Entorhinal and Septal Inputs Differentially Control Sensory-Evoked Responses in the Rat Dentate Gyrus

Abstract. *Averaged sensory-evoked potentials were recorded from the outer molecular layer of the dentate gyrus in naïve rats and in rats conditioned to respond in the presence of an auditory stimulus. Two components (negative peaks) of the potentials were functionally distinguished in terms of responsiveness to unique or conditioned auditory stimuli. Each component was independently generated by a separate input pathway to the dentate gyrus: The perforant path provided an "insignificance" or "unexpected" feature of the sensory stimulus when appropriate, and the septum controlled the development of a second component as a function of the behavioral significance of the stimulus during the acquisition of auditory discrimination behavior. A reciprocal relationship between the peak amplitudes of both components of the average evoked potentials dependent on the relative behavioral significance of the sensory stimulus was observed in all animals during extinction and reconditioning of the sensory discrimination task. The findings indicate that the entorhinal and septal projections to the dentate granule cells are activated differentially by sensory stimuli as a function of their acquired behavioral significance to the animal.*

It has been known for some time (*1*) that the granule cells of the dentate gyrus are innervated by two different major extrahippocampal fiber projections: one from the basal forebrain via the medial septal nucleus (*2*) and the other from the entorhinal cortex via the fibers of the perforant path (*3*). Extensive electrophysiological investigation of these two input pathways has revealed several interesting and important features (*4, 5*). Until recently (*6*), however, there has been no electrophysiological analysis of the functional significance of these pathways in terms of (i) the nature of the information these extrinsic fibers carry to the granule cells and (ii) the consequences of these inputs for granule-cell responsiveness to behaviorally significant environmental events. We have now functionally analyzed the perforant path and septohippocampal fiber systems on the basis of electrophysiological measures of sensory-evoked potentials in the dentate gyrus during behavioral conditioning.

Electrophysiological recordings were obtained from several locations within the dorsal hippocampus of awake, freely moving rats during behavioral conditioning procedures. The apparatus was as described previously (*6*) except that a tone generator circuit with a fast rise-time (1.0 msec) was used to ensure prompt delivery of auditory stimuli (3.5 kHz, 67 dB). Male Sprague-Dawley rats deprived to 80 to 85 percent of their normal body weights were trained to respond in the presence of the tone stimulus with a simple operant response (poking the nose through a photocell beam) to obtain water. Criterion performance (100 percent responding to the tone and fewer than three responses per reinforcement) on this task served as the index of stimulus discrimination (*6*). Rats were surgically prepared for long-term recording by implanting (i) a mount for a removable microelectrode drive (*7*), and (ii) stimulating electrodes in the entorhinal cortex for activating perforant path fibers, in the CA3/CA4 region of the contralateral hippocampus for activating hippocampal commissural fibers, or both. Precise positioning (with a resolution of 50 μm) of the movable recording microelectrode at various points within the CA1 subfield and dentate gyrus in the dorsal hippocampus was possible during the recording session by utilizing well-established electrophysiological criteria (*8*).

From the outer molecular (OM) layer of the dentate gyrus we recorded averaged sensory-evoked potentials (AEP's) to the onset of the tone stimulus during stimulus discrimination learning (*6*). The OM AEP consisted of two separate components (Fig. 1A): a short (15 msec) monophasic negative wave (N_1) and a second, longer (50 to 60 msec) negative peak (N_2) (*9*). The latency to onset for N_1 was 20 msec, while for N_2 it was 45 to 55 msec; the interval between N_1 and N_2 peaks was 50 to 55 msec.

The two components were differentially affected by the conditioned status of the animal. At criterion stimulus discrimination performance, N_2 reached its maximum amplitude and N_1 was reduced or in some cases not present (Fig. 1B). In contrast, when behavioral responding was extinguished, N_1 was much larger and N_2 significantly reduced or absent. This relationship between the amplitudes of N_1 and N_2 and behavioral performance has been verified in other sensory discrimination tasks (*10*). These combined results suggest that N_1 might be related to some unexpected or "unpredictable" feature of the auditory stimulus, whereas N_2 varied directly as a function of the behavioral significance of the stimulus. Direct evidence that N_1 reflected this "unexpected" aspect of the sensory stimulus was obtained from unconditioned (naïve) animals ($N = 7$) to which tones were presented in the same random fashion as during stimulus discrimination training. All seven animals displayed the N_1 component of the OM AEP, but none displayed a consistent N_2 component (Fig. 1C). In naïve animals, however, N_1 was extremely sensitive to the temporal pattern of stimulus presentation. Attenuation or disappearance of N_1 was very rapid when stimuli were presented at regular ("predictable") temporal intervals even as long as 10 seconds.

