Gating of Neuronal Transmission in the Hippocampus: Efficacy of Transmission Varies with Behavioral State

Abstract. *Electrical stimuli were applied to the angular bundle of the freely moving rat, and the neuronal responses were recorded ipsilaterally in the dentate gyrus and the CA_1 field of the hippocampus. The number of neurons responding monosynaptically in the dentate gyrus was relatively small when the animal was alert and not moving but was much greater both during slow-wave sleep and during rapid eye movement sleep. In CA_1, however, the trisynaptic population response was considerably smaller during rapid eye movement sleep and when the animal was alert than during slow-wave sleep. These findings are interpreted in terms of a set of behaviorally dependent "neural gates." Measurement of the synaptic current at the dentate gyrus induced monosynaptically by stimulation of the angular bundle further suggests that the mechanism by which gating occurs at this level is either a tonic inhibitory synaptic influence exerted upon the granule cells during the alert state, a tonic excitatory influence during slow-wave sleep, or both.*

Andersen and colleagues elucidated the major physiological relationships among neurons within the hippocampal formation, as well as the sequential effects produced at various stages in the hippocampal circuitry as a result of stimulation of the perforant pathway (*1*). These studies were carried out in anesthetized preparations in order to isolate the system being studied from variable environmental and behavioral influences. Recent findings have suggested the possibility that neuronal excitability at various sites within the hippocampal formation may be modified in a tonic manner during particular waking and sleeping behaviors. First, theta rhythm, an alternating electrical signal, dominates the slow-wave activity in the hippocampal formation during certain behaviors but is absent during others (*2*). Theta rhythm has been shown in several studies to be accompanied by intracellular potential changes which should be capable of affecting neuronal excitability (*3*). Second, other investigations have revealed that there are noradrenergic and serotonergic pathways innervating the hippocampus and have established that the activation of these pathways can modify the excitability of hippocampal neurons (*4*). Furthermore, the rates of firing of the serotonergic and noradrenergic neurons vary according to the animal's behavioral state (*5*). These two groups of findings imply that tonic influences may be exerted upon hippocampal neurons during certain behavioral conditions and suggested to us the desirability of measuring hippocampal neuronal excitability in unanesthetized preparations (*6*). To accomplish this objective, we have utilized perforant pathway volleys and have recorded the evoked neuronal responses at various stages in the hippocampal formation in freely moving rats during a variety of behavioral states.

Experiments were conducted on 18 adult male Sprague-Dawley rats. Each was implanted under pentobarbital anesthesia with several stimulating and recording electrodes. These included a bipolar stimulating electrode inserted stereotaxically into the angular bundle for activating the perforant pathway (*7*), and a microelectrode device for later insertion of a recording electrode along a preselected vertical track through the dorsal hippocampal formation ipsilaterally (*8*). Several recording macroelectrodes were installed in fixed position bilaterally in the hippocampal formation for monitoring hippocampal slow-wave activity (*9*), and an additional recording macroelectrode was positioned in the ipsilateral entorhinal cortex. All electrode terminals and the indifferent connection (a screw implanted in the right frontal bone) were led to a multipin miniature connector which was sealed to the skull, along with the electrodes, by means of acrylic cement. During the subsequent experiment, a mating connector was attached to the animal on which was mounted a multichannel source-follower amplifier with field-effect transistor input stages. A counterbalanced cable led the signals through a mercury commutator, an arrangement that allowed the animal to move about freely during the experiment within the confines of the test chamber. The various signals were amplified and passed through either a narrow band-pass filter (3 to 20 hertz) for recording ongoing slow-wave activity or through a wide band-pass filter (0.07 hertz to 3 khz) for recording the response to electrical stimulation.

