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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16. By use of fluoresence microscopy in a slight modification of the Falck-Hillarp formaldehyde condensation technique, we observed that 5-HT terminals in the HB and other periventricular zones were completely abolished after the treatment with 5,7-DHT; in contrast, 5-HT–containing neurons in the midbrain raphe were still intact.
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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

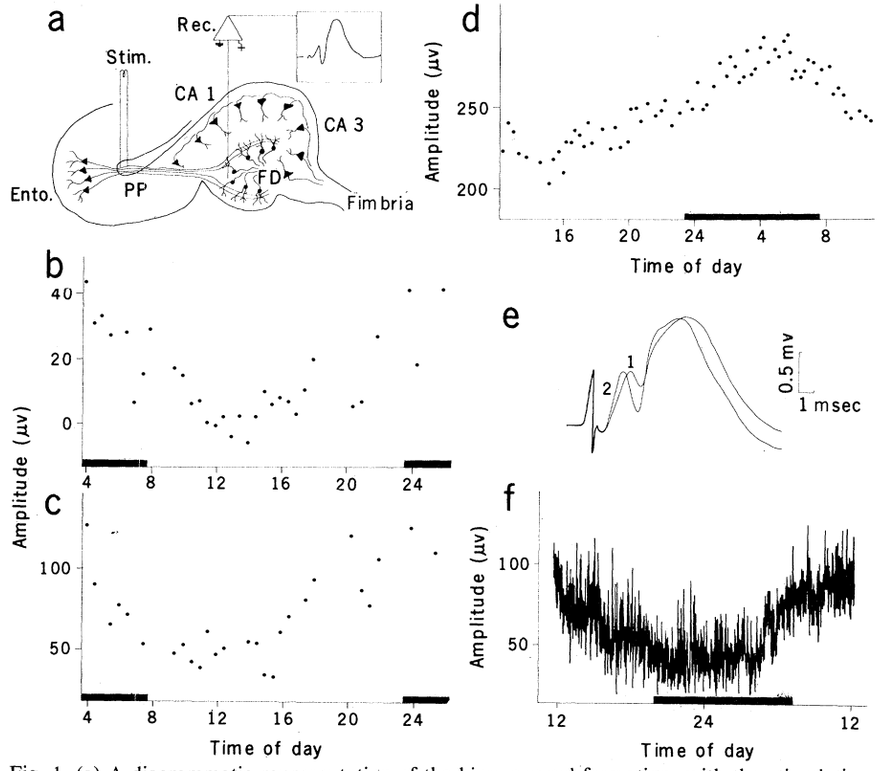

Fig. 1. (a) A diagrammatic representation of the hippocampal formation, with the stimulating (*Stim.*) and recording (*Rec.*) configuration used; *FD*, fascia dentata; *CA 1, CA 3*, Ammon's horn; *Ento.*, entorhinal cortex; *PP*, perforant path. (b and c) The amplitude of a point on the EPSP taken at 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.

the dentate gyrus consisting of three components: (i) a short-latency, small-amplitude deflection representing the propagated action potentials of the perforant path (*13*); (ii) a population excitatory postsynaptic potential (EPSP) (*14*); and (iii) a population action potential which is superimposed on component (ii), representing the firing of many granule cells (termed the population spike). The various components of the evoked response can be identified in the field potential, recorded from an indwelling electrode, which remains stable for many days or weeks (unlike single-cell responses).

Animals were prepared for chronic recording and stimulation as described by Douglas and Goddard (*12*) (Fig. 1a). The perforant path fibers were stimulated with constant-current diphasic square-wave pulses, and the response of the granule cells was recorded at a number of time points over extended periods (up to 48 hours). This was done in several ways in male rats and male squirrel monkeys.

In the first procedure, the evoked response from one rat was sampled approximately once every half-hour over a 24-hour period. Measures of the amplitude of the EPSP and spike are plotted against time in Fig. 1, b and c. The same procedure was used again, starting at a different time of day, in another rat (Fig. 1d). The EPSP and population spike amplitude fluctuated with a circadian rhythm, which was independent of the time of day that testing began. An example of evoked responses taken at two times of day in one rat is shown in Fig. 1e.

The circadian rhythm corresponded to the light-dark schedule in the colony room; the EPSP in our best example being approximately 30 percent larger in the dark phase than in the light phase. To determine whether the cycle was dependent on the level of illumination or general visual stimulation, samples of evoked potentials from rats were taken in the light and in the dark at times corresponding to the rat's normal light period. The amplitude of the potential was characteristic of the time of day tested, and short-term changes in illumination did not affect this characteristic amplitude.

To examine whether the rhythm was endogenous, or whether it was entrained by the light-dark cycle, one rat was blinded by enucleation and tested 1 month later, concurrently with seven sighted rats. The eight rats were tested once every 45 minutes over a single 48-hour period. The amplitude of the evoked potentials from the seven normal animals fluctuated as described above, with the peak amplitude highest in the middle of the dark phase and lowest in the middle of the light phase. The cyclic fluctuation was not abolished in potentials recorded from the blind rat, but its phase was advanced by approximately 4 hours from that of the sighted rats. Hence all environmental factors such as light, temperature, or ambient noise can be rejected as possible causes of the observed fluctuations (although the rhythm is certainly synchronized by light and perhaps other factors in the normal rat). The results from the sighted rats are consistent with the report that multiple unit activity in certain areas of the septum and hypothalamus of female rats is highest during dark periods and lowest when lights are on in the colony room (*15*).

Two squirrel monkeys were also tested over extended intervals. A cycle in the responsiveness of granule cells to perforant path stimulation was again observed, but the phase of the cycle for these animals was opposite to that in the rat; the amplitude was highest during light periods and lowest during darkness. Evoked responses were sampled from the monkeys once every 84 seconds; a representative record for 24 hours is shown in Fig. 1f.

We cannot tell with certainty whether the observed cycle is truly a cycle of synaptic efficacy or an alteration in some other parameter of transmission dynamics, such as the resting potential of the granule cell membrane. A strong case, however, can be presented against the argument that the fluctuation is due to altered sensitivity of the perforant path fibers to electrical stimulation. In some animals prepared for recording, it was possible to record what has been described by Lomo (*16*) as the presynaptic fiber potential [component (i) above]. This provides a measure of the number of active perforant path fibers contributing to the generation of the extracellular EPSP. In one animal we studied in detail the relationship between the EPSP slope and the fiber potential slope over a range of stimulus intensity producing threshold to maximum response. The intensity series was measured at two times of day, corresponding approximately to the maximum and minimum of the EPSP cycle. Although the slope of the EPSP was clearly related to the slope of the fiber potential for the different intensities of stimulation, analysis of covariance showed that fluctuations in the fiber potential slope made no significant contribution to the statistically significant difference in the EPSP slope between the two times of day tested [$F(1, 177) = 15.29$].

The possibility of a circadian cycle of synaptic transmission in the hippocampus is particularly interesting. The integrity of the hippocampal formation is critical to the normal performance of a number of behaviors (*17*) and may be important for normal memory function (*18*). This daily change in synaptic responsiveness covaries with a number of reported behavioral fluctuations (*1–4*), and is maximal at night in the nocturnal rat and maximal during the day in the diurnal monkey. These results raise the possibility that the behaviors that require activity in the dentate granule cells may be emitted with different probabilities at different times of day, even when the eliciting conditions (stimuli) are constant.

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