Since the two components of the OM AEP were reciprocally related to each other as a function of the conditioned behavioral status of the animal, we hypothesized that each might reflect the activation of a different population of afferent fibers to the dentate granule cells. Confirmation that N_1 resulted from sensory activation of the perforant path fibers was provided by depth profile analyses of both components of the AEP's (Fig. 1D). The N_1 component was consistently largest at a point 0.15 mm above the granule cell layer, the region heavily innervated by perforant path axons (*11*). It rapidly declined and reversed to positive as the electrode was advanced ventrally into the granule cell layer and hilus region (*12*). The profile for N_2 differed in that its maximum amplitude was recorded in the perforant path zone and 0.05 mm from the granule cell layer, the region of dense commissural-associational synaptic contacts (*13*).

In order to determine whether N_1 was produced by axons in the perforant path, extensive lesions were placed bilaterally in the entorhinal cortex of three animals that were then tested for OM AEP's in both the naïve and conditioned behavioral states (*14*). Figure 2B shows an example of the complete absence of N_1 in a naïve animal in which the entorhinal cortex had been bilaterally destroyed. The N_1 was severely attenuated or absent in all three of the naïve animals as verified by depth profile analyses (Fig. 1D). When animals with entorhinal lesions were trained to respond discriminatively (at 100 percent) to the same auditory stimulus, only N_2 was present (Fig. 2E). This was verified by the disappearance of N_2 during behavioral extinction and its reappearance during reconditioning.

This finding suggested that another afferent pathway to the dentate gyrus might be responsible for the generation of N_2 during behavioral conditioning. Al-

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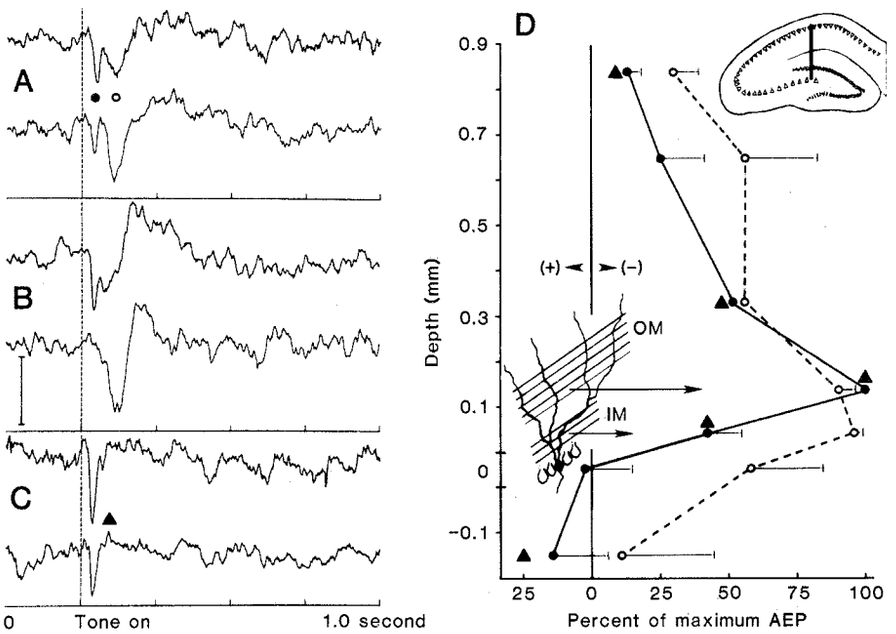

Fig. 1. Sensory-evoked potentials recorded from the dentate gyrus in conditioned and naïve animals. (A) N_1 (dot) and N_2 (circle) components of OM AEP recorded from two different animals during a learned stimulus discrimination task. Average of 50 trials. Tone onset was at 200 msec. Both OM AEP's are shown during the early conditioning phase of the task in order to exhibit both N_1 and N_2. (B) Effects of extinction and reconditioning on N_1 and N_2. Upper trace shows the AEP obtained during 50 trials of behavioral extinction. Lower trace indicates an extreme case in which N_2 is large and N_1 is completely absent during the 50 subsequent reconditioning trials in the same animal. Calibration, 300 μV. (C) The presence of only N_1 recorded from two different naïve animals; tones were presented randomly in the same pattern as during conditioning. The filled triangle refers to the depth profile of the N_1 component shown in (D). (D) Depth profile analysis of the amplitude of N_1 (dots) and N_2 (circles). The electrode track through the dorsal hippocampus is shown in an inset at top right. N_1 (solid line) shows a peak amplitude 0.15 mm from the granule cell layer within the OM layer, where perforant path axons are located (see granule cell inset and long arrow). N_1 reverses to positive at layer of granule cell bodies and in the hilus (−0.1 mm). N_2 shows a different depth profile; the maximum peak is maintained at the inner molecular layer (short arrow) and does not reverse in the granule cell layer. Triangles show a correspondence of the N_1 depth profile recorded in the naïve animal in the lower trace of (C). Each point in the profile (except triangles) represents the average percentage (± standard error of the mean) of the maximum peak amplitudes of N_1 and N_2 recorded over 20 sessions for two animals. The inset drawing of a granule cell depicts regions of maximal peak amplitudes of N_1 (*OM*) and N_2 (*IM*) as shown by arrows.

