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Morphological Characterization of the CA1 Region of the Adult Wistar Rat Hippocampus by Modified Rapid Golgi Staining

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: The CA1 region of the hippocampus plays a crucial role in learning and memory and is characterized by a distinct laminar organization. Detailed visualization of its neuronal architecture is essential for understanding hippocampal function. The present study aimed to characterize the morphology of the CA1 region in adult Wistar rats using a modified rapid Golgi staining technique.

Materials and Methods: Hippocampus from ten adult male Wistar rats were processed using a previously described modified rapid Golgi staining technique. Horizontal sections (120 µm) were examined under light microscopy to evaluate the laminar organization of the CA1 region and the morphology of pyramidal neurons.

Results: The modified rapid Golgi staining technique clearly demonstrated the four layers of the CA1 region, namely the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare. Pyramidal neurons exhibited distinct triangular cell bodies with well-defined apical and basal dendrites, allowing clear visualization of neuronal architecture and dendritic arborization.

Conclusion: The modified rapid Golgi staining technique provides clear visualization of the normal cytoarchitecture and neuronal morphology of the CA1 region, making it a useful tool for neuroanatomical and experimental studies.

Keywords: Hippocampus, CA1, pyramidal neurons, cytoarchitecture, Golgi staining, Wistar rat.

Introduction

The hippocampus is an essential component of the limbic system and plays a pivotal role in learning, memory consolidation, spatial navigation, and cognitive processing. It forms part of the trisynaptic hippocampal circuit and is particularly susceptible to structural and functional alterations associated with ageing, neurodegenerative diseases, epilepsy, cerebral ischemia, and experimental neurological disorders [1,2]. Owing to its functional importance, the hippocampus has been extensively studied in both basic and clinical neuroscience.

Among the hippocampal subfields, the CA1 region is of particular interest because it serves as the primary output region of the hippocampus and is highly involved in memory encoding and retrieval. It is composed of a well-organized laminar architecture consisting of the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare. The principal neurons of this region are pyramidal cells, characterized by triangular cell bodies, prominent apical dendrites extending towards the superficial layers, and basal dendrites projecting into the stratum oriens. The precise organization of these neurons forms the structural basis for synaptic integration and information processing within the hippocampus [3,4].

Histological techniques capable of demonstrating complete neuronal morphology are indispensable for studying the cytoarchitecture of the hippocampus. Among these, the Golgi staining technique remains the gold standard because it selectively impregnates individual neurons, allowing visualization of the neuronal soma, dendrites, dendritic branches, and spines. Although several modifications of the classical Golgi technique have been developed to improve staining quality and reduce processing time, detailed morphological descriptions of the CA1 region using these techniques remain limited [5,6].

Although the normal cytoarchitecture of the hippocampus has been extensively described, detailed qualitative documentation of the CA1 laminar organization using a modified rapid Golgi staining technique remains limited. Therefore, the present study aimed to characterize the morphology of the CA1 region in adult Wistar rats.

In our previous study, we described a modified rapid Golgi staining technique that provided rapid, reproducible, and high-quality neuronal impregnation. Building upon that work, the present study focuses on the qualitative morphological characterization of the CA1 region of the adult Wistar rat hippocampus. The study aims to describe the laminar organization of the CA1 region and the morphology of pyramidal neurons as visualized by the modified rapid Golgi staining technique.

Materials And Methods

Study Design

A descriptive observational study was conducted to evaluate the cytoarchitecture and neuronal morphology of the CA1 region of the hippocampus in adult Wistar rats using a modified rapid Golgi staining technique.

Experimental Animals

The study was carried out on ten adult male Wistar rats weighing 180–250 g, obtained from the Central Animal House, St. John's Medical College, Bengaluru, India. The animals were housed under standard laboratory conditions under a 12:12 h light/dark cycle with free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and were conducted in accordance

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with the CPCSEA guidelines. Every effort was made to minimize animal suffering and the number of animals used.

Tissue Collection

The animals were euthanized by cervical dislocation followed by decapitation. The brains were carefully removed, and the hippocampi were dissected and blocked for histological processing.

Rapid Golgi Staining

The hippocampal tissue was processed using the modified rapid Golgi staining technique previously described by Smitha et al [9]. Briefly, the tissue was fixed in a potassium dichromate-based solution, impregnated with 0.75% silver nitrate under dark conditions, dehydrated, embedded in paraffin wax, and sectioned for microscopic examination.