Experiments were conducted at least 48 hours after surgery and after the acclimation of the animal to the test chamber. The microelectrode was lowered stepwise toward the hippocampal formation, and at each microelectrode position a single stimulus pulse was applied through an isolation unit to the angular bundle (250 μsec duration, 100 to 900 μa intensity), and the response was observed on an oscilloscope. Once the evoked field potential indicated that the electrode tip was situated in a region of interest, the electrode was left in position and then stimulus trials were given at a variety of intensities during each of three behavioral states (*10*). These states were slow-wave sleep (SWS), sleep with rapid eye movements (REM), and wakefulness without any outwardly observed movement (alert). At a given stimulus intensity, approximately 20 trials were carried out during each behavioral state, with behaviors being alternated, on the average, every 10 trials so as to avoid misinterpreting possible temporal changes in response as behavioral changes. Trials were given once per 10 seconds on the average, but in no case more often than once per 5 seconds. This procedure was repeated at a series of stimulus intensities. The evoked responses were recorded on FM magnetic tape, along with slow-wave activity from the microelectrode and macroelectrodes and pulses indicating stimulus onset. Slow-wave activity was recorded in addition on paper polygraph tape, along with a coded indication of each stimulus presentation. Evoked responses from the magnetic tape were subsequently converted into digital form at 100-μsec sampling intervals on a computer, and these records were also processed by computer to yield, for each combination of electrode position, stimulus intensity and behavioral state, the mean potential response, and the standard error of the mean. After the experiment, electrode positions were verified histologically as in previous experiments (*8, 9*).

Figure 1A shows, in schematic form, a coronal section of the dorsal hippocampal formation of the rat, along with sample electrical records. The trisynaptic intrahippocampal chain elucidated by Andersen and colleagues (*1*) may be traced in the figure both anatomically and electrically from the entorhinal cortex to the CA_1 field of the hippocampus. From its source in the entorhinal cortex, the fibers of the perforant pathway converge to form the angular bundle before dispersing to innervate their target cells in the dentate gyrus. Stimulation of the angular bundle is followed, at monosynaptic latency, by a negative-going evoked potential recorded in the molecular layer of the dentate gyrus, reflecting synaptic currents generated in this region by action of the excitatory perforant pathway terminals. This evoked potential is labeled 3 in Fig. 1A. A micro-

electrode placed closer to the granule cell somata will detect a spikelike potential representing monosynaptic activation of a population of granule cells. Figure 1, A2 shows such a spikelike potential. The evoked potential is positive-going in this case, reflecting a small displacement of the recording electrode along the dendrites from the presumed site of action current generation in the granule cell somata. A negative-going spikelike evoked potential is seen within the granule cell layer. To distinguish evoked potentials produced by the extracellular flow of synaptic currents from those arising from extracellular action currents, the term evoked synaptic potential (ESP) will be used for the former and evoked action potential (EAP) for the latter. Thus, Fig. 1, A3 and A2 represent the ESP and EAP, respectively, of the dentate gyrus. The axons of the granule cells project in turn to the CA_3 field via the mossy fibers. Disynaptic ESP's and EAP's may be recorded at CA_3 (not illustrated). The CA_1 hippocampal field is activated trisynaptically via collaterals of CA_3 neurons, the Schaffer collaterals, which form excitatory connections with the dendrites of the CA_1 pyramids. Figure 1, A1 shows a prominent EAP reflecting action potentials evoked trisynaptically in a population of CA_1 cells as a result of angular bundle stimulation.

The ESP's and EAP's illustrated in Fig. 1A are similar to those previously demonstrated in anesthetized preparations of species other than the rat (*1*). Recordings made in the present study in the freely moving, unanesthetized rat revealed that the amplitudes of these field potentials were very much dependent upon the animal's behavioral state. Let us consider the CA_1 EAP shown in Fig. 1, A1. This recording was made during SWS. However, during the states of alert and REM the prominent population spike seen during SWS was almost completely absent even at stimulus intensities that were supramaximal during SWS (two to three times threshold; Fig. 1, C1). Figure 1, B1 is an illustration of the computer-generated mean response at a stimulus intensity of 625 μa. In the leftmost part of the figure the method of measurement of the EAP is indicated. In Fig. 1, C1, EAP amplitudes are plotted as a function of stimulus intensity. It can be seen that information from the perforant pathway proceeds relatively free of restriction to the CA_1 pyramids during SWS, while during the states of alert and REM the effectiveness of transmission is drastically reduced.

