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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-

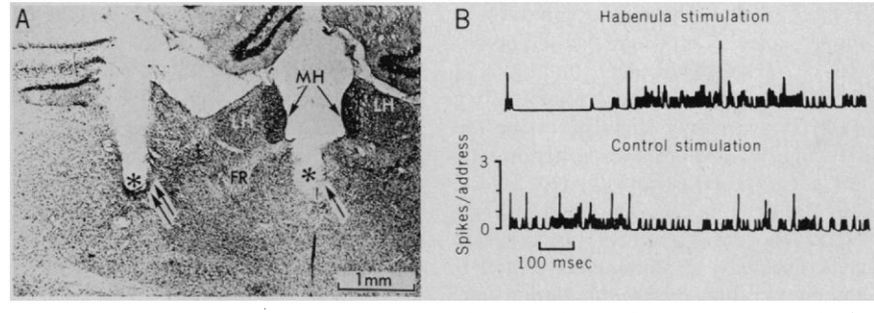

Fig. 1. (A) Photomicrograph of a coronal section of the brain illustrating the tracts (asterisks) of HB stimulation (arrow) and control electrodes (double arrow) in the area of the HB and thalamus, respectively. The distance from the midline HB stimulating electrode to the medial edge of the lateral 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.

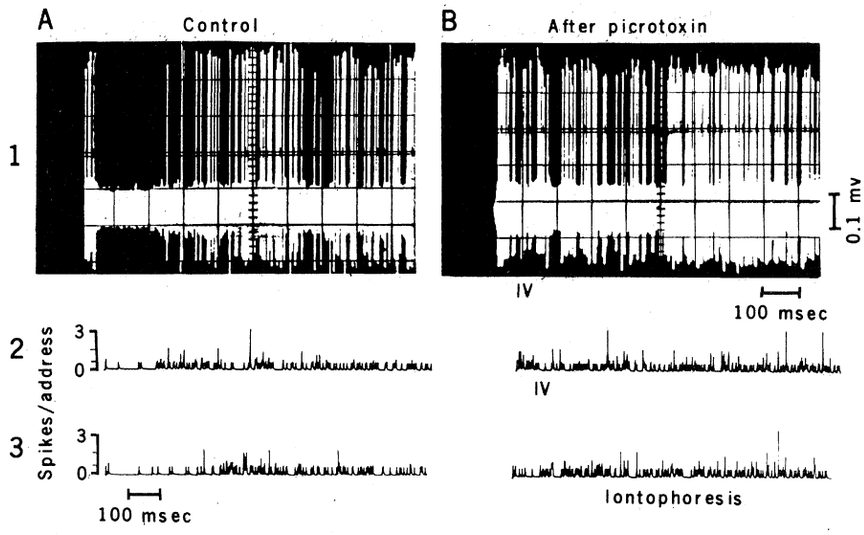

Fig. 2. HB-induced suppression of serotonergic neurons and blockade by picrotoxin. Data are shown for three different serotonergic neurons, numbered 1 to 3. Activity of neurons was displayed either directly on a storage oscilloscope (cell 1) or in the form of poststimulus time histograms (cells 2 and 3). Every picture and histogram consists of at least 150 sweeps which were triggered by the synchronous stimulus-pulse output. Electrical stimulus starts at the beginning of the pictures and histograms. Before administration of picrotoxin (column A), HB stimulation suppressed serotonergic neurons. After administration of picrotoxin (column B) either by intravenous injection (cells 1 and 2) or by iontophoretic application (cell 3), HB-induced suppression was blocked.

riods of suppression lasting 50 to 400 msec in 143 of 158 typical serotonergic cells (90 percent) (Fig. 1B). The number of spikes during the period of suppression (bin 21 to 120) was significantly lower than that toward the end of the poststimulus time histograms (bin 801 to 900) [1.6 ± 2.6 compared to 12.5 ± 5.3 (mean ± standard deviation) 91 cells; $P < .001$; paired t-test]. Of 37 cells in the midbrain raphe area which lacked the characteristic firing pattern of serotonergic neurons, only 19 (51 percent) showed poststimulus suppression. None of the ten cells tested in the midbrain reticular formation were suppressed by the HB stimulation. Latencies of HB-induced suppression were 8 to 35 msec (16.3 ± 6.8; 81 cells). These are comparable to the latencies of antidromic action potentials of HB cells evoked by stimulation of the DRN (*13*). When the stimulating electrode was only slightly outside the HB (Fig. 1A), 35 of 39 serotonergic cells (90 percent) were not affected by the stimulation (Fig. 1B). The difference between the proportion of serotonergic cells responsive to stimulation of the HB and that responsive to stimulation outside it is highly significant ($P < .001$; χ^2 test). Two low cerveau isolé (pretrigeminal) rats were used to study the effect of HB stimulation on DRN cells in the absence of anesthesia. The effect of HB stimulation in these rats was no different from that in rats anesthetized with chloral hydrate. This suggests that chloral hydrate did not contribute materially to the depression produced by HB stimulation.

