Interactive report

The hippocampal lamella hypothesis revisited

Per Andersen*, Anne F. Soleng, Morten Raastad

Department of Neurophysiology, Institute for Basic Medical Sciences, University of Oslo, Pb. 1104 Blindern, 0317 Oslo, Norway

Accepted 28 September 2000

Abstract

We have re-examined the hippocampal lamellar organization of the CA3-to-CA1 connection. Based on a new technique with electrophysiological quantification of Schaffer collateral density, and a review of recent literature, we conclude that the lamellar organization remains a useful concept for understanding hippocampal connectivity. Using a sheet-like hippocampal preparation, containing the whole CA1 region, we mapped the distribution of Schaffer collaterals by two procedures. First, we recorded the amplitude of the Schaffer compound action potential in various parts of CA1 after stimulation of a point in CA3. Second, we charted the CA1 positions from which we could antidromically excite individual CA3 neurones. Although the Schaffer collaterals radiated from their CA3 cells of origin within a wide, fan-shaped area, covering a large part of the septo-temporal extent of CA1, the amplitude of the compound action potential was largest in a slightly oblique, transverse band across the CA1 towards the subicular region.

Keywords: Hippocampus; Lamellar organization; CA3 axon; CA1; Schaffer collateral

1. A preparation for studying connectivity principles

The hippocampal formation is a useful preparation in which to study general neuroscience problems. One reason is that the hippocampal formation demonstrates a number of organizational principles. First, between the narrow strip-formed cortical subdivision of the hippocampal formation, there is a one-directional connectivity between the principal cells of each strip. Second, there is a remarkable stratification of afferent fibres such that their synapses often segregate to specific dendritic regions.

A third principle is the lamellar organization of intrahippocampal fibre systems [2] to indicate that the major part of principal cell axons are oriented parallel to each other and course nearly transversally to the long axis of the hippocampus. Andersen et al. [2] further proposed that the fibre orientation implied that the hippocampal cells were activated in a strip-like fashion, and that the coactivation of a number of cells in such a near-transverse band, called a lamella, could represent a functional unit of the hippocampus.

The lamella hypothesis was criticized, however, because fibre tracings showed a wide, fan-shaped distribution of Schaffer collaterals. These studies were made with fibre staining of anterogradely and retrogradely transported markers [1,7] or by tracing of axonal branches after intracellular marking of individual CA3 cells [8]. These authors noted that the connection between CA3 neurones and CA1 pyramidal cells, the Schaffer collaterals, were heavily branched in a markedly diverging pattern. Thus, a small injection in the CA3 region led to transport of a marker substance in up to 2/3rds of the longitudinal extent of CA1. Further, retrogradely transported markers from a small spot in the subiculum-near part of CA1 were found in longitudinally dispersed CA3 neurones. Thus, Amaral and Witter [1] concluded: “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.”

A similar set of findings emerged from studies with intracellular filling of CA3 neurones with horseradish peroxidase (HRP) and subsequent tracing of branches from such single cells [8]. Axonal branches were found in numerous clusters, spread widely along the longitudinal...
extension of CA1, covering up to two thirds of the entire length.

The conclusion from such studies was that an individual CA3 neurone not only activates CA1 neurones along a narrow transverse lamella, but should be able to influence a broad sector of CA1 neurones comprising more than half of the structure through the fan-like spread of its many Schaffer collaterals.

2. Quantitative connectivity data are wanted

Can the lamella idea survive such a set of well conducted studies? We feel that it can, focussing upon the necessity to apply a numerical analysis of the relevant synaptic connectivity and thus offer a re-interpretation of the reported morphological results. Although these findings do indicate a wide distribution of the CA3-to-CA1 axonal projection, they are not easily quantified. Specifically, this approach can not tell the exact proportion of axonal branches from a single CA3 cell which pass through the various positions of a plane oriented normal to the alvear surface and along the longitudinal axis of the hippocampus. This makes it difficult to estimate the density and distribution of boutons along all axonal branches. It is this synaptic density which constrains the functional efficiency of the afferent system, and not the total width of the axonal distribution.