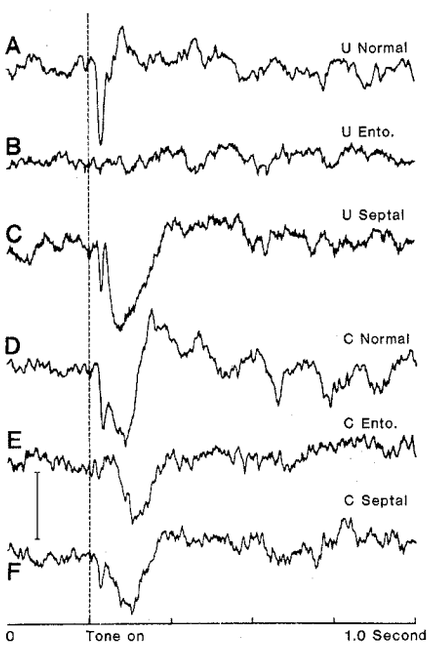

Fig. 2. Selective effects of septal and entorhinal lesions on N_1 and N_2 components of OM AEP's as a function of the conditioned status of the animal. (A) An untrained (*U*) normal animal shows only N_1. (This and subsequent traces represent averages of 50 trials.) (B) Absence of N_1 in a naïve animal sustaining bilateral lesions of the entorhinal cortex. (C) Emergence of N_2 with N_1 in a naïve animal sustaining a lesion of the medial septal nucleus. (D) AEP from a conditioned (*C*) normal animal showing N_1 and N_2 components. (E) Appearance of only N_2 in a conditioned animal with bilateral entorhinal lesion [same as shown in (B)]. Note the absence of N_1 and the slight increase in peak latency of N_2. (F) Presence of N_1 and N_2 in a conditioned animal with a medial septal lesion. The slight reduction in amplitude of N_1 and N_2 (trace C) in the animal with a septal lesion is within the variance range of OM AEP's from normal animals over different recording sessions. Tone onset was at 200 msec; calibration, 300 μV. All potentials from animals with lesions were obtained at least 30 days after surgery. Criterion behavioral performance on the stimulus discrimination task accompanied conditioned OM AEP's shown in (D) to (F).

though depth analyses suggested the commissural-associational projection, the septal input to the granule cells was also a likely source for N_2. Therefore, we tested for OM AEP's before and after discrimination training in three animals sustaining lesions of the medial septum. In all cases the lesions were verified histologically, as well as physiologically, by an absence of the theta rhythm (*5*). Septal lesions selectively influenced the appearance of N_2. Surprisingly, three naïve animals with septal lesions demonstrated both N_1 and N_2 to tones presented randomly in the absence of prior behavioral conditioning. The N_2 in naïve animals with septal lesions was also larger in amplitude than in normal conditioned animals (Fig. 2, C and D); however, these differences in amplitude did not persist once the animals were conditioned (Fig. 2F). The amplitude of N_2 remained larger during behavioral extinction than it did in normal animals. In addition, hippocampal theta rhythm was not responsible for generating the OM AEP. Others have shown that sensory stimulation elicits unitary discharges recorded from the dentate gyrus of unanesthetized rats; these discharges increase after septal lesions are made (*15*). We have now shown that such increased discharge might be mediated by the additional occurrence of N_2 to sensory stimuli in animals with septal lesions.

The appearance of N_2 in the dentate gyrus of naïve animals with septal lesions and its presence in conditioned animals with entorhinal lesions support the hypothesis that N_2 does not depend on intact connections with the entorhinal cortex or the medial septum (*16*). In normal animals, N_2 appeared only after discrimination training and decreased rapidly during extinction [see also (*6*)]. In animals with septal lesions, N_2 did not decrease during extinction. Since N_2 was fully developed in naïve animals with septal lesions, the septal connections are strongly implicated in the regulation of the development of N_2 in normal rats during sensory discrimination learning. Thus, the septum may exert a generalized tonic suppression over other sensory responsive hippocampal circuits (that is, commissural and associational) in naïve animals. Release of this suppressive influence during learning results in the appearance of N_2 to behaviorally significant stimuli.

