

COMMENTARY

THE THREE-DIMENSIONAL ORGANIZATION OF THE HIPPOCAMPAL FORMATION: A REVIEW OF ANATOMICAL DATA

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Abstract—In the early 1970s, Andersen and colleagues proposed that the principal excitatory pathways of the hippocampal formation were organized in a lamellar fashion. This proposition, based heavily on the physiological studies of the proponents, indicated that “a point source of entorhinal activity projects its impulses through the four membered pathway (of the hippocampal formation) along a slice or lamella, of hippocampal tissue oriented normally to the alvear surface” [Anderson P., Bliss V. P. and Skrede K. K. (1971) *Expl Brain Res.* 13, 222–238] and perpendicular to the long axis of the hippocampus. Andersen *et al.* further suggested that, “By means of this lamellar organization, small strips of the hippocampal cortex may operate as independent functional units, although excitatory and inhibitory transverse connections may modify the behavior of neighboring lamellae.”

The “lamellar hypothesis” of hippocampal anatomical organization has had tremendous influence on the conceptualization of hippocampal information processing and was largely responsible for prompting the establishment of the *in vitro* hippocampal slice technology. While the “lamellar hypothesis” was consistent with the known neuroanatomy, subsequent neuroanatomical investigations, using a variety of modern tracing techniques, have invariably demonstrated that all of the major hippocampal projections, except for those arising from the granule cells of the dentate gyrus, are much more divergent than would be consistent with a strict interpretation of the lamellar hypothesis. This has become particularly clear in ongoing studies of the intrinsic hippocampal projections using the recently introduced anterograde tracer, *Phaseolus vulgaris* leucoagglutinin.

Citing the conclusions from several papers dealing with the anatomical organization of the hippocampal formation and using examples from recent *Phaseolus vulgaris* leucoagglutinin mapping studies, the following are demonstrated. (1) That the major hippocampal projections are as extensive and highly organized in the long or septotemporal axis of the hippocampus as in the transverse axis. (2) That at least some of the hippocampal projections, such as the associational projections arising from the dentate gyrus, appear to be specifically organized to integrate distant levels of the hippocampal formation. (3) That the physiological data of Anderson *et al.* can be re-interpreted in the light of these new anatomical data to show how the stimulation and recording protocols used at the time would, in fact, generate the appearance of a lamellar organization.

It is concluded that it is heuristically most reasonable to consider the hippocampal formation as a three-dimensional cortical region with important information processing taking place in both the transverse and long axes. This view of intrinsic hippocampal circuitry may be important for the interpretation of physiological studies of the rat hippocampus and for efforts directed at developing models of hippocampal function.

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1. INTRODUCTION

The hippocampal formation comprises four relatively simple cortical regions (Fig. 1A). These include the

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Abbreviation: PHA-L, *Phaseolus vulgaris* leucoagglutinin.

dentate gyrus, the hippocampus proper (which can be divided into three sub-fields, namely CA3, CA2 and CA1), the subiculum complex (which can also be divided into three subdivisions: the subiculum, presubiculum and parasubiculum) and the entorhinal cortex which, particularly in the rodent, is generally

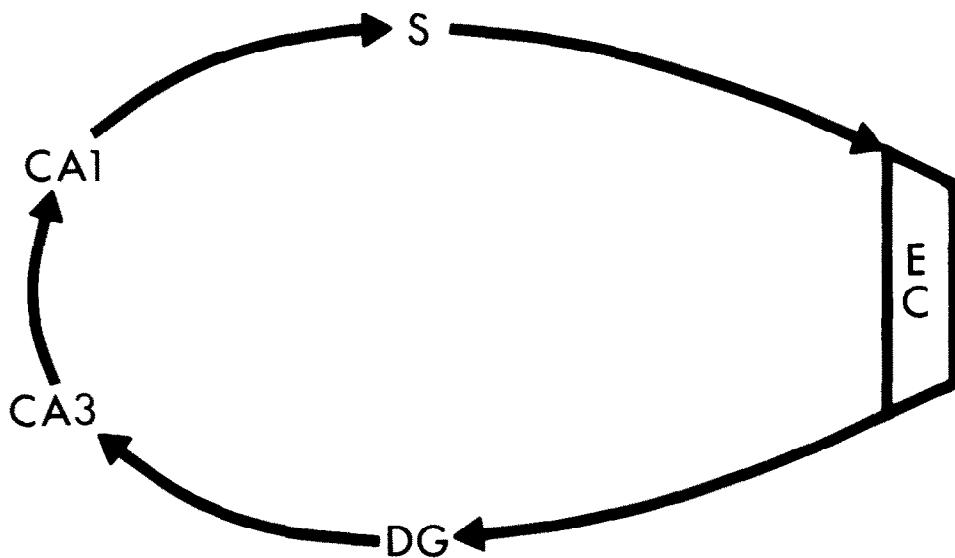
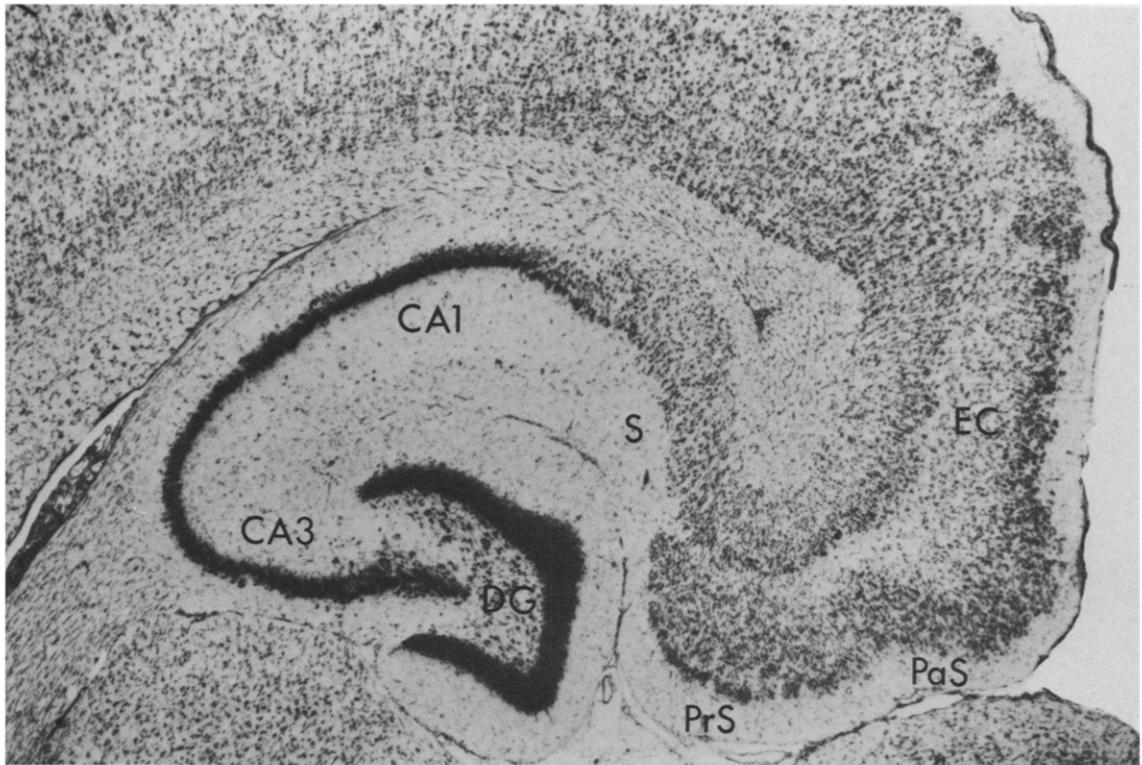


Fig. 1. Top. Photomicrograph of a Thionin-stained horizontal section through the rat hippocampal formation. The hippocampal formation comprises four distinct cytoarchitectonic regions: the entorhinal cortex (EC), the dentate gyrus (DG), the hippocampus proper (which is divided into fields CA3, CA2—not indicated—and CA1) and the subiculum complex, which is subdivided into subiculum (S), presubiculum (PrS) and parasubiculum (PaS). Bottom. A unique feature of the hippocampal formation is that each of the fields are linked by unidirectional excitatory projections. These are schematized in this simplified diagram of the intrinsic circuitry of the hippocampal formation. A more complete circuit summary is shown in Fig. 8.

divided into medial and lateral subdivisions. The three-dimensional shape of the rodent hippocampal formation is relatively complex. It appears grossly as an elongated structure with its long axis bending in a C-shaped manner from the septal nuclei rostro-dorsally to the incipient temporal lobe caudo-

ventrally. The long axis is generally referred to as the septotemporal axis and the orthogonal axis will be referred to as the transverse axis. These are shown diagrammatically in Fig. 2.

We shall return to a more detailed description of the intrinsic circuitry of the hippocampal formation.

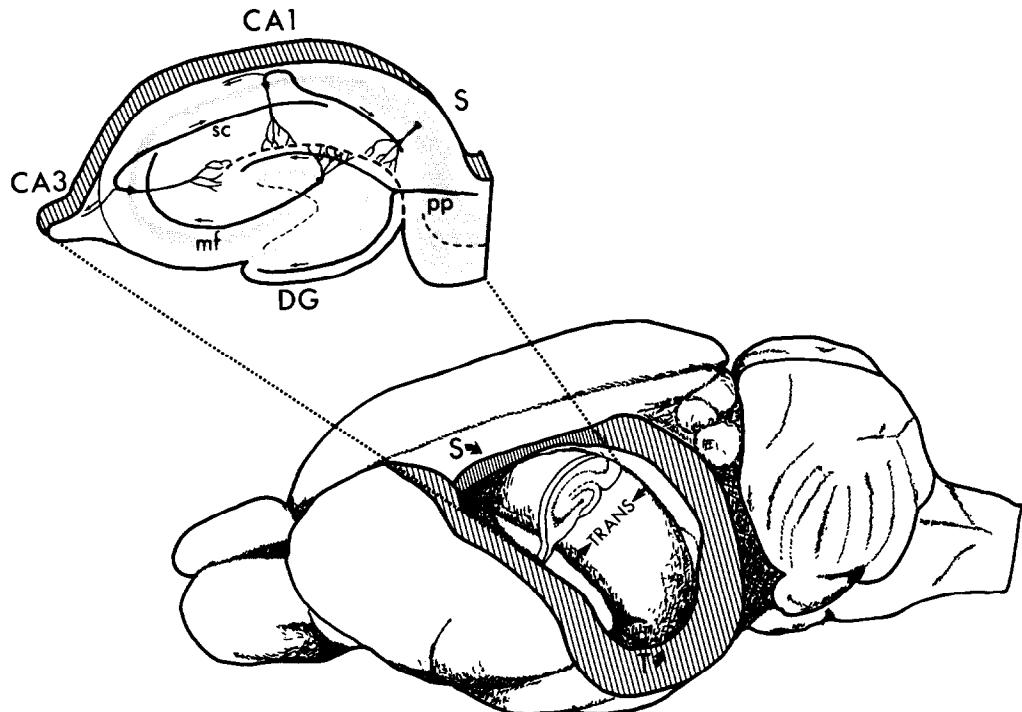


Fig. 2. The position of the hippocampal formation in the rat brain is shown in this drawing of a preparation in which the cortical surface overlying the hippocampus has been removed. The hippocampus is an elongated, C-shaped structure with the long or septotemporal axis running from the septal nuclei rostrally (S) to the temporal cortex (T) ventrocaudally. The short or transverse axis (TRANS) is oriented perpendicular to the septotemporal axis. The major fields of the hippocampal formation (except for the entorhinal cortex) are found in slices taken approximately midway along the septotemporal axis. The slice pictured at top left is a representation of the summary of the major neuronal elements and intrinsic connections of the hippocampal formation as originally illustrated by Andersen *et al.*² (see text for details).

