

Histological and Morphological Analysis of the Hippocampal Subfields in the Adult Rat

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Summary:

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Background: The hippocampus is empirically assumed to have different functions, of which the best known are: the representation of self-location in cognitive dimensions, and the storage and retrieval of memory.

Materials and methods: Eleven adult male albino rats were used. Brain specimens were processed into paraffin blocks, sectioned (10 μm thick), and stained using Haematoxylin and Eosin, and Luxol Fast Blue MBS counterstained with Cresyl Violet. Morphometric analysis was done through eyepiece micrometers.

Results: The lamellae of the hippocampus were morphometrically evaluated in context of embryogenesis and nerve fiber content.

Conclusion: This study confirmed the existence of differences in nerve fiber distribution among the subfields of the hippocampus which is attributed to differences in the embryonic development processes and to the selectivity of the different connections.

Keywords: Rat Hippocampus, Lamellar Morphometry.

Introduction:

The hippocampus is phylogenetically one of the oldest parts of the brain; it resides in the temporal lobe, and forms part of the limbic system. It consists of the complex interfolded layers of the dentate gyrus and cornu ammonis; (1) which develop from morphogenetically discrete components that differ in their proliferative activity: the ammonic neuroepithelium and the primary dentate neuroepithelium. (2) Each of the regions of the hippocampus, including their various subfields, as well as the different lamellae has been empirically assumed to carry out different tasks in hippocampal functions, (3) of which the two best known examples are: the ability to represent current self-location in spatial and other cognitive dimensions, and the involvement in the storage and retrieval of episodic memory (the ability to recall personal, spatial, and temporal context of events). (4) Also, the hippocampus has been implicated in other areas such as learning, motivation, and the control of appetite. (5) In this study we aimed to identify the morphometric differences between the constituent lamellae of the different regions of both, cornu ammonis and the dentate gyrus; that have been found to play different roles in hippocampal circuitry by electrophysiological recording studies, and to analyze these differences in a functional context.

Materials and Methods:

A total of 11 adult male albino rats – above 22 days of age (i.e. 22 hemispheres) were used in the study. Brain specimens were acquired after the animal was anaesthetized by nipping the calvaria and removing the brain en mass; afterwards they were processed into paraffin blocks and sectioned. (6) Fifty serial coronal sections, 10 μm thick, were made out of each block. The slices were then mounted on slides covered with a thin film of tissue adhesive. Sections made out of the rostral part were regarded to advance septally with respect to the orientation of the hippocampus; those obtained from the caudal part were regarded to advance temporally. Sections were then subjected to histological staining using Haematoxylin and Eosin, and the myelin specific Luxol Fast Blue MBS counterstained with Cresyl Violet. Morphometric analysis was done by utilizing eyepiece micrometers of grid and scale types calibrated with a calibration slide.

Results:

Cornu Ammonis

The Alveus: The alveus (figures 1, 2) is about 175-200 μm in width in the proximal CA1 region (close to the subiculum), this is reduced to 100-125 μm in the distal CA1 region (close to CA3 field). Afterwards, it gradually thickens to about 150 μm in field CA3a and 225 μm in fields CA3b and c.

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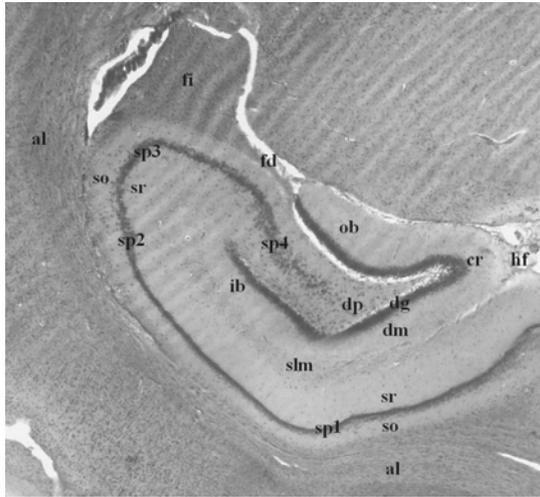


Figure 1a: The hippocampus at a septal level, al: alveus, so: stratum oriens, sp1: stratum pyramidal of field CA1, sr: stratum radiatum, slm: stratum lacunosum-moleculare, dm: moleculare layer of the dentate gyrus, dg: granule cell layer of the dentate gyrus, dp: polymorphic layer of the dentate gyrus, sp2: field CA2 pyramidal layer, sp3: field CA3 pyramidal layer, sp4: field CA4 pyramidal layer, ob: outer blade of the dentate gyrus, ib: inner blade of the dentate gyrus, cr: crest of the dentate gyrus, fi: fimbria, fd: fimbriodentate fissure, hf: hippocampal fissure. H&E, X32