Recordings made in the dentate gyrus revealed that the dependence of transmissional efficacy upon behavioral state was equally specific at this structure and in CA_1. Figure 1, B2 and C2 illustrate sample responses and the stimulus-response relationship during all three behavioral states. In the dentate gyrus the EAP's were distinctly greater in magnitude during both sleep states than during the alert state. Presumably, the relative failure of transmision through the perforant pathway to the granule cells is responsible for the ultimate failure of transmission to the CA_1 pyramids. However, since transmission during REM is equally effective at this stage as during SWS, whatever process interferes with transmission through CA_1 during REM must act either at CA_3 or at CA_1 itself.

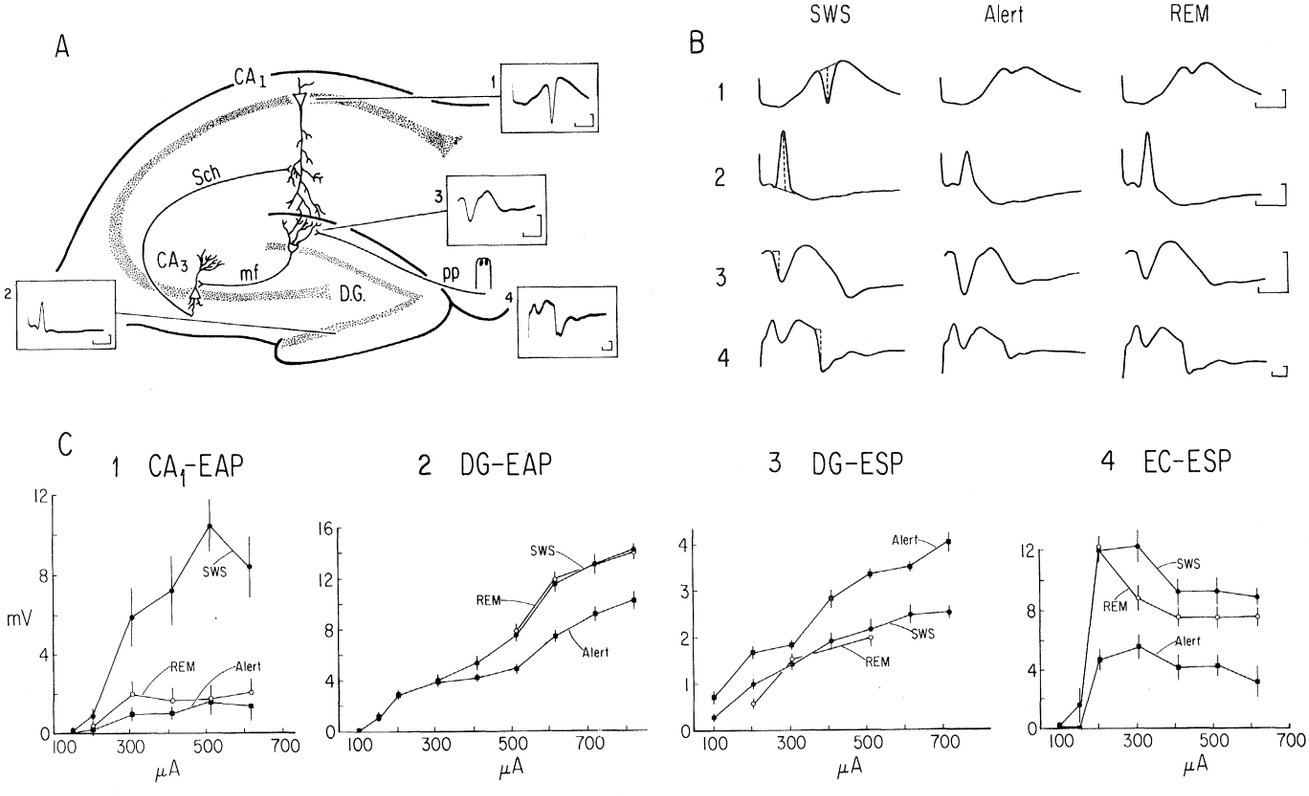

Fig. 1. (A) Coronal section of rat hippocampus illustrating trisynaptic chain. Perforant pathway (*pp*), stimulated at the site of the angular bundle (not shown) activates granule cell of the dentate gyrus (*DG*) which projects via its axon, the mossy fiber (*mf*), to a CA_3 pyramidal cell. The CA_3 neuron innervates a CA_1 cell by the Schaffer collateral (*Sch*). Record 1, evoked action potential (*EAP*) at CA_1; record 2, EAP at DG; record 3, evoked synaptic potential (*ESP*) at DG; and record 4, ESP at entorhinal cortex (*EC*). Time and voltage calibrations: 5 msec and 4 mv (positivity is upward). (B) Computer averages of field potential responses during various behavioral states as shown. 1, EAP at CA_1, stimulating current 625 μa; 2, EAP at DG, 625 μa; 3, ESP at DG, 500 μa; and 4, ESP at EC, 300 μa. Measurement of response amplitudes is indicated by dotted lines. The ESP amplitude at DG was measured at a latency of 3.2 msec from stimulus onset. The ESP amplitude at EC was measured at a latency of 1.7 msec from onset of the late potential. Calibrations as in A. (C) Amplitudes of EAP and ESP plotted as a function of stimulus current. Curves in 1 to 4 correspond to the same recordings as in (B) 1 to 4. Vertical bars indicate 95 percent confidence limits. Closed circles, SWS; open circles, REM; and closed squares, alert.

The mechanism by which transmission through the dentate gyrus is controlled may be inferred from the stimulus-response relationship for the ESP, shown in Fig. 1, B3 and C3. The synaptic current, in contrast to the action current, is greater during the alert state than it is during SWS and REM. This suggests that the granule cells receive tonic inhibitory influences during the alert state which do not operate during SWS and REM, or tonic excitatory influences during SWS and REM which do not operate during the alert state, or both. In either case, the cell membranes are relatively hyperpolarized during the alert state and are relatively depolarized during SWS and REM, with the consequence that an afferent volley of constant size will evoke a larger synaptic current during the alert state, but will be less effective in evoking action potentials in the granule cells.