Abbreviations: DG, dentate gyrus; mf, mossy fibers; pp, perforant path; sc, Schaffer collaterals.

However, the basic connections as determined in the classical Golgi studies of Ramón y Cajal²⁹ and Lorente de Nò,^{25,26} and the degeneration studies of Blackstad,^{5,6} Blackstad *et al.*⁷ and Raisman *et al.*²⁸ can be summarized as follows. The fields of the hippocampal formation are linked by unique and largely unidirectional connections (Figs 1 (bottom) and 2). The dentate gyrus receives its major input from the entorhinal cortex via the so-called perforant pathway. The granule cells of the dentate gyrus project via their mossy fibers to the CA3 field of the hippocampus. Pyramidal cells of the CA3 field give rise to collateralized axons that terminate within CA3 as associational connections and also provide the major input to the CA1 field of the hippocampus, the so-called Schaffer collaterals.

2. THE LAMELLAR HYPOTHESIS OF HIPPOCAMPAL ORGANIZATION

In the early 1970s, the results of a number of physiological studies and the anatomical studies cited above, were combined to provide a coherent hypothesis of the three-dimensional organization of the major intrinsic hippocampal connections.² We will refer to the view promulgated primarily by Andersen and colleagues as the "lamellar hypothesis" of hippo-

campal organization. Before critically reviewing the evidence marshaled in support of this hypothesis, we should summarize its major features. This is best accomplished by reiterating the statements of Andersen *et al.*² in the paper which first explicitly presented the "lamellar hypothesis." They summarize the results of their physiological studies in the rabbit in the following way. "The conclusion of the experiments . . . is that the four pathways successively activated when a stimulus is delivered to the entorhinal area, i.e., the perforant path, the mossy fibers, the Schaffer collaterals, and finally the alvear fibers of CA1, are all oriented in the same direction, namely nearly transversely to the longitudinal axis. Thus, a localized activation of the entorhinal area will lead to a small slice, or lamella, of tissue being activated by the four pathways in succession." They further concluded that, "The hippocampal cortex seems to be organized in parallel lamellae . . . By means of this lamellar organization, small strips of the hippocampal cortex may operate as independent functional units, although excitatory and inhibitory transverse connections may modify the behavior of the neighboring lamellae." According to this view, the hippocampal formation comprises a number of cytoarchitectonically and connectionally stereotyped

slices that are stacked up to form the long axis of the structure. Furthermore, the major flow of neural activity is suggested to be within each slice rather than between adjacent slices (Fig. 2). While not explicitly proposed by Andersen *et al.*,² it was presumed by some that the lamellar organization of the hippocampal formation entailed a physical discontinuity of lamellae much like the separation of ocular dominance columns in visual cortex. While the thickness of the slice was never explicitly stated, the impression given was that there were likely to be many (possibly 10 or more) in the rabbit hippocampal formation.

What were the anatomical and physiological considerations that led Andersen and colleagues to this view? And to what extent has recent anatomical data supported the "lamellar hypothesis"? To provide answers to these questions, we will discuss anatomical information relevant to the organization of each of the links in the chain of hippocampal connectivity. Before beginning this review, however, we must first briefly describe the basic physiological paradigm employed by Andersen and colleagues and then describe recently employed anatomical methodologies that have facilitated a detailed analysis of the three-dimensional organization of hippocampal connections.

3. PHYSIOLOGICAL PROCEDURES

The *in vivo* studies of Andersen and colleagues² were carried out in the dorsal hippocampal formation of rabbits. Basically similar experimental designs were used to study the organization of (i) the perforant pathway from the entorhinal cortex to the dentate gyrus, (ii) the mossy fiber projection from the dentate gyrus to CA3, and (iii) the Schaffer collaterals to CA1. Both orthodromic and antidromic stimulation were applied, and either the effect of stimulation in a single position was measured at multiple recording sites, arranged along the longitudinal or transverse axes of the hippocampal fields, or the effects of stimulating different locations were registered through a single recording electrode. Using these procedures they were able to assess the longitudinal spread of activity, following focal stimulation in each of the hippocampal pathways. As seen in Fig. 7B, for example, two stimulating electrodes were placed in a fixed position in the cell layer of CA3. A single recording electrode was lowered into CA1 at several positions along the long axis of the hippocampus. The amplitude of the population spike was then recorded and plotted relative to the position of the electrode.

4. RECENT ANATOMICAL PROCEDURES AND RESULTS

Because of its complex three-dimensional shape, normal sections of the hippocampus, i.e. those oriented perpendicular to the long axis, are obtained for only a small part of its septotemporal extent in standard coronal or horizontal sections. This situation severely complicates the analysis of the con-

nnections within the hippocampal formation. We have adopted a strategy first described by Gaarskjaer¹² that obviates this problem. In short, following an appropriate survival after injection of neuronal tracer, the fixed hippocampal formation is dissected from the brain and gently extended before histological processing. In this way the extended hippocampus can be positioned such that normal sections are obtained from much of the septotemporal extent of the structure. This procedure greatly facilitates analysis of the three-dimensional organization of the intrinsic connections.

The description of hippocampal connections, like those in other brain regions, has historically been influenced by the images provided by available neuroanatomical techniques. A variety of technical parameters, such as the sensitivity of the tracer and the potential for artifactual labeling (such as the fiber-of-passage problem that plagued the degeneration methods), condition the amount and final interpretation of anatomical information available at different times. The recently introduced anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L)¹³ has several advantages over older anterograde tracing techniques. First, when PHA-L is iontophoretically injected into the hippocampus, neural systems, including cell bodies, axons, collaterals, varicosities and terminal ramifications, are labeled in their entirety in a Golgi-like fashion. Second, the iontophoretic injection of PHA-L results in small, restricted injections than can be precisely delineated; there is little or no background precipitate that is associated with the injection site. Third, iontophoretically injected PHA-L does not generally label fibers passing through the injection site.

The results of recent PHA-L experiments using the extended hippocampal preparation, while confirmatory of much of the available data on hippocampal connectivity, have provided the most clear cut picture to date of the three-dimensional organization of the intrinsic hippocampal connections. As we will now show, these data indicate that the "lamellar hypothesis" does not accurately portray the anatomical organization of the hippocampal formation.

4.1. Entorhinal cortex and the perforant path

As pointed out in the Introduction, the Golgi studies of Ramón y Cajal²⁹ and Lorente de Nò,^{25,26} and the degeneration studies of Blackstad^{5,6} and Raisman *et al.*²⁸ demonstrated that the entorhinal cortex is the origin of a strong projection (the perforant pathway) to the dentate gyrus and hippocampus. Both techniques demonstrated fibers that emanate from the entorhinal cortex, continue into the underlying white matter and form a relatively compact bundle that Ramón y Cajal called the angular bundle. The fibers of the perforant path travel dorsally and bend sharply into the transverse plane, perforating the pyramidal layer of the subiculum along its long axis and ultimately entering

the dentate gyrus and hippocampus. Perforant path fibers distribute within the molecular layer of the dentate gyrus, stratum lacunosum-moleculare of the hippocampus and the molecular layer of the subiculum.^{15,27,37,46}

For the present purposes, we will only deal with the fibers that innervate the molecular layer of the dentate gyrus. At this point it is necessary to introduce a few additional descriptive terms concerning the dentate gyrus. There are a number of terminologies applied to the various portions of the V- or U-shaped dentate gyrus. We will refer to the portion that lies adjacent to the hippocampal fissure as the supra-pyramidal blade and the opposite blade as the infrapyramidal blade; the connecting portion will be referred to as the crest. It is also important to note that the boundary between the molecular layer of the dentate gyrus and stratum lacunosum-moleculare of the hippocampus is the obliterated hippocampal fissure. In the Golgi studies it was noted that fibers of the perforant path cross the hippocampal fissure in the region of the crest, bifurcate and extend ramifying branches into the supra- and infrapyramidal blades of the molecular layer.

Andersen *et al.*² cite a personal communication from Jeune concerning experimental neuroanatomical studies on the organization of the perforant path projection as evidence supportive of the "lamellar hypothesis." This work was published in a complete form by Hjorth-Simonsen and Jeune in 1972.¹⁵ Data from 18 experimental cases, in which lesions were placed in the entorhinal cortex and degeneration in the dentate gyrus was mapped, were presented in the paper. With even the smallest lesions, degeneration was observed in no less than 30% of the septotemporal extent of the dentate gyrus and in many cases the degeneration covered as much as 70%. The authors summarized their findings by stating, "On the basis of the evidence presented . . . , it is justified to conclude that the perforant path is organized according to a *level-to-level pattern* of localization. The more dorsal the lesion of the entorhinal cortex, or of the fields traversed by the fibers, the farther the ensuing terminal degeneration extends in a septal direction." The picture that emerges from this paper is that there is a rough topography of the entorhinal projection to the dentate gyrus. But there is no direct evidence presented that focal patches of entorhinal cortex give rise to narrow bands of fibers that project to "slices or lamellae" of the dentate gyrus, unless the slices are conceived of as several millimeters wide.

With the introduction of the autoradiographic tracing technique,⁹ it became possible to study the distribution of perforant path projections arising from more restricted parts of the entorhinal cortex. In

the rat, both Steward³⁷ and Wyss⁴⁹ provided evidence that even small injections of labeled amino acids resulted in widespread transport of the isotope along the long axis of the dentate gyrus. Wyss⁴⁹ in particular drew attention to the divergent nature of the projections and stated that his "results would suggest that some further reservations (with respect to the lamellar organization of the perforant pathway) are appropriate."

One might argue that the perforant pathway is indeed organized in a lamellar fashion but because it is technically difficult to place small injections of anterograde tracers in the rat entorhinal cortex, the entorhinal projection to a single lamella cannot be discretely labeled. This problem can be obviated by placing small injections of anterograde tracers into the entorhinal cortex of the cat or monkey, where the absolute size of the entorhinal cortex is larger than in the rat and a discrete projection should be more readily obtainable. However, in experiments conducted both in the cat and the monkey, even small injections result in widespread terminal labeling along the long axis of the dentate gyrus.^{45,48} Thus, studies using the autoradiographic technique in a variety of species consistently indicate that the perforant pathway is not organized in a lamellar fashion.