In normal animals, the dependence of the reciprocity between N_1 and N_2 on the conditioning history of the tone stimulus supports our different lesion effects in designating two separate functional roles

for the septal and entorhinal projections to the dentate gyrus. Seemingly, both the septal and entorhinal inputs to the dentate gyrus relate to the behavioral significance of sensory stimuli. The functional characteristics of these two inputs make it possible for the granule cells to respond differentially to such stimuli. The nature of this response depends on the position of the sensory stimulus along an abstract continuum anchored at one end by "unexpectedness" or lack of behavioral significance and at the other by features that provoke maximum expectation of biologically significant events through associative learning. In essence, such a system would be capable of discriminating the degree of familiarity of any sensory stimulus, a function previously suggested to explain many of the similarities in memory disruption in humans and animals resulting from hippocampal damage (*17*).

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Imipramine: Effect of Ovarian Steroids on Modifications in Serotonin Receptor Binding

Abstract. *Long-term administration of imipramine caused a decrease in serotonin$_2$ receptor binding in rat brain cerebral cortex, an effect that was abolished by ovariectomy. In contrast, ovariectomy had no effect on imipramine-induced decreases in hippocampal serotonin or in cerebral cortical and hippocampal β-adrenergic receptor binding. Administration of estradiol or progesterone separately or in combination reestablished the effect of imipramine treatment on cortical serotonin$_2$ receptors. These results suggest that ovarian steroids may play an important, but subtle, role in the neurochemical and perhaps clinical response to this drug.*

Affective illness is thought to be related to a dysfunction in monoamine activity in the central nervous system (*1*). This hypothesis is supported by the finding that clinically effective antidepressants, such as imipramine, modify monoaminergic transmission (*1*, *2*). Recent studies suggest that antidepressants share a common action in that long-term administration leads to a dose- and time-dependent decrease in the number of β-noradrenergic and serotoninergic receptor sites in brain (*3*). Of particular interest is the fact that these changes in neurotransmitter receptor binding and function correspond, in a temporal fashion, with the delay observed in the onset of the therapeutic response to these drugs. Thus, antidepressant-induced neurotransmitter receptor alterations may be a useful index for measuring the therapeutic efficacy of this drug class.

Steroid hormones may also be involved in some types of affective disorders (*4*). Ovarian hormones may be important in this regard since depression is often associated with physiological states related to ovarian hormone secretion such as menstruation, parturition, and menopause (*4-6*). In addition, abnormalities in hormone secretion and activity have been noted in depressed patients (*7*).

We have examined whether there is a relation between ovarian hormones and the neurochemical effects of imipramine. The results indicate that the imipramine-induced decrease in serotonin, but not β-adrenergic, receptor binding depends on the presence of these steroids and suggest that the response to imipramine may be altered in patients suffering from ovarian hormone deficiencies.

Sprague-Dawley rats (125 g) were ovariectomized bilaterally and 7 days after surgery imipramine treatment was initiated. Imipramine was administered intraperitoneally at 10 mg/kg once daily for 21 days. In replacement experiments, groups of animals received daily subcutaneous injections of 17β-estradiol (40 μg/kg), progesterone (4 mg/kg), the combination of estradiol and progesterone, or vehicle (ethanol and safflower oil) only, beginning at 2 days after surgery and continuing for 26 days. On day 6 of hormone replacement treatment, imipramine administration was initiated and continued for 21 days. All the animals were killed 48 hours after the last injection, which was 30 days after ovariectomy.

The animals were killed by decapitation and their brains were rapidly removed, dissected, and stored at −20°C. Cerebral cortical and hippocampal serotonin$_2$ (5-HT$_2$) receptor binding was analyzed by using [^{3}H]spiroperidol (New England Nuclear; 23 Ci/mmole) as a ligand (*8*); β-adrenergic receptor binding was assayed by using [^{3}H]dihydroalprenolol ([^{3}H]HDHA; New England Nuclear; 49 Ci/mmole) as a ligand (*9*). For these assays, brain membranes were incubated with either 0.3 n*M* [^{3}H]spiroperidol or 0.5 n*M* [^{3}H]DHA in the presence or absence of unlabeled serotonin (10^{-1}*M*) for the former or *dl*-propranolol (10^{-5}*M*) for the latter. Binding assays were terminated by rapid filtration under vacuum over Whatman glass fiber filters with three 5-ml rinses in cold buffer. The filters were counted by liquid scintillation spectrometry. Protein concentrations were determined by the method of Lowry (*10*). Specific receptor binding is de-

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