It is difficult to ascertain that suppression of firing of 5-HT neurons induced by HB stimulation is due to a selective activation of HB-raphe pathways. However, our HRP study (*4*) showed that within the thalamic and hippocampal area the lateral HB is the only nucleus containing cells that project to the raphe. It is not likely, therefore, that current spread to adjacent thalamic nuclei (such as the periventricular system) would have a direct effect upon 5-HT cells in the midbrain raphe. In one series of experiments, the fasciculus retroflexus and adjacent areas [the path taken by HB-raphe projections (*6*)] were destroyed bilaterally by radiofrequency lesions. In these 15 rats the effect of HB stimulation was almost totally abolished after 5 to 8 days; only 9 of 87 cells (10 percent) were suppressed by HB stimulation. The proportion of serotonergic cells suppressed by HB stimulation after fasciculus retroflexus lesions is significantly less than that for those cells under normal conditions ($P < .001$; χ^2 test). When the lesions were made unilaterally, HB-induced suppression still occurred. Although our results suggest that suppression of 5-HT neurons produced by HB stimulation is mediated by a direct HB-raphe pathway, the possibility of a polysynaptic pathway cannot be ruled out by our data.

The interpretation of our results is complicated by the fact that some DRN cells project to the HB region (*2*) and that antidromic stimulation of serotonin fiber pathways can produce recurrent inhibition of DRN cells (*10, 12*). In the present study eight serotonergic neurons showed antidromic responses to stimulation of the HB region. To rule out the possibility of recurrent inhibition, 5,7-dihydroxytryptamine (5,7-DHT), which is relatively selective in destroying 5-HT fibers and terminals (*14*), was injected into a lateral ventricle (*15*). This resulted in the disappearance of 5-HT terminals in the HB as well as in other periventricular zones but had no effect on 5-HT cells (*16–17*). In five rats treated with 5,7-DHT 3 to 5 days before testing, there was little or no reduction in HB-induced suppression in 22 DRN cells tested. This result argues against the possibility that the effect produced by HB stimulation is due to recurrent inhibition.

As a first step toward determining the identity of possible inhibitory neurotransmitters in the HB-raphe pathway, we applied γ-aminobutyric acid (gaba) and glycine by iontophoresis onto serotonergic cells in the DRN. We confirmed (*17*) that serotonergic cells were inhibited by iontophoretic application of either gaba or glycine and that the inhibition was selectively blocked by the gaba antagonist picrotoxin and the glycine antagonist strychnine, respectively. However, depression of serotonergic cells produced by HB stimulation was either suppressed or totally abolished by intravenously administered picrotoxin (4 to 6 mg/kg in seven cells tested in seven rats) (Fig. 2, cells 1 and 2) but not by strychnine (0.4 to 0.7 mg/kg in six cells tested in six rats). Although intravenously administered picrotoxin readily suppressed the HB-induced depression by serotonergic cells, conceivably this effect could have resulted from some indirect action (such as an enhanced excitability of serotonergic neurons caused by a suppression of inhibition from other afferent pathways). However, local, iontophoretic application of picrotoxin with low ejection currents (20 na) over long intervals (5 to 12 minutes) was effective in reducing both the suppression induced by HB stimulation (Fig. 2B, cell 3), and the inhibition produced by gaba iontophoresis in the absence of an obvious increase in the firing of four serotonergic neurons. These results suggest that gaba is the inhibitory transmitter of the HB raphe pathway. Since HB-induced suppression has never diminished spontaneously, its blockade by picrotoxin cannot be attributed to either neuronal fatigue or acute habituation. The suggested presence of gaba-ergic pathways in the raphe is consistent with the report

that the midbrain central gray has a high glutamate decarboxylase activity, a marker for gaba neurons (*18*).

In conclusion, our data indicate that HB stimulation markedly suppresses neurons in the midbrain raphe nuclei and that this effect might be mediated by a direct HB-raphe gaba-ergic pathway. These results provide the first physiological support for the concept that the lateral HB serves a pivotal role in funneling information from the limbic forebrain to the "limbic midbrain area" (*5*).