There are some suggestions that the Schaffer axonal distribution is not uniform within the longitudinal dimension of the axonal tree. For example, Sik et al. [13] stained a single CA3 cell which, within a set of transverse sections covering about 1.8 mm along the longitudinal axis, had more than 15 000 boutons. This high number suggest to us that a major part of the total axonal tree is likely to be found within this transverse band, possibly with lower densities on either flank. A similar impression emerges from the plots of Schaffer collateral branches of Li et al. [8]. The electron microscopical reconstruction technique gives relatively short lengths of the individual axonal branches, so the longitudinal distribution was estimated by the density of such twigs. In addition to a certain accumulation of axonal stubs along a transversally oriented region from the stained cells, there were distinct peaks with higher axonal branch density at various positions along the longitudinal dimension. The number of twigs were, on the whole, less in the clusters found on either flank compared to those in the median region. All in all, the axonal pattern is not unlike the amplitude distribution of synaptic signals in the original lamella paper (see Fig. 1D.C of ref. [2]).

3. A new approach

Although we feel that the discrepancy between the results from modern morphological studies and the lamella

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Hippocampal compound action potentials and conduction velocities. (A) Averages of action potentials recorded from the Schaffer collaterals (Sch), longitudinal association fibres (Ass) and the fimbria (Fim). The open circle and star label two components of the action potentials in the latter. B. The mean and standard deviation (sd) and error (sem) of the apparent conduction velocities for the same fibre categories. The signals were recorded after all excitatory synaptic activity were blocked by CNQX and AP5.
hypothesis is less than expressed by some authors, we appreciate that more precise data on Schaffer collateral fibres and their properties is desirable. Therefore, we now present a set of new data with direct recording of the density of Schaffer collateral branches and of the excitatory synaptic signals generated by these fibres. These observations are made in a set of horizontal slices in vitro, covering the entire CA1 and with possibility for stimulation of CA3 cells giving rise to these signals. Our results substantiate the findings of wide axonal branching of the Schaffer collaterals, but give quantitative data, showing that the heaviest distribution of such branches is distributed along a vector running somewhat obliquely to the longitudinal axis and with gradually falling branch densities on either side. Further, the Schaffer collaterals generate synaptic potentials which have the same amplitude distribution as the axonal signals. In other words, both the Schaffer collaterals and the excitatory synaptic signals they generate have the same distribution as in the original lamella description.

In order to record the action potentials along the Schaffer collateral branches, we have made 500–900 μm thick horizontal tissue slices (parallel to the CA1 pyramidal layer) after the hippocampus was isolated and unbent into a slightly curved, banana-shaped form. Such slices contain the entire CA1 region from the septal nuclei till the temporal tip, and from the CA3 to the subicular border. A strip of CA3, containing from a third to one half of the CA3 region, is attached to the CA1 part. The volume of this CA3 strip varied in different preparations.

These horizontal slices were mounted in a chamber in a manner to secure good access of the oxygenated perfusion fluid to all sides of the tissue and with the top close to the fluid surface. The temperature was kept at 22–25°C except for short, 1–2 min periods at 35–37°C in order to test the signals at more physiological temperatures. After stimulation of a small group of CA3 neurones, extracellular field potentials were recorded from the CA1 region. In addition, antidromic field and single cell discharges were evoked by stimulation of Schaffer collateral branches within CA1.

4. Schaffer collateral signals

Following blockade of excitatory synaptic potentials with cyanouquinoxaline (CNQX, 10 μM) and amino-phosphono-pentanoic acid (AP5, 50 μM), the remaining signal was due to conduction along axons. The signals were usually triphasic positive–negative–positive, although the last component was often quite small (Fig. 1A). Following stimulation of a small group of CA3 cells, axonal action potentials were recorded from several positions, the main subdivisions being the fimbria, the longitudinal association path within CA3 and the Schaffer collaterals in CA1. The various branches conducted at different velocities (Fig. 1A,B). Because we do not know the actual length of the various branches, we can only give apparent conduction velocities as calculated from the surface measurements of the recording points. Real conduction speeds must await reconstruction of the fibres in question. Such studies are feasible but time-consuming. There were two classes of CA3 axon collaterals in the fimbria (Fim), having apparent conduction velocities (at 23°C) of 0.99 and 0.37 m/s, respectively. Most likely, these represent fibres with and without myelin. The mean apparent conduction velocity of longitudinal association fibres (Ass) were 0.39 m/s, whether they were recorded in the septal or temporal direction or from the str. radiatum or str. oriens. The average conduction velocity of Schaffer collaterals was lowest, measuring 0.25 m/s, and with distinct variations between different parts of the axonal tree.