Since iontophoretic injections of PHA-L produce very small injection sites, this method is ideal for demonstrating discrete projections, even in the rat. In a recent series of experiments (Witter and Jorritsma-Byham, unpublished observations), small injections of PHA-L were placed into several regions of the rat entorhinal cortex. The injections involved cells in layer II, and the hippocampi were processed in the extended fashion described above. In all cases, terminal labeling was noted along a substantial portion of the long axis of the dentate gyrus. The trajectories of fibers originating from these injection sites to their destinations in the dentate gyrus have proven to be quite complex. At least some follow the classical trajectory, in which they immediately cross the obliterated hippocampal fissure after perforating the subiculum. Others, however, first travel in the stratum lacunosum-moleculare of the hippocampus, before either crossing the hippocampal fissure or extending around the tip of the suprapyramidal blade of the dentate gyrus to enter the molecular layer. Since the labeled perforant path fibers only perforate through a restricted septotemporal extent of the subiculum, it appears that the fibers that travel in the stratum lacunosum-moleculare of the hippocampus must contribute greatly to the longitudinal extent of the terminal field within the dentate gyrus.

The projection of the entorhinal cortex to the dentate gyrus can be seen in a representative PHA-L experiment (Fig. 3) in which the injection is located in the lateral subdivision of the entorhinal cortex, and involves mainly cells in layers II and III.* The diameter of the injection is on the order of 700 μm . In this particular case, the length of the entorhinal

*Results of retrograde tracing studies in the rat have indicated that the perforant pathway projections to the dentate gyrus originate mainly from cells in layer II of the entorhinal cortex.^{33,34,38}

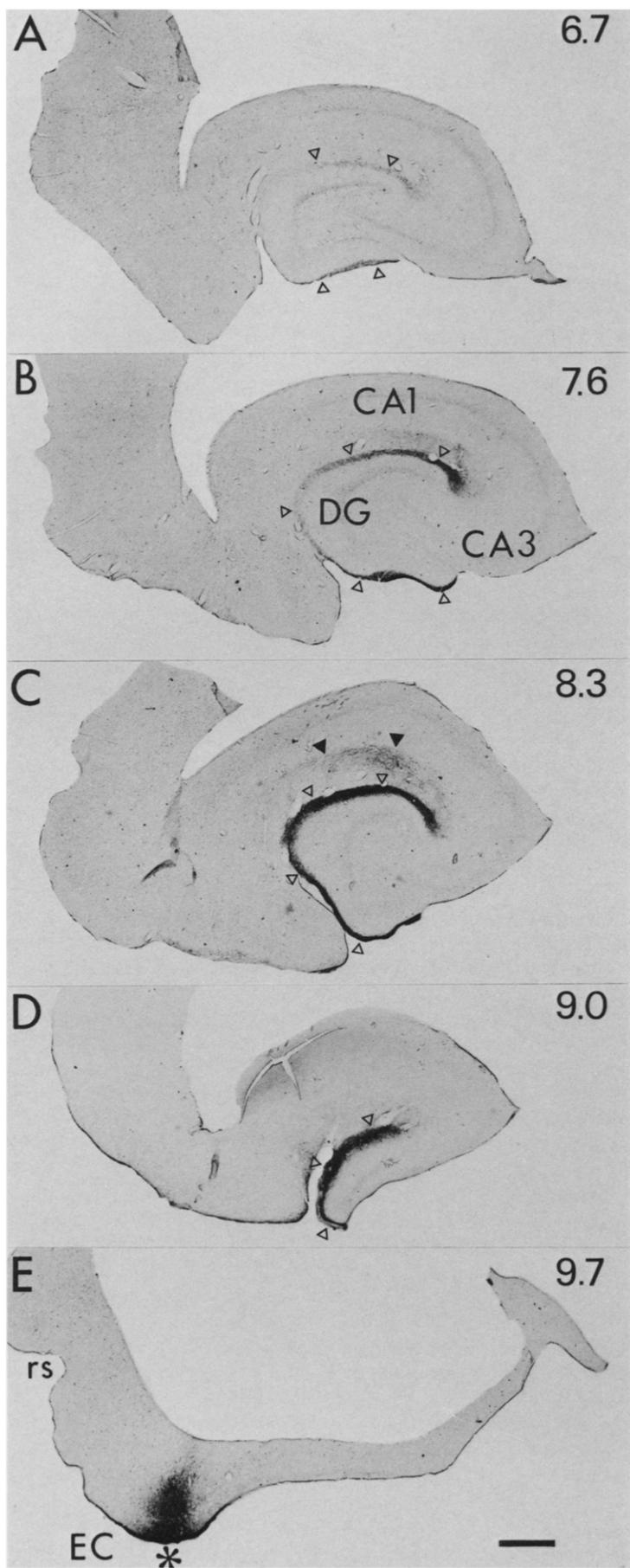


Fig. 3.
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cortex is approximately 7.2 mm, and the length of the dentate gyrus is about 10.2 mm. While the density of the terminal field varies at different septotemporal levels of the dentate gyrus, it nonetheless extends for approximately 4.5 mm along the septotemporal axis. A length of some 2.5 mm shows a consistently high density of termination. Therefore, a conservative estimate would lead to the conclusion that 10% of the length of the entorhinal cortex projects heavily to 25% of the length of the dentate gyrus. If the "lamellar hypothesis" were correct, one would expect that 10% of the dorsoventral extent of the entorhinal cortex would map onto no more than 10% of the septotemporal extent of the dentate gyrus. The data derived from the PHA-L experiments, therefore, are consistent with the autoradiographic data, and indicate that the perforant path projection is more divergent than would be predicted according to the lamellar hypothesis.

The observation that widespread projections along the septotemporal axis of the dentate gyrus originate in focal regions of the entorhinal cortex could be explained by two different organizational patterns. First, individual cells of a local population might project to restricted but different portions of the dentate gyrus. Thus, the entire ensemble of neurons would project to a widespread portion of the dentate gyrus. Alternatively, individual neurons in the entorhinal cortex could originate highly collateralized axons so that each neuron distributes to several levels of the dentate gyrus. By using an experimental design in which two fluorescent retrograde tracers are injected into different levels of the septotemporal axis of the dentate gyrus, the true projectional scheme can be determined. Experiments in the monkey hippocampal formation, using this particular experimental approach, have provided evidence that entorhinal cortical cells do give rise to collaterals that innervate distant septotemporal levels of the dentate gyrus.⁴⁸ Furthermore, this study as well as retrograde tracing studies in the rat^{33,34} and in the cat⁴⁵ have consistently shown that a relatively restricted injection of a retrograde tracer into a single level of the dentate gyrus leads to retrogradely labeled cells in a long, rostrocaudally oriented zone of the entorhinal cortex. Injections of anterograde tracers anywhere within similarly oriented zones within the entorhinal cortex result in overlapping terminal patterns in the dentate gyrus (see Refs 45, 48; Witter and Jorritsma-Byham, unpublished observations).

From the anatomical data presented above, it appears that the projection from a "focal point" in

the entorhinal cortex diverges to an extensive portion of the long axis of the dentate gyrus. As a consequence of this divergence, the projections originating from several "focal points" in the entorhinal cortex converge at a particular level of the dentate gyrus. The anatomical organization of the perforant path projection, therefore, is inconsistent with the conclusion derived from electrophysiological studies that "a point source of entorhinal activity projects its impulses . . . along a slice, or lamella, of hippocampal tissue . . .".²

If, as the anatomical data appear to indicate, the entorhinal projection to the dentate gyrus projects in a far more divergent manner than suggested by the "lamellar hypothesis," how can the physiological findings obtained by Andersen *et al.*² and Lomo²⁴ be explained? Both workers stimulated the perforant path fibers and recorded population spikes in the dentate gyrus that were spatially restricted. Lomo²⁴ summarized his results by stating that, "*Provided the stimulating electrode was located close to the hippocampal fissure* where the perforant path enters the dentate area, it was consistently found that the incoming perforant path fibers divide the dentate area into a series of parallel segments." It is the positioning of the stimulating electrode in this experimental protocol (which was similar to the procedure of Andersen *et al.*,² see Fig. 4) that may provide the answer. Lomo²⁴ in fact showed that when the perforant path was stimulated more laterocaudally (closer to the angular bundle) activation of the dentate gyrus was more widespread.

One scenario that could accommodate the findings of Andersen *et al.*² is the following. If the fibers that perforate the hippocampal fissure to enter the molecular layer of the dentate gyrus terminate within a relatively restricted septotemporal segment of the dentate gyrus, then stimulation of these fibers near the hippocampal fissure would give the appearance of a lamellar activation (available anatomical information does not provide the basis for predicting the extent of longitudinal dispersion of perforant pathway fibers once they enter the dentate gyrus). However, it is clear that the fibers that cross the hippocampal fissure to enter the dentate gyrus are collaterals of axons that contribute a far more widespread pattern of termination within the dentate gyrus. Why doesn't the antidromic activation of these fibers lead to a more widespread activation of granule cells? It is important to remember that Andersen *et al.*² were recording population spike activity which results from integration of temporal and spatial

Fig. 3. Bright-field photomicrographs of representative sections through the rat hippocampal formation arranged from septal (A) to temporal (E). In this experiment, a PHA-L injection (asterisk) was placed into the entorhinal cortex (EC). Anterogradely labeled fibers and terminals are seen in the molecular layer of the dentate gyrus (open arrowheads) as well as in stratum lacunosum-moleculare of the hippocampus (solid arrowheads). The numbers at the right of each panel indicate the distance in millimeters of each section from the septal pole of the hippocampus. rs, rhinal sulcus. Calibration bar equals 500 μm (Witter and Jorritsma-Byham, unpublished observations.)