Deadwyler *et al.* (*11*) reported the existence of a physiological pathway projecting back from the CA_3 zone of the hippocampal formation to the ipsilateral entorhinal cortex. Figure 1, A4, shows a record from the entorhinal cortex in response to stimulation of the angular bundle that is similar to the response previously found to direct stimulation of CA_3, except that the late negative potential is about 3 msec greater in latency in the present experiment. This response presumably represents the same process of entorhinal cortex activation via CA_3 (*11*). The magnitude of this ESP was also found to vary according to the animal's behavioral state, as shown in Fig. 1, B4 and C4. The difference in the ESP between SWS and the alert state seen in the entorhinal cortex is similar to that which has already been shown to occur in the EAP's both in the dentate gyrus and in CA_1. During REM, the response in the entorhinal cortex is large, just as during SWS. This is what occurs also in the dentate gyrus, but is in contrast to what happens in CA_1 where the response during REM is small. This suggests that the relative ineffectiveness of transmission from perforant pathway to CA_1 that occurs during REM is due to some process that acts at the CA_1 level and not at CA_3.

We have shown that at various synapses in the hippocampal formation the effectiveness of neuronal transmission is greater during some behavioral states than during others. This behavioral influence may be conceptualized as a gating process which operates at several critical hippocampal junctures. In the dentate gyrus the mechanism by which gating is effected appears to be either an excitatory influence which is tonically active during SWS and REM or an inhibitory influence which is tonically active during the alert state (*12*). Our findings do not distinguish which of these two mechanisms occurs. However, a substrate for an inhibitory mechanism may be provided by other findings. There are extensive noradrenergic and serotonergic terminations in the dentate gyrus (*4*). These transmitters are known to produce inhibition of neuronal firing rates in the hippocampus, and their neurons of origin fire more rapidly during the alert state than during SWS (*4*, *5*). These findings, taken together, are compatible with the inhibitory mechanism of behavioral gating. However, further experiments are necessary to resolve this point.