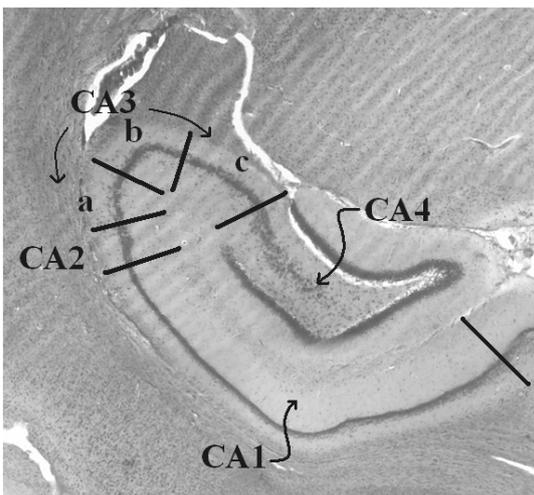


Figure 1b: The hippocampus at a septal level, the lines and arrows demarcate the division of Cornu Ammonis into fields CA 1-4; and field CA3 in to a, b, and c.

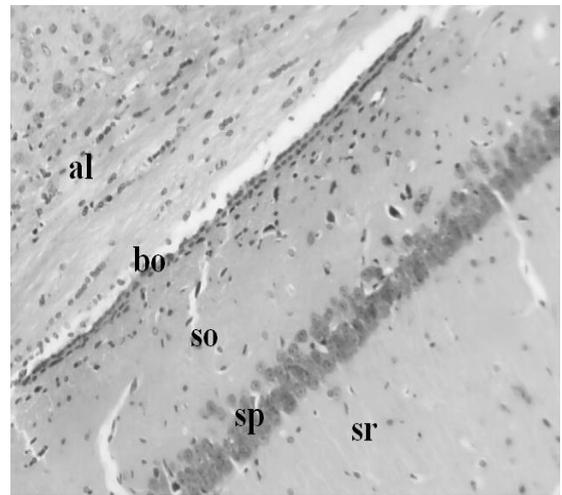


Figure 2: The field CA1. al: alveus, bo: border zone, so: stratum oriens, sp: pyramidal layer, sr: stratum radiatum. H&E, X100

The Stratum Oriens: Stratum oriens (figure 2) starts in field CA1 proximal region with a width of 75-100 μm which increases to 125 μm in the distal part of the same field; afterwards it decreases back to 100 μm in field CA3a and 75 μm in fields CA3b and c (figure 1).
The Stratum Pyramidale: In the proximal part of field CA1 (figures 1, 2), the pyramidal cell layer is 50 μm in thickness. In the distal part, the layer becomes 25-40 μm thick. Throughout fields CA3a and b (figure 1), the stratum is 50-65 μm in width. In region CA3c, the layer increases in width to 75-100 μm . Field CA4, as a result of its position between the blades of the dentate gyrus, does not demonstrate a clear lamellar pattern rendering an accurate measurement of its width not feasible.

The Stratum Radiatum: The stratum radiatum, as its name implies, has a radial streaking pattern that is very clear especially in field CA1 (figure 3), less in field CA2, CA3a, b, and even less in field CA3c. This pattern extends between the strata pyramidale and lacunosum. Beyond CA3c region, the pattern of streaking becomes disorganized and starts to appear mesh-like – in several directions. In proximal CA1 field (figure 2), the layer is about 150-200 μm in width and gradually increases to 175-225 μm in the distal end of field CA1. At field CA2 it becomes reduced to 75 μm as the pyramidal layer increases in width. Throughout field CA3 (figure 1), the width of the layer increases from 75 μm to 100 μm and then back to 75 μm at the end of CA3c region where the arrangement starts to become disorganized.

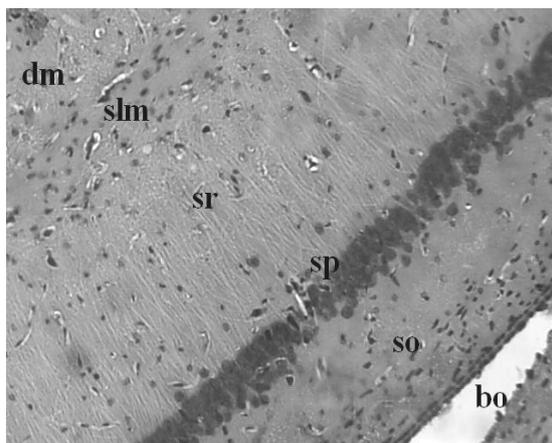


Figure 3: The streaking of stratum radiatum in field CA1, formed by the extension of apical dendrites of pyramidal cells deep into stratum lacunosum-moleculare. bo: alveus/oriens border, so: stratum oriens with interneurons concentrated at the border area, sp: stratum pyramidale, sr: stratum radiatum, slm: stratum lacunosum-moleculare fused with the molecular layer of the dentate gyrus (dm). H&E, X100