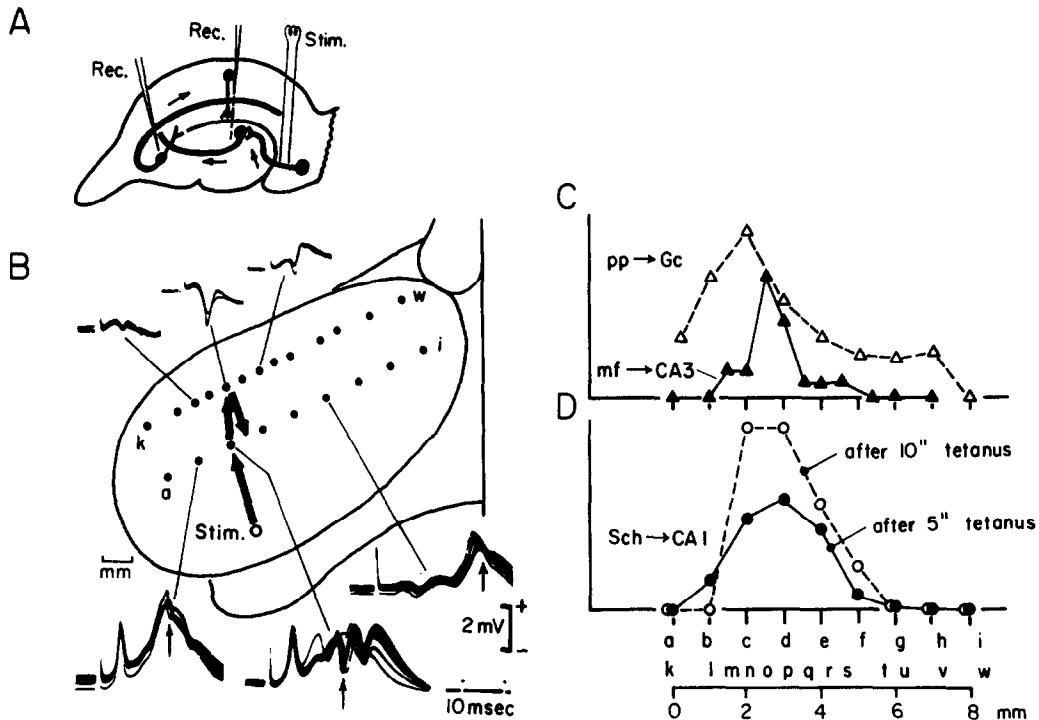


Fig. 4. Reproduction of Fig. 7 from Andersen *et al.*² which shows monosynaptic, disynaptic and trisynaptic activation of hippocampal neurons. A. This diagram shows the orientation of electrodes for simultaneous recording of potentials resulting from perforant path stimulation (Stim.). B. This diagram shows the septotemporal location of a stimulating electrode (Stim.) located in the perforant path fibers. The location of recording electrode positions in CA1 are indicated by dots a-i and in CA3 by dots k-w. The extracellular potentials recorded from several of the electrode positions are shown as insets. C. The open triangles indicate the magnitude of the synaptic wave recorded in the dentate gyrus along tracks a-i. The filled triangles show the magnitude of population spikes recorded in CA3 along tracks k-w. D. The magnitude of population spikes in CA1 recorded from tracks a-i in response to 10/s perforant path stimulation (open circles) or 5/s stimulation (closed circles). See text for discussion.

synaptic activation. If one assumes that these collaterals terminate with lower density at progressively greater distance from the stimulation point, the synaptic effect on distant granule cells may be insufficient to generate sufficiently synchronous activation to produce detectable population spikes. Thus the widespread anatomical divergence of the perforant path projection might have been difficult to appreciate with the electrophysiological techniques employed by Andersen *et al.*²

Perhaps the ultimate resolution of this issue will come with the focal stimulation of small populations of entorhinal cortical cells while evoked activity is recorded along the full septotemporal extent of the dentate gyrus. A study of this kind has not yet been done. Probably the first paper in which entorhinal stimulation was used to activate the hippocampus was published in 1940 by Renshaw *et al.*³¹ In this paper, however, the spread of the perforant pathway along the longitudinal axis of the dentate gyrus was not studied. To our knowledge, the only paper that deals with this point is a study in the cat,⁴⁴ where focal stimulation of the superficial layers of the entorhinal cortex resulted in activation of at least half of the septotemporal extent of the dentate gyrus.

These data are suggestive that the anatomical picture of highly divergent entorhinal projections could be detected physiologically if appropriate experimental procedures were followed.

4.1.1. Summary. All experimental anatomical studies of the organization of the perforant path projection to the dentate gyrus, including those using the PHA-L method, consistently demonstrate that the projection is highly divergent though organized in a roughly topographic or "level-to-level" manner. If the smallest projection field of a localized region of the entorhinal cortex defines the size of a lamella, then it would extend for something on the order of 2.5 mm in the septotemporal axis of the dentate gyrus. Furthermore, the perforant path projection is also convergent onto the dentate gyrus. Relatively large portions of the entorhinal cortex provide overlapping projections to particular segments of the dentate gyrus. Both of these organizational features of the perforant path are inconsistent with a major conclusion of the "lamellar hypothesis" that "a localized activation of the entorhinal area will lead to a small slice, or lamella, of tissue being activated."¹² The physiological data collected by Andersen *et al.*,² Lomo²⁴ and others,³⁰ which appear to indicate a

lamellar organization of the perforant path projection, may be the result of the positioning of the stimulating electrode and the experimental protocols employed. This, coupled with their interpretation of the picture provided by the Golgi studies of Lorente de Nò,²⁶ that "is suggestive of a parcellation of the hippocampal formation whereby each section or segment perpendicular to the long axis is connected with corresponding subdivisions of the entorhinal area by the perforant path fibers" (Lomo²⁴), and the lack of detailed experimental mapping studies of the perforant path, makes the original proposition of the "lamellar hypothesis" of entorhinal-dentate interactions perfectly reasonable. However, the current understanding of the anatomical organization of the perforant path projection no longer supports the lamellar concept, and it would appear that the organization of physiological interactions between the entorhinal cortex and the dentate gyrus are in need of re-evaluation.

4.2. Dentate gyrus and the mossy fibers

As noted above, the dentate gyrus is a major recipient of the perforant path projection from the entorhinal cortex. It, in turn, provides a prominent input to the CA3 field of the hippocampus. The dentate gyrus is divisible into three layers: the molecular layer, in which the perforant path fibers terminate; the granule cell layer, which is populated by the principal cell type, the granule cell; and a deep or polymorphic layer which is populated by a variety of neuronal types. The granule cells give rise to distinctive axons, the mossy fibers, which collateralize in the polymorphic layer before entering the CA3 field where they form en passant synapses on the proximal dendrites of the pyramidal cells.^{7,8,12,12a} The cells of the polymorphic layer give rise to at least two systems of associational connections that end within the dentate gyrus.

The mossy fibers were extensively studied by the classical Golgi anatomists and the general distribution of mossy fibers was well established by Golgi, Ramón y Cajal and Lorente de Nò. The first experimental study of this fiber system was conducted by Blackstad *et al.*⁷ who used the Fink and Heimer degeneration method to map the distribution of the mossy fibers in the rat hippocampus. The results of these experimental studies, appearing a year or so prior to the publication of Andersen *et al.*,² were cited

as strong evidence in support of the "lamellar hypothesis." Subsequently, Gaarskjaer,^{12,12a} using the degeneration technique with the extended hippocampal preparation, and Swanson *et al.*,⁴² using the autoradiographic technique, re-analysed the course and distribution of the mossy fibers.

The results of both the Golgi and the experimental studies are consistent with the interpretation that the mossy fibers are mainly organized in a lamellar fashion (see review by Gaarskjaer^{12a}). Bands of mossy fibers are principally oriented transverse to the long axis of the hippocampal formation and the bands arising from each septotemporal level of the dentate gyrus only minimally overlap those arising from other septotemporal levels. In both the degeneration and autoradiographic studies, the major portion of the mossy fiber trajectory does not extend far outside of the band of lesioned or isotopically labeled granule cells. The only exception to this general rule occurs at the transition from CA3 (and CA2) to CA1. As Swanson *et al.*⁴² demonstrated quite clearly in their autoradiographic studies, mossy fibers, especially at septal levels of the hippocampal formation, make an abrupt turn caudally as they approach the CA1 field and travel parallel to the long axis of the hippocampus for as much as 2 mm. These data are consistent with the view of Ramón y Cajal and Lorente de Nò that the mossy fibers first travel transversely and then bend to take a more longitudinal trajectory. At mid and temporal levels of the hippocampal formation, however, the extent of the temporally directed component of the mossy fiber projection is rather meager.

The band-like nature of the mossy fiber projection is demonstrated in Fig. 5. Sections from a representative PHA-L experiment in which granule cells of the infrapyramidal blade were labeled are illustrated. Labeled granule cells were observed for approximately 600 µm in the septotemporal axis. Two distribution patterns can be observed in this case: a diffuse plexus of collaterals that innervates the polymorphic layer of the dentate gyrus, and the major bundle of labeled mossy fibers that occupies the narrow stratum lucidum just superficial to the pyramidal cell layer that innervates the CA3 pyramidal cells. Septally, both patterns of labeling end approximately 450 µm above the septal limit of the injection. Temporally, labeling of the mossy fibers, including the temporally directed distal component, extends for no more than 600 µm. Thus, this 600-µm injection labels a terminal

Fig. 5. Dark-field photomicrographs of representative sections through the rat hippocampal formation arranged from septal (A) to temporal (E). The PHA-L injection in this case involved the infrapyramidal blade of the granule cell layer of the dentate gyrus (black arrows in panel C). The labeled mossy fibers contribute a dense plexus to the polymorphic layer (hilus) of the dentate gyrus (open white arrows). The main mossy fiber bundle projects to the CA3 field mainly in the stratum lucidum located just above the pyramidal cell layer (filled white arrows). The temporally directed tail of the mossy fiber bundle is indicated with an asterisk in panels D and E. The injection site is located in panel C which is marked as level 0 on the right side. Distances either septal (A and B) or temporal (D and E) in millimeters from the focus of the injection are indicated on the right of these panels. Calibration bar equals 500 µm. (Amaral, unpublished observations.)