The behaviorally specific gating that we have shown to occur in the hippocampal formation controls the passage of information both into the hippocampal formation and from it to extra-hippocampal structures. This gating apparently underlies central nervous system processes that occur during waking behavior, SWS, and REM.

JONATHAN WINSON
The Rockefeller University, New York 10021

CHARLES ABZUG
Department of Physiology, University of Maryland School of Medicine, Baltimore 21201

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6. Changes in neuronal excitability with behavioral state have previously been shown to occur within both sensory and motor systems [for example, E. V. Evarts, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **19**, 828 (1960); M. M. Gassel, P. L. Marchiafava, O. Pompeiano, *Arch. Ital. Biol.* **102**, 471 (1964); F. Michel, *J. Physiol. (Paris)* **57**, 266 (1965); G. Carli, K. Diete-Spiff, O. Pompeiano, *Arch. Ital. Biol.* **105**, 52 (1967); T. Allison and G. D. Goff, *ibid.* **106**, 41 (1968); E. Favale, A. Seitun, A. Tartaglione, M. Tondi, *Arch. Int. Physiol. Biochim.* **80**, 649 (1972)]. These studies have been carried out by means of stimulation of either central or peripheral structures and recording of either evoked field potentials or unitary responses.
7. The animal was positioned in a stereotaxic device so that the skull flat plane (passing through bregma and lambda and parallel to the interaural line) was level, and the stimulating electrode was implanted 7.2 mm posterior to bregma, 4.1 mm lateral to the midline, and 3.5 mm below the surface of the skull.
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10. Recordings were made from between one and three locations in the hippocampal formation of each animal.
11. S. A. Deadwyler, J. R. West, C. W. Cotman, G. Lynch, *Exp. Neurol.* **49**, 35 (1975).
12. There are other routes by which the perforant pathway can affect the various subdivisions of the hippocampal formation in addition to the trisynaptic pathway of Andersen and co-workers (*1*). These include, but are not limited to, a direct entorhinal input to hippocampal subfields other than the dentate gyrus [A. Hjorth-Simonsen and B. Jeune, *J. Comp. Neurol.* **144**, 215 (1972); A. Hjorth-Simonsen, *ibid.* **146**, 219 (1972)] and a recurrent pathway from CA_3 and CA_4 pyramids to the granule cells of the dentate gyrus [J. Zimmer, *J. Comp. Neurol.* **142**, 393 (1971); D. I. Gottlieb and W. M. Cowan, *J. Comp. Neurol.* **149**, 393 (1973)]. Our findings do not exclude the possibility that such pathways participate in the effects we have recorded.
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Infrared Reflectance in Leaf-Sitting Neotropical Frogs

Abstract. *Two members of the glass-frog family Centrolenidae (*Centrolenella fleischmanni, C. prosoblepon*) and the hylid subfamily Phyllomedusinae (*Agalychnis moreletii, Pachymedusa dacnicolor*) reflect near-infrared light (700 to 900 nanometers) when examined by infrared color photography. Infrared reflectance may confer adaptive advantage to these arboreal frogs both in thermoregulation and infrared cryptic coloration.*

Many arboreal members of the glass-frog family Centrolenidae and tree-frog family Hylidae are green, and thus cryptically colored when viewed in visible light (400 to 700 nm). Infrared color photography (*1*) reveals that two centrolenids (*Centrolenella fleischmanni, C. prosoblepon*) and two phyllomedusine hylids (*Agalychnis moreletii, Pachymedusa dacnicolor*) also reflect light in the near-infrared region (700 to 900 nm). This is, to our knowledge, the first report of infrared reflectance in neotropical frogs. Since photosynthetic leaf surfaces also reflect infrared, these animals are virtually indistinguishable from the leaves on which they sit, both in visible and near-infrared light ranges. All other