Sectioning and Mounting

The impregnated hippocampal tissue was sectioned horizontally at a thickness of 120 µm using a sliding microtome. The sections were collected in 70% ethanol, cleared in xylene, mounted on glass slides using DPX mounting medium, and cover slipped.

Microscopic Examination

The stained sections were examined under an Olympus BX51 light microscope (Olympus, Japan) using low- and high-power objectives. Digital images were captured using a CCD camera (JVC, Tokyo, Japan; MBF CX9000) attached to the microscope. Image acquisition was performed using Image-Pro Plus version 5.1.1 (Media Cybernetics, USA) and Neurolucida version 9 (MBF Bioscience, USA).

Morphological Evaluation

The CA1 region was identified based on standard anatomical landmarks. The laminar organization of the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare was examined qualitatively.

Statistical Analysis

As the present study was descriptive in nature, no statistical analysis was performed. The observations are presented as qualitative descriptions supported by representative photomicrographs.

Results

The technique provided excellent visualization of the cytoarchitecture of the CA1 region of the adult Wistar rat hippocampus. The neuronal cell bodies, dendritic processes, and laminar organization were distinctly demonstrated with minimal background staining. The four principal layers of the CA1 region, namely the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare, were clearly identified (Figure 1).

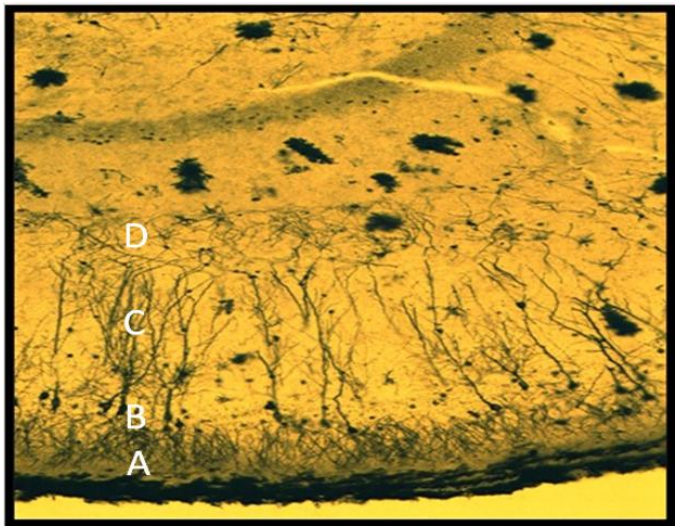


Figure 1: Low-power photomicrograph showing the laminar organization of the CA1 region of the hippocampus A- stratum oriens, B- stratum pyramidale, C- stratum radiatum & D- stratum lacunosum-moleculare

The stratum oriens consisted of a relatively cell-sparse layer situated adjacent to the alveus. It contained scattered interneurons and the basal dendrites of

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pyramidal neurons extending from the stratum pyramidale. The basal dendrites exhibited variable branching patterns and were readily visualized by the Golgi stain (Figure 2).

The stratum pyramidale formed a compact and continuous layer composed of closely arranged pyramidal neurons. The neuronal cell bodies were triangular to pyramidal in shape with a prominent apical dendrite arising from the apex of the soma and extending toward the superficial layers. One or more basal dendrites projected into the stratum oriens. The nucleus and primary dendritic processes were clearly delineated in well-impregnated neurons (Figure 2).

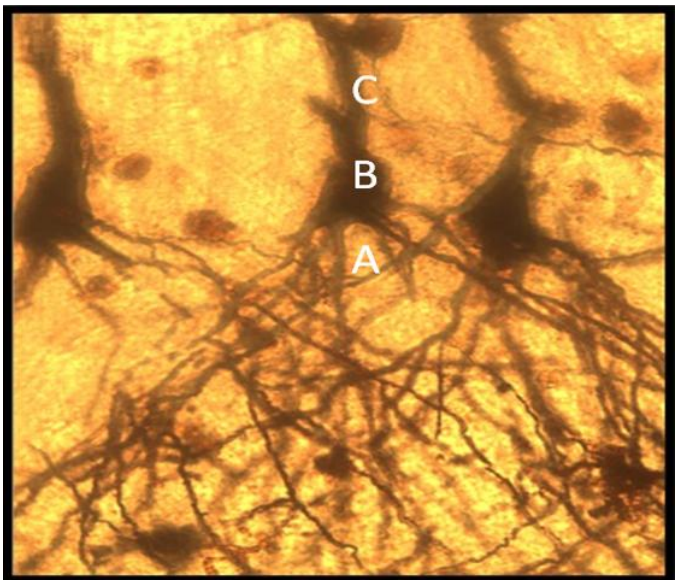


Figure 2: A- Stratum oriens showing basal dendrites of CA1 pyramidal neurons, B- Stratum pyramidale demonstrating closely packed pyramidal neurons with triangular cell bodies & C- Stratum radiatum showing the apical dendrite

The stratum radiatum appeared as a broad layer containing the apical dendrites of CA1 pyramidal neurons. These dendrites exhibited extensive branching, forming a dense dendritic network. Individual dendritic processes could be followed over considerable distances, and higher-order branches were clearly visible in well-stained neurons.