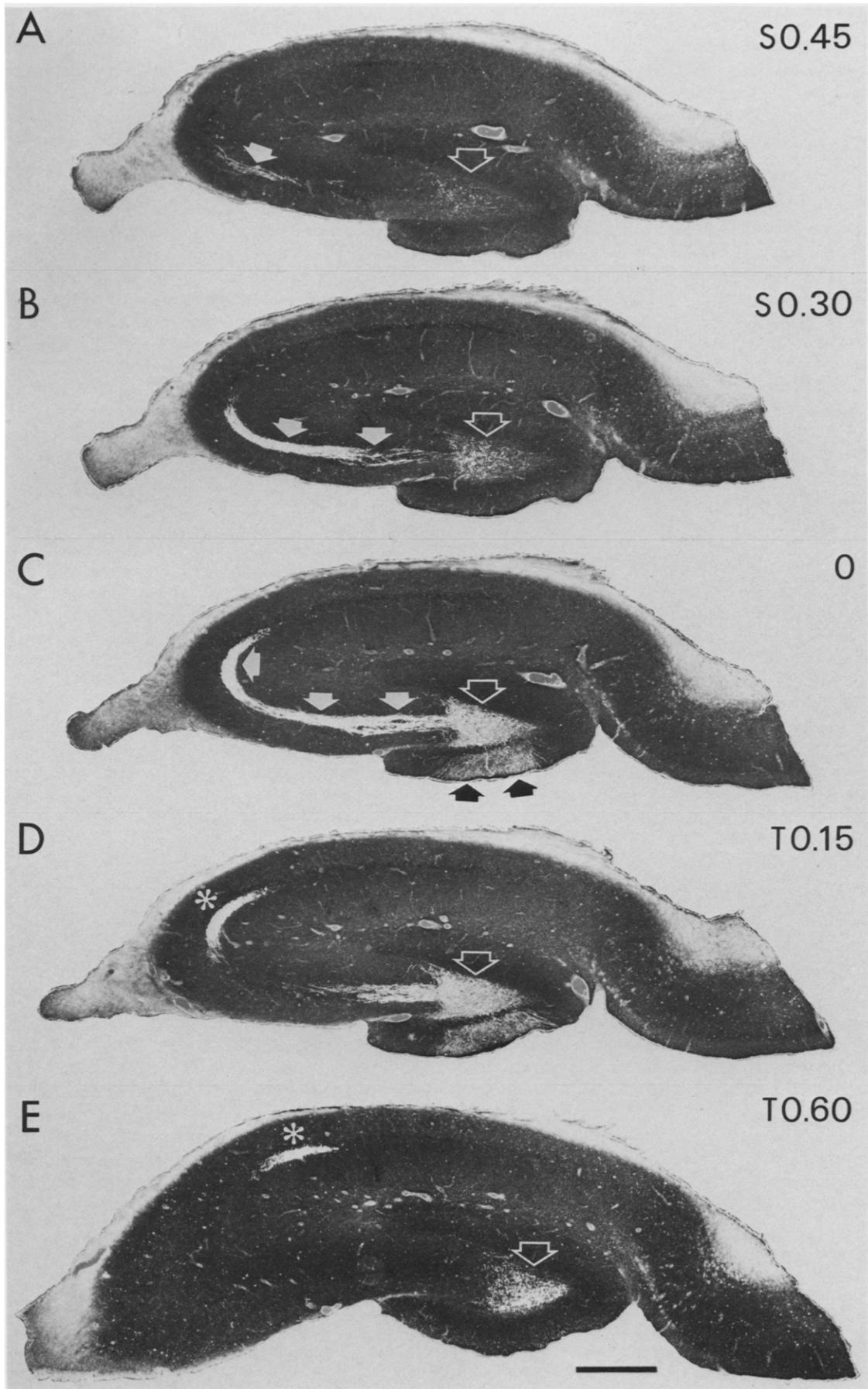


Fig. 5.

field of approximately 1650 μm . While it might be argued that even the mossy fiber projection is somewhat divergent and thus non-lamellar, the impression is that this system is indeed organized to innervate relatively thin slices of the hippocampus. Given this anatomical data alone, it would be quite understandable to suggest that different septotemporal levels of the hippocampal formation are isolated processing units. However, as we will now show, the mossy fiber projection is an anomaly in the system of hippocampal projections. It is the only fiber projection that could credibly be considered to be organized in a lamellar fashion.*

4.3. Dentate gyrus and the associational projection

While the distribution of the mossy fiber projection from the dentate gyrus to the CA3 field of the hippocampus may be viewed as supportive of the "lamellar hypothesis," the organization of the associational projection arising from the polymorphic region provides perhaps the strongest evidence against the "lamellar hypothesis." We should point out that the anatomical demonstration of the associational projection of the dentate gyrus pathway occurred⁵⁰ after the "lamellar hypothesis" was first presented. Thus, the influence of this longitudinally oriented projection on the lamellar organization of the hippocampus was not directly dealt with in the paper of Andersen *et al.*²

The polymorphic region of the dentate gyrus is composed of a variety of neuronal cell types, many of which appear to have locally ramifying axonal plexuses.^{1,29} As noted above, the granule cells give rise to an extensive collateral axonal plexus that terminates on many of the cell types of the polymorphic region.^{8,32} Zimmer⁵⁰ first demonstrated that the inner third of the molecular layer of the dentate gyrus was innervated by fibers of ipsilateral origin. He believed that this projection originated either from the cells of the polymorphic region (which he labeled CA4) or

from the CA3 pyramidal cells closest to the dentate gyrus. Swanson *et al.*⁴² confirmed the ipsilateral associational projection to the dentate gyrus using the autoradiographic tracing technique. Moreover, they noted that this projection appeared to be massively divergent in its extent along the long axis of the dentate gyrus. They pointed out that relatively focal injections of the dentate gyrus at mid septotemporal levels, "result in labeling of fibers throughout the septal two thirds of the dentate gyrus . . ." This finding confirmed the observation of Zimmer⁵⁰ that the degeneration in the molecular layer of the dentate gyrus extended substantially beyond the levels of the dentate gyrus directly involved by the lesion.

During the early stages of the experimental analysis of the so-called "ipsilateral associational projection" of the dentate gyrus, there was substantial controversy concerning the cells of origin. Subsequently, however, studies by Hjorth-Simonsen and Laurberg,¹⁶ Laurberg²² and Laurberg and Sorensen²³ greatly clarified the picture. In short, the associational projections to the dentate gyrus appear to arise nearly exclusively from the cells of the polymorphic layer of the dentate gyrus and not from the pyramidal cells of field CA3 of the hippocampus. Moreover, the polymorphic cells of the dentate gyrus do not project to any field of the hippocampus, i.e. they do not give rise to Schaffer collaterals. Both the associational projections within CA3 and the Schaffer collateral system to CA1 arise exclusively in the CA3 (and CA2) fields of the hippocampus. Our recent PHA-L analyses of the dentate and hippocampal projections are entirely consistent with these conclusions.

In a recent series of PHA-L experiments, the organization of the ipsilateral associational projections of the dentate gyrus and hippocampus have been further analysed.¹⁷ An experimental case in which the injection involved cells both of the polymorphic layer of the dentate gyrus and of the tip of the CA3 pyramidal cell layer is illustrated in Fig. 6. We shall return to this case below during the description of CA3 projections but, for the moment, we will focus on the projections to the molecular layer of the dentate gyrus. The injection site, located approximately 3600 μm from the septal pole (at a mid septotemporal level), extends for approximately 450 μm in the septotemporal axis. We have consistently found that the associational region of the molecular layer (the inner one-third) is not heavily labeled at the level of the injection site. Rather, there is more diffusely organized axonal labeling in the outer two-thirds of the molecular layer.† In this case, the first hint of an associational projection in the septal direction begins approximately 1200 μm from the septal limit of the injection site. The density of terminal labeling in the associational zone, which is first apparent approximately 2400 μm from the septal pole, continues to increase at progressively more septal levels and is maintained until the septal pole of the dentate gyrus.

*In considering the extent of lamellar organization in the dentate gyrus, it is perhaps important to note the study of Struble *et al.*³⁹ that dealt with the three-dimensional organization of presumably inhibitory basket cells in the dentate gyrus. Serial sections stained by the Golgi method were analysed to determine the transverse and longitudinal extent of the axonal plexuses arising from the basket cells. In the suprapyramidal limb of the dentate gyrus, basket cell axons extended, on average, 450 μm in the transverse plane and approximately 1100 μm along the long axis of the dentate gyrus. Thus, even inhibitory interneurons appear to give rise to axonal collaterals that project over a substantial distance along the long axis of the dentate gyrus.

†At least some of the fibers and terminals associated with this diffuse labeling in the outer portion of the molecular layer arise from a population of somatostatin-immunoreactive neurons located in the polymorphic layer.³ This projection extends for approximately 0.5–1 mm septal and temporal to the injection site and the terminal pattern is largely complementary to the projection to the inner third of the molecular layer.

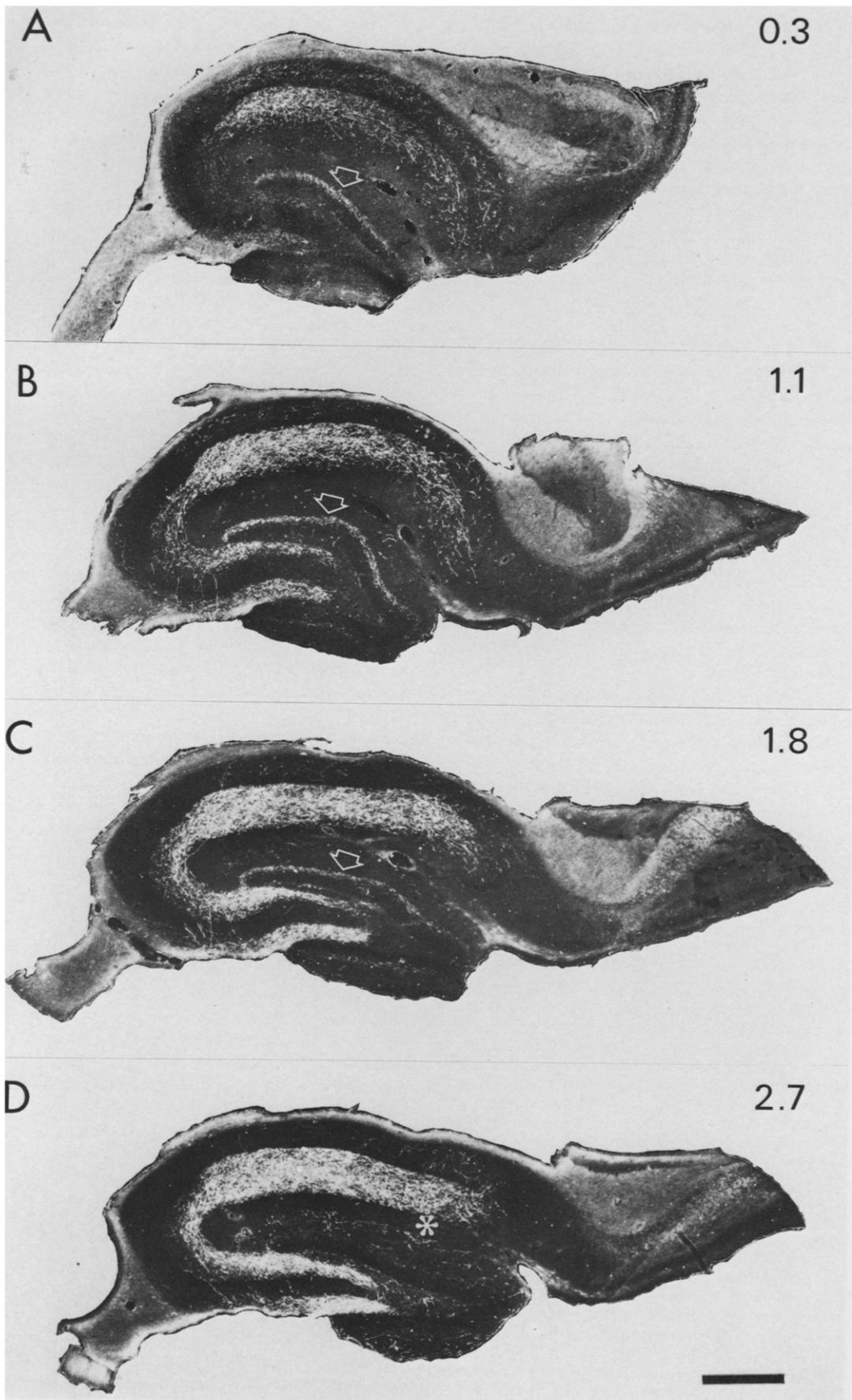


Fig. 6.