The CA1 area from which we could record Schaffer collateral-associated signals had a form like a broad V with the sharp end at the point of stimulation (Fig. 2B). Within this sector, however, the signals differed greatly in magnitude. The largest amplitude were found in a line towards the subiculum, deviating slightly towards the temporal direction from a plane transverse to the longitudinal axis (Fig. 2A,B). With increasing distance from the stimulated CA3 region, the amplitude diminished. The form of the amplitude versus position formed a ridge sloping towards the subicular border and with a similar slope on the septal and temporal flanks. When an average distribution was made from a set of such experiments, the angle of this ridge deviated 18 degrees from the transverse direction, tilted in the temporal direction (Fig. 2A). By recording at various distances from the fimbria, axonal action potentials were recordable all the way to the subicular border. A comparison shows that the distribution of action potentials in the Schaffer collateral system is very close to that of synaptic signals in the anesthetized rabbit (Fig. 2C,D). In the latter, however, the width of the band showing population spikes were considerably narrower.

Conduction along the Schaffer axonal tree could also be analysed by antidromic activation of single CA3 cells. Stimulation within the fan-like distribution area of the Schaffer collaterals could activate CA3 neurones antidromically with the expected constant latency and all or none appearance. The same cell could be antidromically excited by stimulation inside a broad V-shaped region, having a width of up to 4 mm, nearly half of the longitudinal extension of the rat hippocampus, and reaching all the way to the subicular border. This pattern supports the picture gleaned from the orthodromic activations, namely that a CA3 neurone emits a fan of axonal Schaffer collaterals with a considerable longitudinal extension and where many reach the subicular border. From a given stimulation spot in CA1 several neighbouring CA3 single units could be recorded simultaneously. In spite of the same stimulation and recording electrodes, the antidromic latencies often showed a surprising difference. Thus
neighbouring cells have axons with greatly varying conduction velocities or path lengths.

Naturally, the latency of the action potentials increased with the distance from the stimulated CA3 site. Unexpectedly, however, the apparent conduction velocity differed among the various branches of the Schaffer axonal tree. Measuring a subset of transversally oriented Schaffer collaterals, their average conduction velocity was 0.22 m/s (Fig. 3, middle). Fibres in a septal subset of the axonal tree conducted at a similar speed of 0.23 m/s (Fig. 3, left side). However, the Schaffer branches taking a more temporal direction conducted at an apparently faster speed of 0.28 m/s (Fig. 3, right side).
m/s (Fig. 3, right side). The calculation of conduction velocities was hampered by the uncertainty about the exact axonal lengths. Thus, whether these observations mean that the temporally directed branches of the Schaffer collaterals are thicker, or less tortuous than the transversally or oblique septally oriented subgroup is not known. No available information allows an estimate of the possible twists, bends and branching in the Schaffer axonal tree.

Whatever the explanation for the various apparent conduction velocities, the net result is that a fibre volley emanating from a CA3 cell will not spread in a circular fashion but with a somewhat flattened wave-front, delivering its simultaneous excitatory synaptic influence along a longitudinal strip of CA1 cells.

When synaptic field potentials were recorded, their amplitude distribution formed a pattern similar to that of the axonal signals (Fig. 2B), again repeating the slightly oblique distribution pattern with the largest amplitudes found along a line pointing in a subicular direction and at about 20 degrees temporal to a transverse plane. Stimulation of two neighbouring sites of the CA3 region caused a summation of signals with the largest amplitudes along a line between the two ridges of maximal responses to each stimulated site alone.

