serve a pivotal role in funneling information from the forebrain to the midbrain raphe.*

Serotonin (5-hydroxytryptamine or 5-HT) is concentrated within neurons of the brainstem raphe nuclei (*1*). Through its extensive efferents (*2*), the serotonergic system has been implicated in a wide variety of physiological and behavioral functions including sleep, temperature regulation, pituitary hormone release, and psychotogenic drug actions (*3*). However, relatively little is known about the afferent connections of the raphe nuclei (*4*). By a retrograde tracing method in which horseradish peroxidase (HRP) was iontophoretically ejected into the midbrain raphe nuclei, we identified afferent inputs to the rat midbrain raphe nuclei from the prefrontal cortex, the preoptic region (both medial and lateral) of the hypothalamus, the medial forebrain bundle, the lateral habenula (HB), the brainstem reticular formation, the nucleus of the solitary tract, and a number of other areas (*4*). Of all brain areas that send afferents to the midbrain raphe nuclei, the lateral HB contained the highest density of HRP-reactive neurons. The existence of dense HB-raphe projections suggests that cells in the HB might have an important role in regulating or modulating the neuronal activity of serotonergic cells in the midbrain raphe nuclei. This suggestion is in accord with previous anatomical studies (*5–7*) indicating that the lateral HB may serve a pivotal role in funneling information from limbic and striatal forebrain to the "limbic midbrain area." In the present study we investigated the influence of electrical stimulation of the HB on midbrain raphe cells and explored possible neurotransmitters that might mediate these effects. We found that HB stimulation markedly suppresses the firing of raphe cells; this result provides a physiological basis for considering the HB as a major link connecting the forebrain to the midbrain raphe.

Under chloral hydrate anesthesia (400 mg/kg intraperitoneally), 70 Sprague-Dawley rats (200 to 300 g) were implanted with a concentric stimulating electrode in the midline adjacent to the HB nuclei [A, 3430 to 3990 μm; L, 0 μm; H, 0 μm (Fig 1A, arrow) according to König and Klippel (*8*)]; in seven rats an additional control electrode was put into the thalamus at the same frontal plane as the HB electrode but 2 mm lateral to the midline (Fig 1A, double arrow). Micropipettes filled with 2*M* sodium chloride saturated with fast green FCF (*9*) were lowered through a burr hole into the midbrain raphe nuclei. Detailed methods for differential recording of single unit activity and microiontophoresis have been described (*10*). At the end of each experiment the site of the electrode tip was marked by iontophoretic ejection of fast green (*9*). The site of the stimulating electrode tip was marked by small lesions made by passing a 0.05-ma positive direct current for 15 seconds. Animals were perfused with 10 percent buffered formalin. Serial frozen sections (50 μm) were cut and stained with cresyl violet.

A total of 282 cells were recorded from midbrain raphe nuclei during HB stimulation and during iontophoresis of suspected transmitters and their antagonists. In most cases cells were recorded from the dorsal raphe nucleus (DRN); only a few cells were recorded from the median raphe nucleus. Five serotonergic cells were identified during single unit recording by their antidromic responses to stimulation of the major ascending 5-HT pathway in the ventromedial tegmentum of the anterior midbrain. The rest of the raphe cells were tentatively identified by their wave forms and firing patterns: biphasic action potentials (a predominant positive wave, then a negative wave) and a slow, regular spontaneous discharge rate (0.5 to 3 spikes per second). These characteristics have been demonstrated by combined single unit recording and fluorescence histochemical methods (*11*) and by electrophysiological methods [antidromic stimulation of 5-HT pathways (*10, 12*)] to be specific for 5-HT–containing neurons but not for neurons not containing 5-HT in the adjacent central gray or reticular formation.

Electrical stimulation of the HB (0.1 to 0.5 ma; pulse width, 0.5 to 1 msec) produced a marked poststimulus suppression of serotonergic and other neurons in the midbrain raphe nuclei. Repeated HB stimulation at 1 hertz reliably yielded pe-

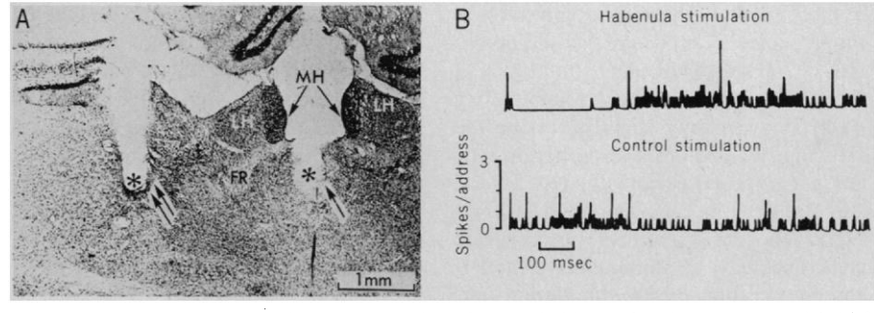

Fig. 1. (A) Photomicrograph of a coronal section of the brain illustrating the tracts (asterisks) of HB stimulation (arrow) and control electrodes (double arrow) in the area of the HB and thalamus, respectively. The distance from the midline HB stimulating electrode to the medial edge of the lateral HB is about 0.5 mm and the distance between the control electrode and the lateral edge of the lateral HB is about 1 mm. If the stimulating current was less than 0.5 ma, stimulation of the control electrode usually produced little or no effect on raphe cells (B); *FR*, fasciculus retroflexus; *LH*, nucleus habenulae lateralis; *MH*, nucleus habenulae medialis. (B) Representative poststimulus time histograms showing that HB stimulation produced a marked poststimulus suppression while control electrode stimulation on the same raphe neuron had no effect. Sweeps were initiated by the synchronous stimulus-pulse output fed directly from the stimulator to a Nicolet computer; bin width, 1 msec.