The stratum lacunosum-moleculare formed the outermost layer of the CA1 region. This layer was relatively less cellular and consisted predominantly of the terminal arborization of apical dendrites and fine neuronal processes. The transition between the stratum radiatum and stratum lacunosum-moleculare was well defined.

The technique enabled complete visualization of the morphology of CA1 pyramidal neurons, including the neuronal soma, apical and basal dendrites, and dendritic arborization. The selective impregnation of individual neurons facilitated clear observation of neuronal architecture without obscuring adjacent cells (Figure 3).

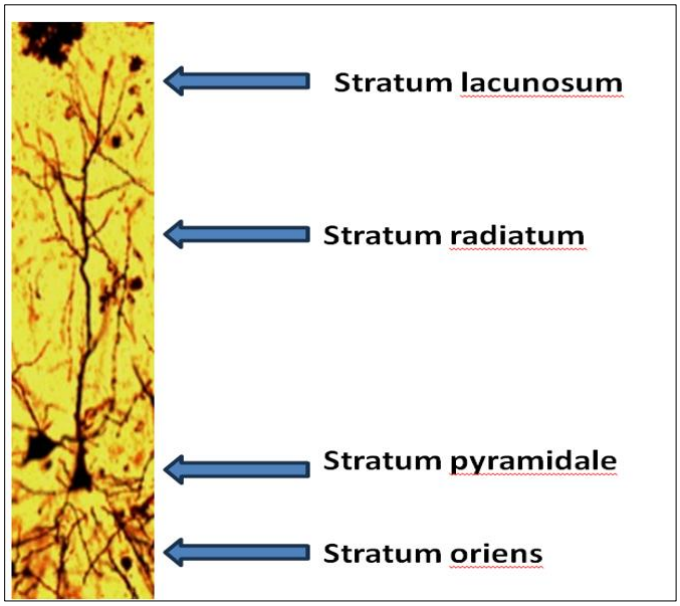


Figure 3: High-power photomicrograph of a CA1 pyramidal neuron showing the soma, apical dendrite, basal dendrites, and dendritic branches

To further demonstrate the quality of neuronal impregnation achieved by the technique, representative CA1 pyramidal neurons were digitally reconstructed using Neurolucida software. The neuronal soma, apical dendrite, basal dendrites, and higher-order dendritic

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branches were clearly visualized, permitting accurate digital reconstruction of the neuronal architecture (Figure 4A). The high-quality impregnation obtained with the technique provided excellent delineation of the dendritic arborization, indicating its suitability for neuronal reconstruction and future quantitative morphometric studies.(Figure 4B)