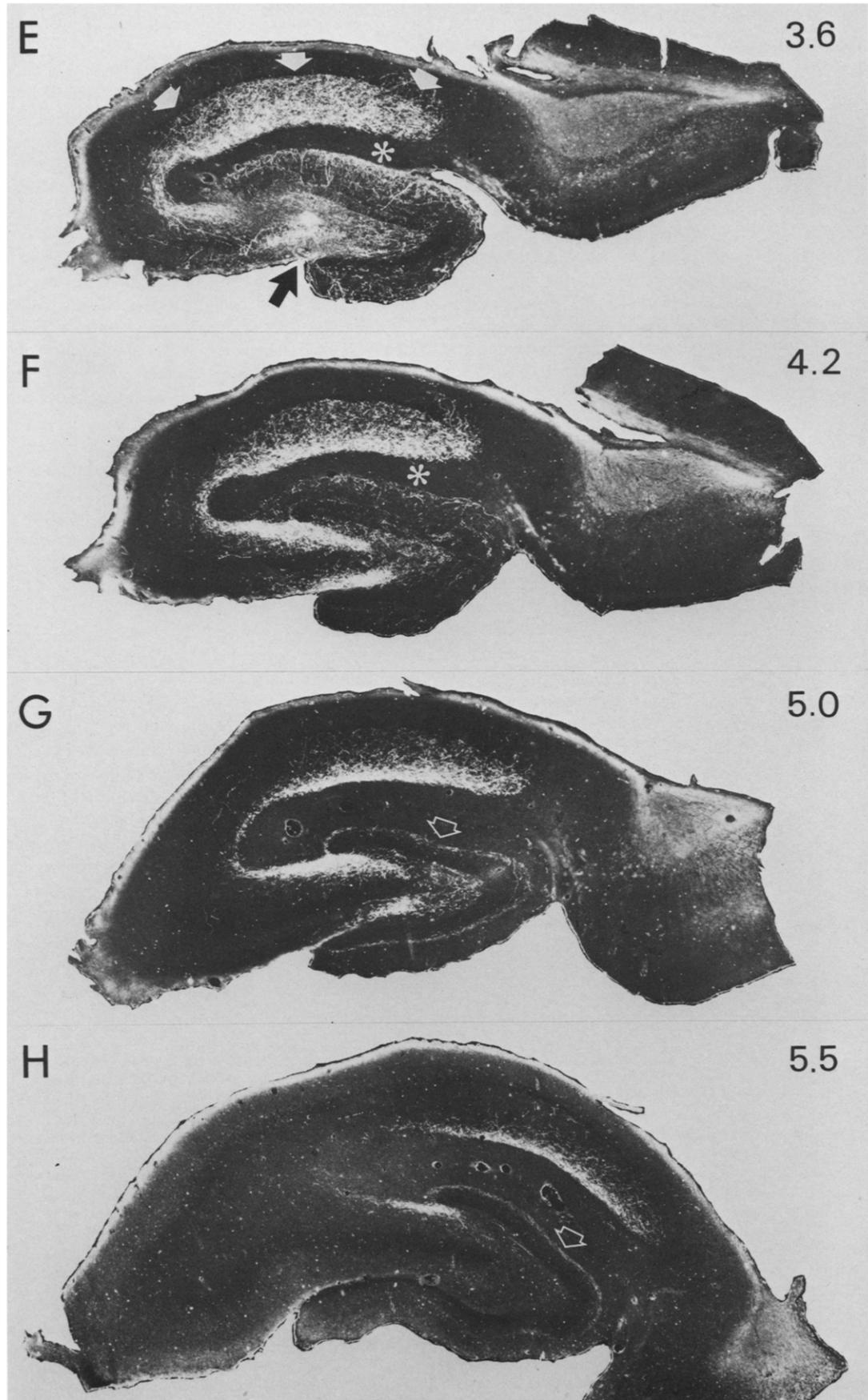


Fig. 6. Contd

In the temporal direction, the first hint of an associational projection is seen approximately 300 μm away from the temporal edge of the injection and the densest labeling is seen some 2000 μm from the temporal edge of the injection site. To summarize, the projection in this case extends from the septal pole approximately 2400 μm temporally, then there is a gap of about 1950 μm that contains the injection site and the projection continues for an additional 2250 μm temporal to the injection site. The total septotemporal extent of the associational projection in this case is approximately 6600 μm .

This case is typical of many PHA-L injections that involve the polymorphic region of the dentate gyrus and it highlights one important organizational feature of the associational projection that was not appreciated in the earlier experimental studies. The consistent finding in the PHA-L experiments is that the associational projection is either not present or is very weak at the level of the injection site. The projection begins to innervate the molecular layer at a distance of 300–1000 μm away from the injection site and the heaviest terminal labeling can occur as much as 2 or 3 mm distant to the first indication of the projection. Thus, the associational projection is not organized to provide feedback to granule cells in the same level in which the polymorphic cells are located but to levels as far as several millimeters from the cells of origin. *It would appear, therefore, that the ipsilateral associational projection of the dentate gyrus is specifically organized to promote integration of information along the long axis of the dentate gyrus. This prominent longitudinal flow of information is antithetical to the concept that individual hippocampal lamellae are functionally independent.*

*The CA2 field makes up a small portion of the pyramidal cell layer of the hippocampus and shares many of the connectional characteristics of CA3. To simplify the present discussion, we will include CA2 as the most distal portion of field CA3 and not deal directly with the differences in connectivity between the two fields.

4.4. Hippocampus: CA3 connections

The hippocampus proper is generally subdivided into three fields (CA3, CA2 and CA1) according to the nomenclature suggested by Lorente de Nò.^{26*} The principal cell type, the pyramidal cell, is the major component of the pyramidal cell layer. Deep to the pyramidal cell layer is the stratum oriens, into which the basal dendrites of the pyramidal cells descend and which contains a number of non-pyramidal neurons. The largely acellular region superficial to the pyramidal cell layer, where the apical dendrites of the pyramidal cells are located, is divided into a deeper stratum radiatum and a more superficial stratum lacunosum-moleculare. In CA3, the region just above the pyramidal cell layer contains the mossy fibers from the dentate gyrus and is called the stratum lucidum.

The pyramidal cells of CA3 have highly collateralized axons that contribute associational projections that terminate within CA3, and give rise to the major projection to CA1, the so-called Schaffer collaterals.^{40,41} As in the other connections discussed above, the existence of projections from CA3 to itself and from CA3 to CA1 was already appreciated in the Golgi studies of Ramón y Cajal²⁹ and Lorente de Nò.²⁶ In fact, Lorente de Nò²⁶ gave the name "longitudinal association bundle" to the CA3 fibers that remained within the field. It was his view that this bundle traveled primarily parallel to the long axis and linked different levels of the hippocampus, whereas he thought that the Schaffer collaterals linked the CA3 and CA1 fields of the same hippocampal level. The extent and topographic organization of the CA3 projections have only recently been studied in any detail and recent PHA-L studies have uncovered a hitherto unappreciated orderliness of the terminal distributions arising from different portions of the CA3 field. We shall return to a description of these PHA-L studies after a brief review of experimental studies of the CA3 projections conducted over the last 15 years.

Fig. 6. Dark-field photomicrographs of representative sections through the rat hippocampal formation arranged from septal (A) to temporal (H). The distance in millimeters of each section from the septal pole of the hippocampus is indicated on the right side. The PHA-L injection in this case (black arrow in panel E) involved both the tip of the CA3 pyramidal cell layer and the polymorphic layer or hilus of the dentate gyrus. This illustration is intended to summarize aspects both of the distribution of association connections arising from the hilus of the dentate gyrus and of the Schaffer collateral projection to CA1 arising from the CA3 pyramidal cells. The classical associational connection of the dentate gyrus innervates the inner one-third of the molecular layer. Note that at the level of the injection (E) and for some distance on each side of the injection (D and F), there is little or no indication of a projection to the inner third of the molecular layer. There is, however, a diffuse projection to the outer two-thirds of the molecular layer (asterisks). At greater distances from the injection, both septally and temporally (A–C, G–H), the density of labeling in the associational zone becomes progressively greater (open arrows). The projections of the CA3 cells to CA1 (the Schaffer collaterals) have an equally widespread distribution. At the level of the injection, the highest density of fiber and terminal labeling (white arrows) is located superficially in stratum radiatum and distally in CA1, i.e. near the subiculum border. At more septal levels, the area of highest terminal and fiber labeling shifts both towards CA3 and more deeply in stratum radiatum and ultimately involves stratum oriens. At more temporal levels, the area of highest labeling shifts superficially in stratum radiatum and distally in CA1, i.e. closer to the subiculum border. Calibration bar equals 500 μm .
(Based on results described in Ref. 17.)

Hjorth-Simonsen¹⁴ used the Fink and Heimer degeneration method in rats where the commissure had been sectioned neonatally to analyse the intrinsic connections of the hippocampus. While the Golgi studies indicated that the CA3 projections terminated in the stratum radiatum and perhaps stratum lacunosum-moleculare, Hjorth-Simonsen¹⁴ found that the projections from CA3 to CA3 and from CA3 to CA1 terminated extensively both in the stratum oriens and stratum radiatum but not at all in stratum lacunosum-moleculare. Moreover, he noted that the degeneration seen in CA1 extended for 1.5–2.0 mm septal to the lesion and 2.1–3.2 mm temporal to the lesion. He pointed out that the 4–5 mm band of degeneration he observed in his experimental studies produced by focal lesions of the CA3 field appeared to be at variance with the physiological data provided by Andersen *et al.*,² that indicated that the Schaffer collateral system is organized in a lamellar fashion. Hjorth-Simonsen¹⁴ also pointed out that the distinction between the Schaffer collaterals and the longitudinal association projections is arbitrary since both arise from the same populations of neurons and both have extensive transverse and longitudinal distributions of their terminal fields.

Swanson *et al.*⁴² used the autoradiographic technique to study the intrinsic hippocampal projections of the rat. While confirming the earlier findings of Hjorth-Simonsen,¹⁴ they also indicated that the intrinsic connections of CA3 are even more extensive than earlier reported. An experiment with a small injection of [³H]amino acids into field CA3 at a mid-septotemporal level, for example, resulted in anterograde transport that extended to the septal pole of the hippocampus and innervated fully two-thirds of the septotemporal extent of fields CA3 and CA1. Injections of CA3 that were focused in the temporal third of the hippocampus resulted in more limited anterograde transport that nonetheless involved the temporal third of the CA fields. Swanson *et al.*⁴² concluded their findings concerning the CA3 projections to CA1 by stating, "It is also evident from our material that while there is a general septo-temporal organization in this system, it cannot be conceived of as being arranged in a series of narrow transversely oriented bands. Thus, even quite small injections at different septo-temporal levels within the regio inferior (CA3) nearly always result in a very broad zone of transported label to the regio superior (CA1) . . ." They emphasized the equally extensive nature of the CA3 projections to other levels of CA3 (the longitudinal association bundle) and concluded that, "The importance of this bundle is that it clearly interrelates widely separated zones of the regio inferior along its longitudinal axis."