5. Possible functional consequences of the lamellar organization

The original lamella concept was derived from studies of anaesthetized rabbits. The direction of the engaged axons was estimated from antidromic and orthodromic field potentials. Characteristically, orthodromically activated synaptic potentials were recorded in a nearly transverse strip with a width of a few millimeters. Depending upon the stimulus strength, the number of excited axons would vary and, consequently, the width of the excited strip of tissue.

Recently, Hampson et al. [6], recorded from groups of CA3 and CA1 neurones in awake, behaving rats. Cells with similar response properties and correlated activity were found to aggregate in 0.6–0.8 mm broad, roughly
transversally oriented bands in both CA3 and CA1. Between the two regions, CA1 cells with similar functional characteristics were offset in the temporal direction by 0.2–0.4 mm relative to their CA3 counterparts, roughly corresponding to the angled orientation of the lamellar main axis. This finding that cells oriented along a lamellar plane are coactivated in a physiological situation appears as a vindication of the lamellar idea as having a functional role.

A comment may be directed at the ’strict interpretation of the lamellar hypothesis’ used by Amaral and Witter [1]. Neither the figures nor the text in [2] appear to be in disagreement with the results of the subsequent morphological labelling experiments. Although there was a definite direction along which the various afferent fibre systems showed maximal postsynaptic responses (the lamella direction), there were also relatively broad shoulders on either side. This was more evident for the synaptic signals than for the population spike, signalling cell discharges (open and closed circles, respectively, in Fig. 2D).

The lamellar organization should be understood in a population context. A strip of CA1 cells will be most heavily engaged synaptically by an activated CA3 cells, but with a flank of more weakly excited cells on either side. Given appropriate input to neurones at these flanks, they could be brought to discharge. Thus, the lamella hypothesis does not require that the excitation is confined to a thin strip. Rather, when referring to Schaffer collaterals, it expresses the main excitatory synaptic influence area of a CA3 neurone.

Although there is much evidence for the proposal by O’Keefe and Nadel [11] that the hippocampus provides the animal with a cognitive map necessary for spatial navigation, we seem to be far from knowing the actual cellular connectivity and discharge patterns at the basis for this map [4,10,12]. An interesting principle emerged from work by Deadwyler et al. [3] that spatial retrieval seems to involve the activation of ensembles of CA1 neurones. Albeit sufficient for a simple recognition task, the activity of a cell ensemble in a small strip of hippocampal tissue may not be sufficient for a complex task like learning a new spatial constellation. By local inactivation of various parts of the hippocampus, Moser and Moser [9] concluded that spatial tasks probably requires neuronal ensembles distributed over more than half of the dorsal hippocampus. In a landmark-based navigation task Gothard et al. [5] found that CA1 units could be bound to multiple reference frames, also suggesting the collaboration of widely spaced neurones.

The fan-like distribution of the CA3-to-CA1 axons suggests that two or more CA3 neurones, separated by a certain longitudinal distance, may create a special convergence pattern which could explain the binding of widely separated CA1 neurones in a retrieval task. When activated in isolation, each CA3 neurone will distribute its excitation to a band of CA1 neurones in a ridge-like fashion. The most effective excitation will take place along the lamellar orientation with smaller effects to either side. However, when two neurones, spaced some distance apart, discharge simultaneously, the summation of the two synaptic influences will be largest at some intermediate position. With multiple CA3 neurones being synchronously active, several small areas of intense convergence may appear in the CA1 matrix, determining the detailed pattern of discharges. With asynchronous CA3 discharges, the situation becomes even more complex. In such cases, the timing of the various CA3 discharges will clearly determine the transverse location of the cells which will receive the most intense synaptic influence. A simple pictorial analogy would be to describe the spread of a single CA3 action potential through the axonal tree as a wavefront into the CA1 area, somewhat similar to waves emanating from a small opening in a stone pier. The effect of two simultaneously active, but longitudinally separated CA3 neurones would look like waves coming out through two neighbouring pier openings, forming an interference line. The direction of this interference line will be a function of the timing of various CA3 discharges. Only for synchronously discharging CA3 cells would the converging excitation coincide with the lamellar direction. Remembering the widely differing CA3 cell antidromic latencies, determination of the exact interference pattern requires detailed knowledge about the conduction along the axonal tree of individual CA1 neurones.

References