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Circadian Rhythm of Synaptic Excitability in Rat and Monkey Central Nervous System

Abstract. *Synaptic responses in hippocampal granule cells to stimulation of their afferent fibers from the entorhinal cortex fluctuate with a 24-hour period. The phase of this cycle for rats and monkeys depends on whether the animal is naturally nocturnal or diurnal. In a rat blinded by enucleation, the rhythm persists but drifts out of phase with the rhythm of sighted controls.*

The periodic nature of animal behavior has prompted research into the mechanism or mechanisms underlying these fluctuations. The most obvious of these periodic behaviors include the sleep-waking cycle (*1*), feeding behavior (*2*), general activity (*3*), and reproductive behaviors (*4*). Performance of avoidance tasks is also best 24 hours after training (*5*), and optimal performance of some appetative tasks fluctuates rhythmically (*6*). Mammalian systems show circadian fluctuation in the concentrations of putative transmitter substances (*7*) as well as of hormones from the adrenal cortex, the pituitary, and the median eminence of the hypothalamus (*8*). Furthermore, single neurons, such as the parabolic burster neuron of *Aplysia* (*9*), show circadian oscillations. The adaptive significance of these rhythms and their importance to a general understanding of behavior has been emphasized (*10*).

The present study, which demonstrates circadian rhythmicity in the dynamics of synaptic transmission in the fascia dentata of the hippocampus, grew out of a larger study of the long-term modifiability of synaptic efficacy in the dentate gyrus (*11, 12*). Because the experiments required prolonged recording of synaptic responses, it was important to determine whether the efficiency of granule cell synapses showed any regular fluctuation over time. When the fibers of the entorhinal cortex are stimulated in awake moving animals, it is possible to record an extracellular field potential in

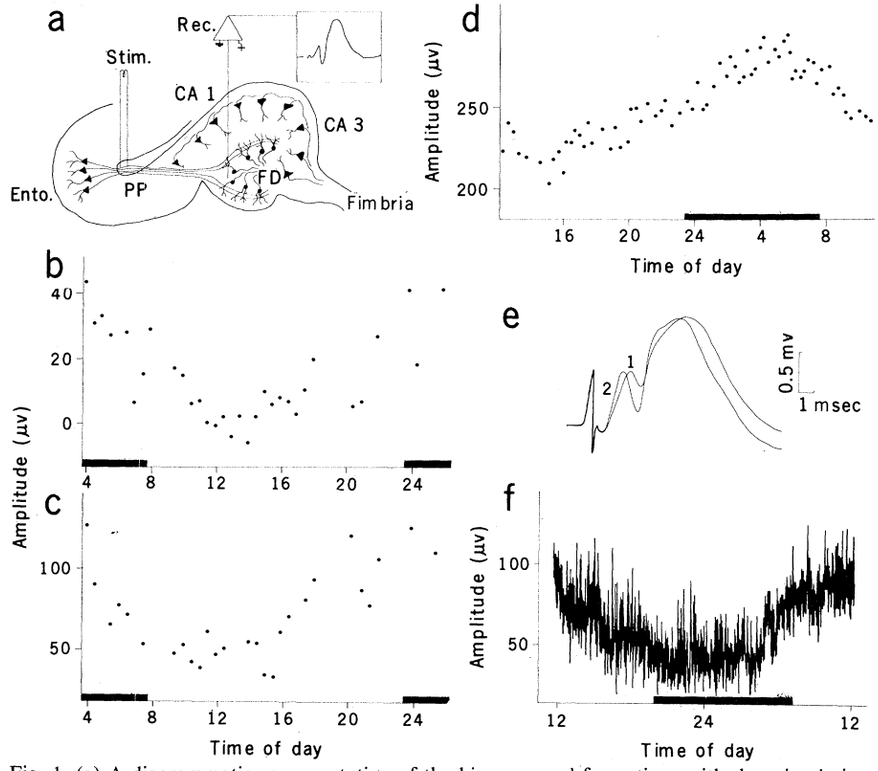

Fig. 1. (a) A diagrammatic representation of the hippocampal formation, with the stimulating (*Stim.*) and recording (*Rec.*) configuration used; *FD*, fascia dentata; *CA 1, CA 3*, Ammon's horn; *Ento.*, entorhinal cortex; *PP*, perforant path. (b and c) The amplitude of a point on the EPSP taken at a fixed latency (3 msec after stimulus) (b) and the population spike (6 msec after stimulus) (c) from the evoked response in rat are largest at times corresponding to the rat's normal dark period (dark horizontal bars represent colony dark intervals). (d) The EPSP amplitude of another rat is shown starting at a different time of day. (e) Examples of an evoked response in the afternoon (1500) (curve 1) and at night (0200) (curve 2) are shown. (f) The amplitude of the EPSP (2.5 msec after stimulus) from one squirrel monkey is shown over a 24-hour period.