Laurberg²² and Laurberg and Sorensen,²³ using degeneration methods as well as retrogradely transported tracers, clarified a number of issues concerning the CA3 projections. They clearly demonstrated, for example, that cells of the polymorphic region of

the dentate gyrus do not give rise to projections to either CA3 or CA1. They also provided a more detailed analysis of the topographic patterns of the CA3 projections that had been hinted at in the work at Hjorth-Simonsen.¹⁴ CA3 projections that extended septally to the lesion site, for example, were observed to preferentially terminate in the deep part of the stratum radiatum whereas projections located temporal to the lesion site terminated more heavily in the superficial portion of the layer.

We have recently re-examined the organization of the CA3 projections in the rat using the PHA-L method and the extended hippocampal preparation.¹⁷ For the present purposes, we will review results relevant to the Schaffer collateral system since data for this projection were presented in the paper of Andersen *et al.*² While the existence of the longitudinal associational projection of CA3 was known at that time, the magnitude and distribution of the projection had not been experimentally studied when the Andersen paper appeared. Suffice it to say that our PHA-L experiments indicate that the CA3 to CA3 projection is at least as divergent as indicated by the study of Swanson *et al.*⁴²

In relation to the Schaffer collateral system, the PHA-L experiments have confirmed most of the findings reviewed above. Because a large number of experiments have been conducted with discrete injections of virtually all septotemporal and transverse regions of the CA3 field, certain of the gradients of organization that were suggested in the previous studies have been more clearly uncovered. The following organizational principles have proven to be consistent across animals and independent of the septotemporal level that the injection has involved. The major organizational features of the CA3 to CA1 projection are illustrated in Figs 6 and 7.

Cells in all portions of CA3 give rise to projections to CA1. The first gradient of connectivity that became clear from the PHA-L studies deals with the distribution of fibers and terminals relative to the transverse location of the injected CA3 cells. Despite the widespread belief that CA3 axons travel parallel to the pyramidal cell layer throughout the transverse extent of CA1, this did not prove to be the case. At the level of the injection, fibers arising from CA3 cells located close to the dentate gyrus distribute preferentially to the distal portion of CA1, i.e. near the subiculum border, where they terminate in the superficial portion of stratum radiatum. CA3 cells located progressively closer to the CA1 border project preferentially to parts of CA1 that are progressively closer to CA3 and to deeper portions of stratum radiatum (and into stratum oriens).

The second major gradient of CA3 connectivity deals with the pattern of termination along the septo-temporal axis of CA1. First, in all cases, the greatest density of fiber and terminal labeling is located a distance of 1 mm or more away from the injection site. CA3 cells located close to the dentate gyrus tend to

project further and more heavily in a septal direction. Cells located near the CA1 border, in contrast, tend to project further and more heavily in a temporal direction. In no case was there convincing evidence that the CA3 to CA1 projection is strongest within the hippocampal level of origin. Thus, contrary to the expectations of the "lamellar hypothesis" the Schaffer collaterals are preferentially connecting a particular septotemporal level of field CA3 with a different septotemporal level of field CA1. Moreover, from any one level of CA3, cells located in different transverse regions of CA3 project preferentially to different septotemporal levels of CA1.

The second aspect of the longitudinal component of the Schaffer projection is that the area of greatest fiber and terminal labeling within CA1 changes in an orderly fashion as one progresses septally or temporally from the injection site. Thus, regardless of the transverse position of the injection site within CA3, the terminal labeling in CA1 tends to be located closer to the subicular border and more superficially in stratum radiatum as progressively more temporal levels are approached. Conversely, at more septal levels, the fiber and terminal labeling moves closer to the CA3/CA1 border and shifts to a deeper location in stratum radiatum and into stratum oriens.

These patterns of labeling are shown in Fig. 6, which illustrates a PHA-L experiment in which the injection has involved the portion of CA3 close to the dentate gyrus. The injection is located at a mid septotemporal level and extends for approximately 450 μm . At the level of the injection site, labeling in CA1 is heaviest near the subicular border, and superficially in stratum radiatum (there are few, if any, labeled fibers and terminals in stratum oriens). The projection in CA1 extends to the septal pole of the hippocampus. Progressing septally from the injection site, the area of heaviest fiber and terminal labeling shifts within CA1 towards the CA3 field and is found deeper in stratum radiatum and ultimately encompasses stratum oriens as well. Progressing temporally from the injection site, the area of labeled fibers and terminals becomes more restricted in CA1 to the region just adjacent to the subiculum and to the most superficial portions of stratum radiatum. The entire septotemporal extent of the labeled fibers in CA1 in this case is something on the order of 6.6 mm or approximately 90% of the length of the hippocampus.

As with the perforant path projection, it is of interest to speculate how the apparently lamellar organization of the Schaffer collateral system could have been derived by Andersen *et al.*² given the overwhelming anatomical evidence for the apparently more widespread distribution of the fibers. Once again, a plausible answer may lie in the particular experimental protocol employed by Andersen *et al.*² As shown in Fig. 7A and B, stimulating electrodes were placed into the CA3 region at two septotemporal levels (Stim 1 and Stim 2). The position of these electrodes, (as shown in panel A) was relatively

close to the CA1 border. A single recording electrode was lowered into CA1 at several septotemporal levels at approximately a midpoint along the transverse extent of the field.

From the gradients described above, it would be expected that stimulation of CA3 close to the CA1 border would produce the greatest effect at a level temporal to the stimulation site. Andersen *et al.*² found, in fact, that the highest amplitude population spike was observed some 1–2 mm temporal to the stimulating electrode (Fig. 7B). At the level of the stimulating electrode, there was little or no indication of evoked activity. Since the stimulated CA3 cells would be expected to project just across the border into CA1 at the same level (Fig. 7D), it is possible that the recording electrodes were simply too distal in CA1 to record the activated cells. The reason that Andersen *et al.*² observed a more limited septotemporal effect of their stimulation may also relate to the positioning of the recording electrode. Remember that the pattern of termination of CA3 projections to CA1 shifts in the transverse axis at different septotemporal levels. Septally from the injection it shifts towards CA3 and temporally it shifts towards the subiculum. Thus, if the recording electrodes were actually placed at the same mid tranverse location in CA1 at the several septotemporal levels analysed, the activated region of CA1 may have been distal to the recording electrodes far temporally from the stimulation site and proximal to the recording electrodes septal to the stimulation site. One would predict, therefore, that by adopting a different experimental procedure in which electrodes are placed at different transverse positions within CA1 at different septotemporal levels, a much broader zone of significant population spikes would be observed.

4.5. Other intrinsic connections

In addition to the connections already discussed, a number of other intrinsic hippocampal projections have been described since the publication of Andersen *et al.*² The topographic organization of many of these connections has yet to be intensively studied. A more complete summary of the intrinsic connections of the hippocampal formation is illustrated in Fig. 8. The additional illustrated projections can be briefly summarized as follows. Unlike the CA3 cells, CA1 pyramidal cells do not appear to give rise to an extensive projection to other levels of CA1 but project in a columnar fashion to the subiculum.^{10,11,43} A relatively weaker CA1 projection is also directed to the deep layers of the entorhinal cortex.⁴² The subiculum projects both to the pre- and parasubiculum and to the entorhinal cortex.^{4,11,18,21,35,36} The presubiculum and parasubiculum also give rise to a major projection to the entorhinal cortex that terminates in layers III and II, respectively.^{18,35} Finally, there appear to be intrinsic projections in the entorhinal cortex that link various parts of the region and link the deep layers with the more superficial layers.^{19,20,47}

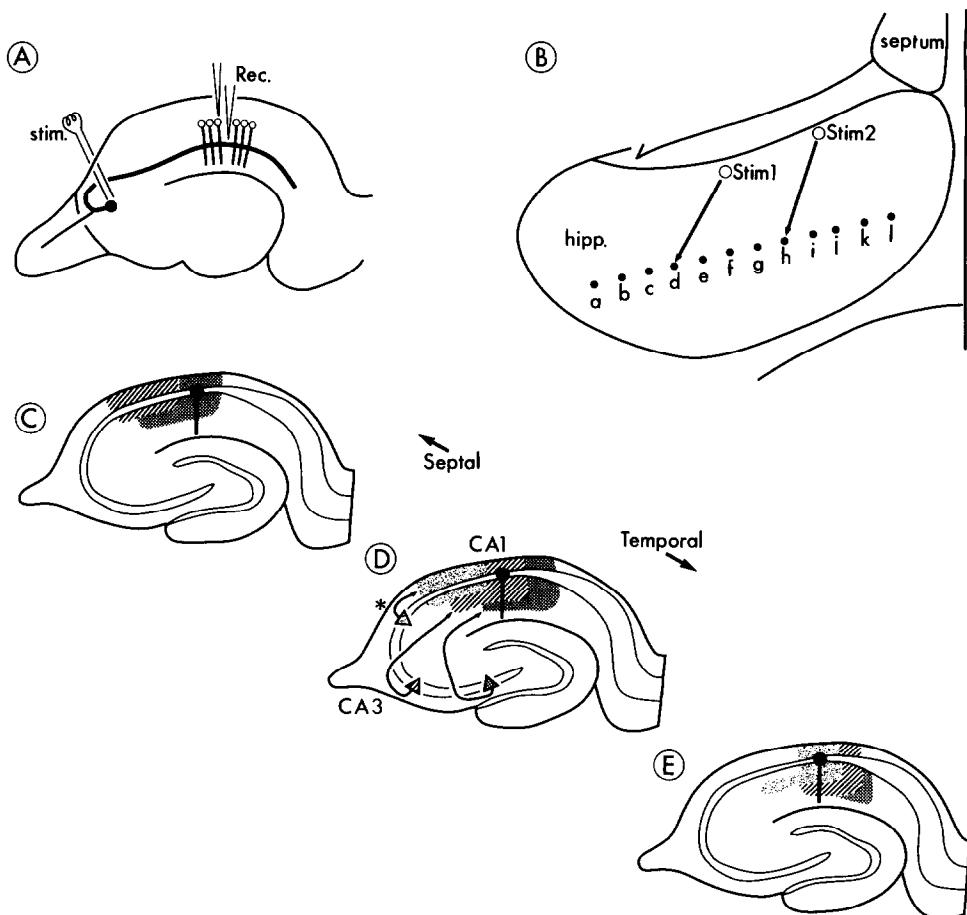


Fig. 7. Panels A and B are redrawn from Fig. 3 of Andersen *et al.*² Panel A shows the position of stimulating and recording electrodes used for recording the activation of CA1 pyramidal cells following stimulation of CA3. Panel B shows the position of the largest population spike in CA1 resulting from CA3 stimulation at the indicated positions (Stim 1 and Stim 2). Recordings were made along tracks indicated by dots a-l. Panels C-E summarize certain aspects of the gradients of termination of the CA3 projection to CA1. Panel D indicates that different portions of the pyramidal cell layer project most heavily to different parts of the CA1 field (the shading pattern in the CA3 cells matches the region of termination in CA1). CA3 cells located near the dentate gyrus project distally in CA1 (near the subicular border) and superficially in stratum radiatum. CA3 cells located close to the CA1 border, in contrast, project just across the border into CA1 and most heavily innervate stratum oriens and the deep portion of stratum radiatum. Regardless of the origin of a CA3 projection, at progressively more septal levels (panel C) the terminal field shifts proximally in CA1 and deeper in stratum radiatum, and at more temporal levels (panel E) the terminal field shifts distally in CA1 and superficially in stratum radiatum. Because of these shifting terminal patterns, it is conceivable that stimulation of one point in CA3 (asterisk in panel D) and recording at one transverse level of CA1 at different septotemporal levels (indicated by the single neuron in panels C-E) would lead to a significant population spike being recorded from a relatively limited septotemporal extent of the hippocampus (indicated in panel E by the coincidence of the CA1 neuron and the shading pattern indicating the terminal field of the stimulated CA3 neuron). However, with a two-dimensional matrix of recording sites that sample several transverse and septotemporal levels of CA1, a much broader region of population spike activity might be expected.