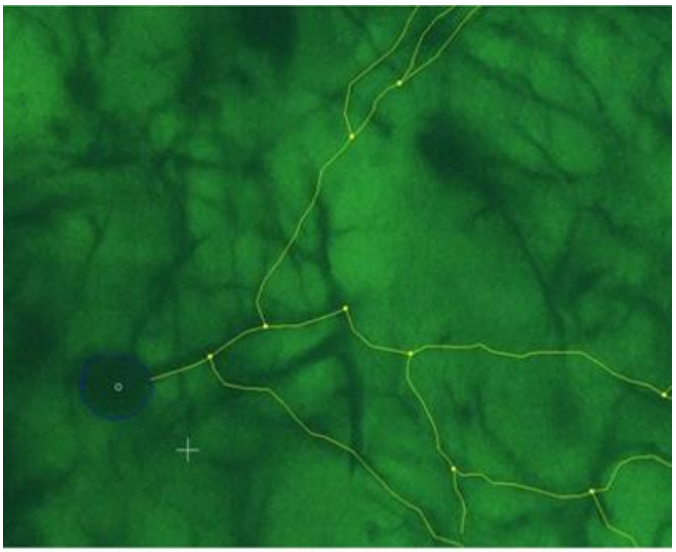


Figure 4A: Original Golgi-stained neuron with Neurolucida tracing superimposed

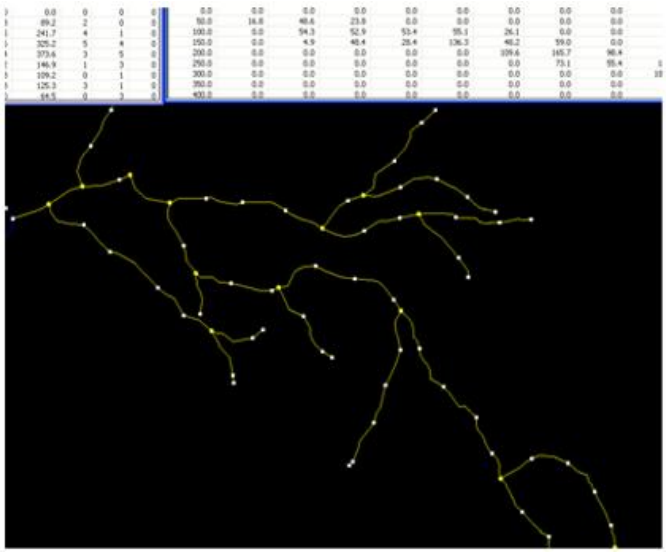


Figure 4B: Final Neurolucida reconstruction showing the complete dendritic arborization

Discussion

The present study qualitatively characterized the cytoarchitecture and neuronal morphology of the CA1 region of the adult Wistar rat hippocampus using a modified rapid Golgi staining technique. The technique provided clear visualization of the laminar organization of the CA1 region together with the morphology of pyramidal neurons and their dendritic arborization.

The hippocampus plays a central role in learning, memory consolidation, and spatial navigation, with the CA1 region serving as one of its principal output areas. The orderly arrangement of pyramidal neurons and their dendritic architecture forms the structural basis for synaptic integration and information processing within the hippocampal circuitry [1–4]. In the present study, the characteristic laminar organization of the CA1 region, comprising the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare, was clearly demonstrated, consistent with previous descriptions of hippocampal architecture [5,6].

The modified rapid Golgi staining technique enabled complete visualization of CA1 pyramidal neurons, including the neuronal soma, apical dendrites, basal dendrites, and higher-order dendritic branches. Similar morphological characteristics have been described in earlier studies, where CA1 pyramidal neurons were identified as the principal excitatory neurons responsible for integrating afferent inputs from multiple hippocampal and cortical pathways [10,11]. The selective impregnation of individual neurons allowed detailed assessment of neuronal morphology with minimal background staining.

Representative neuronal reconstruction using Neurolucida software further demonstrated the quality of neuronal impregnation obtained with the modified rapid

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Golgi staining technique. The clear delineation of the neuronal soma and dendritic arborization permitted accurate digital reconstruction of individual neurons. Although quantitative morphometric analysis was beyond the scope of the present study, these findings indicate that the staining technique is suitable for future investigations involving dendritic morphometry, neuronal reconstruction, and Sholl analysis.

Golgi staining remains one of the most valuable neurohistological techniques because it selectively impregnates a limited number of neurons, enabling complete visualization of neuronal architecture without overlap [7,8]. Since the modified rapid Golgi staining protocol has been reported previously by our group [9], the present study demonstrates its application for detailed morphological evaluation of the CA1 region rather than methodological validation.

The present study was designed as a qualitative morphological investigation and therefore did not include quantitative morphometric analysis. Future studies incorporating Neurolucida-based morphometry, three-dimensional neuronal reconstruction, and experimental models may provide further insight into structural alterations associated with ageing, neurodegenerative disorders, and other neurological conditions [10,13].

Overall, the present findings provide baseline morphological data on the normal CA1 region of the adult Wistar rat hippocampus. The study demonstrates that the modified rapid Golgi staining technique is a reliable method for qualitative neuroanatomical evaluation and provides a suitable platform for future quantitative studies of hippocampal neuronal architecture.

Conclusion

The present study provides a qualitative characterization of the normal cytoarchitecture and neuronal morphology of the CA1 region of the adult Wistar rat hippocampus using a modified rapid Golgi staining technique. The study clearly demonstrated the laminar organization of the CA1 region and the characteristic morphology of pyramidal neurons with well-preserved dendritic arborization. In addition, the high-quality neuronal impregnation permitted digital reconstruction of representative neurons, indicating that the technique is suitable for both qualitative neuroanatomical evaluation and future quantitative morphometric studies. These findings provide baseline morphological data for investigations of hippocampal development, ageing, neuroplasticity, and neurological disorders.

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