The Stratum Lacunosum-Moleculare: This layer measures about 50 μm in width in the proximal part of field CA1. Gradually it thickens to 75 μm at the distal part and then up to 100 μm at CA3a region. As a result of the curved shape of field CA3, it becomes difficult to mark the exact span of the layer and hence accurate measurement was not feasible (figures 1, 3).
The Dentate Gyrus: (figure1) The granule cell layer measures 50-75 μm in thickness in the outer and inner blades, as well as in the crest region. The molecular layer measures 125 μm in thickness in the inner blade, it increases to 150 μm in the crest region, and further to 175 μm in the outer blade. The polymorphic layer, situated between the granule cell layer and CA4 field, has actually a triangular shape instead of a strip-like layer which makes its measurement inaccurate. Tables 1 and 2 summarize the morphometric findings.

Table (1) Hippocampal lamellar width in different fields of Cornu Ammonis. Measurements are in micrometers.

Cornu Ammonis	CA1		CA2	CA3		
	proximal	distal		a	b	c
The Alveus	175-200	100-125	-	150	225	225
Stratum Oriens	75-100	125	-	100	75	75
Stratum Pyramidale	50	25-40	-	50-65	50-65	75-100
Stratum Radiatum	150-200	175-225	-	75	100	75
Stratum Lacunosum-Moleculare	50	75	-	100	-	-

Table (2) Hippocampal lamellar width in the Dentate Gyrus.

Dentate Gyrus	Outer Blade	Crest	Inner Blade
Molecular Layer	175	150	125
Granular Layer	50-75	50-75	50-75
Polymorphic Layer	-	-	-

*Measurements are in micrometers.

Discussion:

The hippocampus has been the focus of numerous investigations attempting to elucidate the mechanisms underlying the specificity of connections within the mammalian central nervous system. These connections show a distinct field organization which, when taken together with the known organization of the afferent and efferent connections, strongly suggest that the different fields of the hippocampus represent functionally distinct entities, (3) and that a systemic attempt to analyze the cytoarchitectonic fields which have been recognized on the basis of the morphology and arrangement of the pyramidal, granule, and other interneurons would be rewarding.

The hippocampus has a mainly lamellar structure; therefore an entire hippocampus can be assembled by stacking together many slices, i.e. the system is easily scalable up in the computational model. (7)

The Alveus: The alveus is the innermost layer for the efferent fibers. The width of this layer is greater in both ends of cornu ammonis (175-200 μm in CA1 field and 225 μm in field CA3c); this clearly correlates with the fact that these are the main projection ports of the hippocampus (CA1 field through the subiculum and CA3 field through the fimbria). (8)

The Stratum Oriens: The stratum oriens functions as a passage for nerve fibers into and out of the alveus. The width of this layer in field CA1 was greater than in field CA3. The contribution of field CA3 pyramidal cells to the efferent fibers leaving through the alveus is less than that from the pyramidal cells of field CA1. (8) In addition, a large portion of the commissural fibers from the hippocampus and entorhinal cortex of the contralateral hemisphere passes through the stratum oriens. The alvear path from the medial entorhinal cortex appears to be restricted in its distribution to the stratum oriens of field CA1. (8)

The Stratum Pyramidale: The differences in the thickness in the different fields of cornu ammonis may be exemplified by a number of facts. Observations made on methacrylate sections of rat embryos (9) suggest that the stratum pyramidale of field CA1 forms before that of field CA3, and it is influenced by the alvear pattern of development. The alignment of CA1 field is regionally related to the Schaffer's collaterals of field CA3 projection to field CA1. The pyramidal cells destined to settle in field CA3 and to