5. CONCLUSIONS

The “lamellar hypothesis” was a theoretical explanation of the intrinsic organization of the hippocampal formation that was born from the physiological studies of Andersen and colleagues but relied heavily on the anatomical literature for support. Since 1971, when the “lamellar hypothesis” was first explicitly presented, the organization of the intrinsic hippocampal connections has been investi-

gated in several neuroanatomical studies. We have reviewed the results of some of these and related them to more recent results in our own laboratories using the PHA-L technique and the extended hippocampal preparation. *The overwhelming consensus in all these studies is that aside from the mossy fibers, none of the intrinsic connections of the hippocampal formation is organized in a lamellar fashion.* Quite the opposite organization seems to be the rule. Projections in the long, or septotemporal axis, are as prominent and

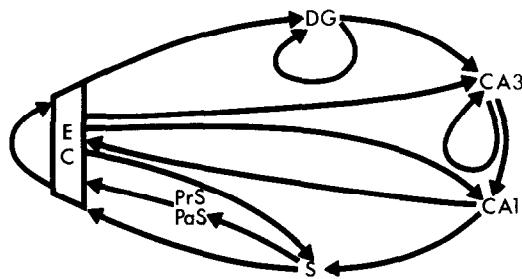


Fig. 8. Summary diagram of intrinsic circuits of the hippocampal formation. See text for description of these projections. Abbreviations: DG, dentate gyrus; EC, entorhinal cortex; PrS, presubiculum; PaS, parasubiculum; S, subiculum.

well organized as those which run in the transverse axis. Some connections, such as the associational projection of the dentate gyrus, appear to be specifically disposed for integration of distant levels of the dentate gyrus along the septotemporal axis. The transverse and longitudinal organization of the major intrinsic hippocampal connections are illustrated schematically in Fig. 9.

Two plausible interpretations could be drawn from the conflicting views provided by the anatomical data and the physiological studies of Andersen and colleagues. First, it might be suggested that the anatomically demonstrated divergence of the intrinsic hippocampal circuitry does not reflect the true functional or physiological operation of the hippocampal system. Perhaps intrinsic inhibitory mechanisms act to channel information through the hippocampal circuit in a lamellar fashion. This remains a viable alternative but physiological studies are needed to determine how local circuit interactions might serve to limit the widespread distribution of information that the anatomical organization indicates. A second interpretation, however, is that the flow of information along the septotemporal axis is an important component of normal hippocampal function that could be appreciated physiologically given appropriate experimental paradigms. The experimental paradigms employed by Andersen *et al.*,² Lomo²⁴ and others, conditioned as they were by existing anatomical views that the hippocampal circuit was indeed lamellar, might have given undue significance to the transverse flow of information. We have attempted to provide reasonable explanations based on our current view of hippocampal anatomy, as to why those studies produced evidence apparently in support of the "lamellar hypothesis." We suggest, however, that if other physiological paradigms had been used that were more sensitive to connections along the septotemporal axis, a picture of hippocampal physiology that is more consistent with the anatomy would have been obtained.

We have purposely quoted from several of the articles that we reviewed to indicate the relatively widespread acknowledgement of the inadequacy of the

"lamellar hypothesis" to account for hippocampal anatomy. Thus, a critique of the "lamellar hypothesis" is not particularly novel or revolutionary. However, despite the extremely meager anatomical support for the "lamellar hypothesis," it nonetheless maintains substantial influence over the way neurobiologists view information processing in the hippocampal formation. We believe that clinging to the lamellar concept of hippocampal function is fast becoming detrimental to further advances in understanding structure/function relationships in this system. And as computational modeling becomes more of an integral component in the analysis of hippocampal function, *it becomes imperative to promote a view of hippocampal anatomy that realistically reflects the three-dimensional organization of its circuitry.*

We have attempted to highlight two basic aspects of hippocampal circuitry that are incompatible with the "lamellar hypothesis." The first is that all of the links in the hippocampal circuit, save the mossy fibers, are much more divergent along the long axis than is consistent with the point-to-point flow of information implied by the lamellar hypothesis. This is clearly illustrated by the perforant path projection to the dentate gyrus. Cells in the entorhinal cortex give rise to highly collateralized axonal projections that distribute for some distance along the septotemporal axis of the dentate gyrus. The trajectory of these fibers appears complex and in need of substantially more study. Some collaterals perforate the subiculum and distribute in a transverse direction in the molecular layer of the dentate gyrus. The long extent of these collaterals is not yet clear but may be rather restricted. Other collaterals in either the stratum lacunosum-moleculare or in the angular bundle course along the septotemporal axis of the hippocampal formation before entering the dentate gyrus. Taken together, these collaterals form an impressive longitudinal component of the perforant path projection. Thus, information originating focally in the entorhinal cortex will be widely dispersed along the septotemporal axis of the dentate gyrus.

The second aspect of hippocampal organization that is incompatible with the "lamellar hypothesis" is that certain of the hippocampal connections, such as the long associational projection of the dentate gyrus and the Schaffer collateral projection from CA3 to CA1, are highly organized in a gradient fashion to provide dispersion of information from a particular level in the hippocampal formation to much of its septotemporal extent. The associational projection arising from the polymorphic layer of the dentate gyrus, for example, does not return information to the granule cells at the level of origin, but rather relays information to granule cells located several millimeters away from the origin. This fiber system appears to be organized to integrate different hippocampal levels rather than to isolate them. We believe, therefore, that the hippocampal formation should be viewed as a series of simple cortical regions that are

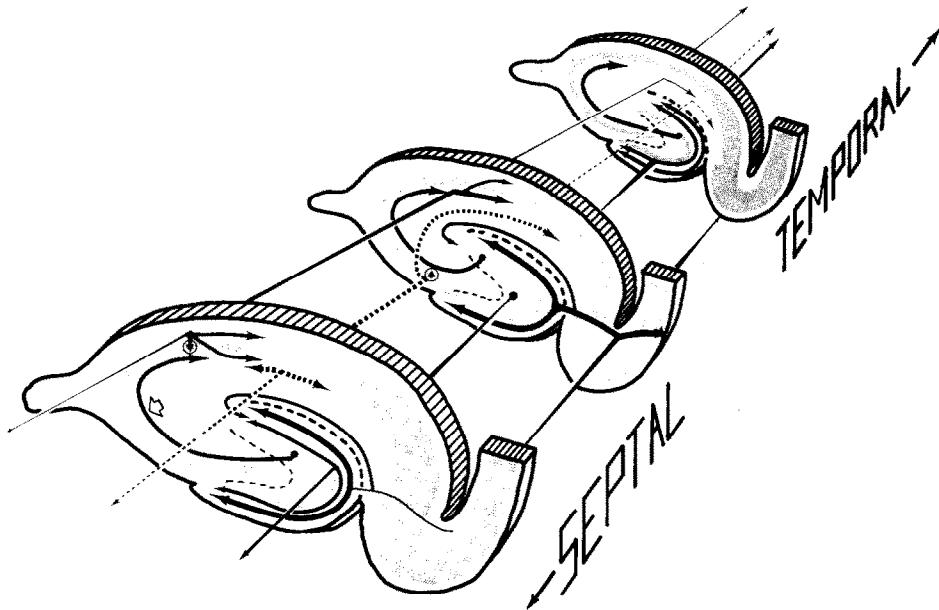


Fig. 9. Summary diagram of the three-dimensional organization of intrinsic hippocampal connections. The perforant path projection (red) originating at one position in the entorhinal cortex (red star) diverges to innervate a broad band of the dentate gyrus and hippocampus. The mossy fiber projection (black—indicated by an open arrow), in contrast, projects onto the CA3 field of the hippocampal formation in a relatively lamellar fashion. Fibers of the major associational projection arising from cells of the hilus of the dentate gyrus (blue) do not innervate the level of origin or the immediately adjacent 0.5–2 mm of dentate gyrus. The projection terminates in the inner third of the molecular layer beginning approximately 1 mm away from the origin and increases in density for 2–4 mm septally and temporally from the level of origin. The Schaffer collateral projection from CA3 to CA1 (green) also projects divergently along the septotemporal axis and is organized according to several orderly gradients. CA3 cells located close to the dentate gyrus (dotted green lines—arrowhead pointing upwards) project superficially in stratum radiatum and distally in CA1 (closer to the subiculum). Projections from these cells terminate most heavily at a level approximately 1 mm septal to the cells of origin. At progressively more septal levels from the level of origin, the distribution of fibers and terminals shifts proximally in CA1 and deeper in stratum radiatum. At progressively more temporal levels, the fiber and terminal labeling shifts distally in CA1 and superficially in stratum radiatum. Projections from CA3 cells located closer to CA1 (solid green lines—arrowhead pointing downwards) initially terminate most heavily in stratum oriens and deeply in stratum radiatum. The fiber plexus is densest proximally in CA1. The distribution of fibers and terminals follows the same gradient of changes described above at septal or temporal levels from the level of origin.

organized for information flow both in the transverse and in the septotemporal axes. By this view, processing of information for a particular task will take place simultaneously over much of the septotemporal axis of the hippocampal system rather than in a thin isolated slice.

One obvious question is whether the non-lamellar view of intrinsic hippocampal circuitry presented in this Commentary has any implications for work conducted with the *in vitro* hippocampal slice preparation. Our view is that it is likely to have more impact on the planning and interpretation of slice studies than on the use of this experimental approach. The placement of electrodes or the orientation in which the slice is cut may be reconsidered on the basis of the anatomical conclusions presented in this Commentary. However, it is apparent from these anatomical conclusions that a 350- μm slice is not likely to contain the cells of origin for many of the fibers that travel through or terminate within the slice. Nonetheless, stimulation of these cut axons, at least for the short time course of the typical slice experi-

ment, obviously produces a relatively normal physiological effect. Therefore, it appears that the presence of intact projections is not mandatory for the generation of useful data with this preparation. Thus, the *in vitro* preparation will obviously continue to generate useful information concerning the cellular physiology of the hippocampal formation. However, the *in vitro* slice preparation does not allow for the simultaneous evaluation of information flow along the transverse and septotemporal axes of the hippocampal system. Therefore, the *in vitro* hippocampal slice cannot be viewed as an adequate model of information processing in the *in vivo* hippocampal formation.

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