be contacted by the granule cells axons (the mossy fibers), will have to await the formation of the granule cell layer of the dentate gyrus. (10) Various studies have shown that there is different functional significance of fields CA3 and CA1 pyramidal neurons. This is confirmed through experiments that demonstrate differences in the physiological processes of fields CA1 and CA3 pyramidal cells. Field CA3 is one of the most epileptogenic structures of the brain. Studies have demonstrated that kainite-induced experimental epilepsy in rats and human cases of epilepsy are associated with sprouting of the mossy fibers of the dentate granule cells and selective loss of pyramidal neurons, notably in the CA3 and CA4 areas of cornu ammonis. (11) It has been found that, in developing rats, CA1 field neuronal number was significantly reduced by condensed alcohol treatment, while CA3, CA4, and the dentate gyrus populations were not reduced with any alcohol treatment. (12) The Stratum Radiatum: The shift of streaking pattern of the stratum radiatum in field CA4 into a mesh-like organization marks the end of Cornu Ammonis. This is compared to the beginning of stratum radiatum which marks the beginning of cornu ammonis from the end of the subiculum which has no such layer. The stratum radiatum has been shown, in this study, to have the same thickness as the alveus in the proximal CA1 region, and thicker than both the alveus and the stratum oriens in the distal region of field CA1 (175-225 μm vs. 100-125 μm). The same finding applies to field CA3. It was also shown that the stratum radiatum is thicker in field CA1 than in field CA3; particularly in the distal region of CA1. This is due to the fact that this region contains Schaffer's collaterals from all three subregions of field CA3, while the distal region contains only the collaterals originating from field CA3c. (8) Experimentally induced lesions have shown that, in field CA1, only commissural fibers have been found to terminate in this stratum. In the other fields, there is also a considerable number of afferents from the septum. (13) The Stratum Lacunosum-Moleculare: The stratum lacunosum consists principally of horizontal fibers; these are derived in part from the Schaffer's collaterals and hence the layer is presumed to be mainly in fields CA1 and CA2. Thus, the stratum lacunosum-moleculare in field CA3 (100 μm thick) consists mainly of the myelinated fibers of the perforant path from the entorhinal cortex that are in contact with the outermost branches of the apical dendrites. (8) The Dentate Gyrus: The juxtgranular half of the stratum moleculare of the dentate gyrus contains commissural afferents from the opposite side, while the perforant temporo-ammonic fibers fill the outer half of this layer. (14) The molecular layer in the outer blade is thicker than in the inner blade. This may be due to a larger number of axons running transversely through the subiculum as the perforant

path to reach the surface and hence enter the molecular layer of the outer blade of dentate gyrus in the commencement of field CA1 than those reaching the inner blade. (13) It has been shown that there was no difference among the outer, inner blades, and the crest regions of the dentate gyrus as concerning the width of the granule cell layer. Studies based on methacrylate sections and thymidine radiograms (9) have shown that the primary dentate neuroepithelium is distinguished from the ammonic neuroepithelium during early phases of embryonic development in its location, shape, and cellular dynamics. These observations suggest that the site of origin and settling of the granule cells of the dentate gyrus are determined neither by the afferent perforant path and commissural fibers, nor by the alignment of cornu ammonis pyramidal cells. The lamination in the rat hippocampus is most likely based on the time of arrival of the respective afferents in the target areas. (15) The regional differences in the thickness of the various hippocampal subfields are also related to the regional differences in the termination of the efferent and afferent connections. This may be exemplified by reference to 2 hippocampal pyramidal cells from fields CA1 and CA3. The basal dendrites of the CA1 pyramidal neuron receive afferents from the medial entorhinal cortex, whereas those of field CA3 pyramidal neuron receive septal afferents. The apical dendrites of field CA1 pyramidal neuron receive the Schaffer's collaterals at the level of the stratum lacunosum, while those of field CA3 receive the mossy fibers from the dentate granule cells at the level of the stratum lucidum and septal afferents at the stratum radiatum. (16) It was not possible to assign a rigid classification to fields CA2 and CA4. The former, seen to share -to some extent- the connections of both of the neighboring fields CA1 and CA3; whereas the latter, for topographical reasons, does not present a distinct lamellar organization. In conclusion, this study confirmed the existence of differences in nerve fiber distribution among the different subfields of the hippocampus. This can be attributed to differences in the embryonic development processes as well as to the selectivity of the different connections, whether hippocampal afferents or efferents. The unique lamellar organization of the hippocampus has been evaluated. The different subfields of the hippocampus, whether of cornu ammonis or the dentate gyrus, were found to differ in their criteria, basically because of the density of the afferent or efferent fibers of each layer. This was found to be initially governed by the chronological order of arrival of the afferents or extension of the efferents, in addition to the mode of development of the adjacent lamellae, as seen in cornu ammonis field CA1 and in the alveus. The discrepancy in the thickness of the different subfields of the hippocampus merits the

search for factors such as the composition of the extracellular matrix and the capillary density, and other factors which may play a role in the distinct hippocampal lamellar organization.

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