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Intramuscular innervation of rectus extraocular muscles revisited histologically

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ABSTRACT

Aim: This study aims to explore the intramuscular innervation patterns of rectus extraocular muscles (REOMs) comprehensively. By supplementing existing knowledge using histological techniques to enrich current research findings, we endeavor to provide a holistic perspective on the complexities of REOM innervation.

Material and methods: Ten REOMs (superior, inferior, medial, and lateral) were collected from adult human cadavers of both sexes. All samples were carefully dissected to preserve muscle integrity and architecture and proceeded using H&E staining.

Results: Within each REOM, the intramuscular innervation is characterized by a dense network of motor sub-branches. Muscular sub-branches are distributed evenly within each REOM's mid-length cross-section.

Conclusions: In conclusion, the intramuscular innervation of the rectus extraocular muscles is a highly complex and organized system crucial for precisely controlling eyeball movements.

1. Introduction

Understanding the innervation of the rectus extraocular muscles (REOMs) is pivotal in elucidating their precise motor control within the remarkably intricate human visual system [1,2]. With their precision, these muscles play a crucial role in directing the gaze by coordinating precise movements of the eyeballs [3]. Their ability to swiftly adjust the position of the eyes ensures optimal visual acuity and depth perception, making them indispensable for daily tasks ranging from reading to driving.

The innervation of REOMs involves a sophisticated network of cranial nerves and neuromuscular junctions orchestrating their movements with remarkable accuracy [4]. Each rectus muscle receives specific motor innervation from the oculomotor nerve (CN III) and the trochlear nerve (CN IV), ensuring coordinated action to achieve smooth, binocular vision [5]. The abducens nerve (CN VI) innervates the lateral rectus muscle, enabling abduction of the eyeball. In contrast, the oculomotor nerve innervates the remaining medial, superior, and inferior rectus muscles to control adduction, elevation, and depression movements, respectively [6].

Beyond their motor functions, the REOMs also contribute significantly to maintaining ocular stability and alignment [7]. Dysfunction in their innervation can lead to various clinical conditions such as strabismus (ocular misalignment), diplopia (double vision), and impaired eyeball movements, profoundly affecting visual perception and quality of life [8].

In recent years, advancements in neuroanatomical imaging techniques and neurophysiological studies have provided more profound insights into the intricate neural circuitry governing REOM innervation [9]. These insights enhance our understanding of normal ocular motor function and pave the way for innovative therapeutic strategies to treat neurogenic disorders affecting eyeball movement control [10].

This study aims to explore the intramuscular innervation patterns of REOMs comprehensively. By supplementing existing knowledge using histological techniques to enrich current research findings, we endeavor to provide a holistic perspective on the complexities of REOM innervation. A thorough understanding of REOMs' innervation pattern potentially promises to improve treatment modalities and enhance overall patient outcomes in ophthalmology and neurology.

2. Material and methods

Sample Collection: Ten REOMs (superior, inferior, medial, and lateral) were collected from adult human cadavers of both sexes. All samples were carefully dissected to preserve muscle integrity and architecture.

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Histological Processing: Each muscle sample was fixed in 10 % buffered formalin and washed in phosphate-buffered saline (PBS). The samples underwent a thorough dehydration process using increased ethanol solutions and embedding in paraffin wax. Serial sections of 7 μm thickness were obtained using a rotary microtome and mounted on glass slides, ensuring the accuracy of our results.

Hematoxylin and Eosin Staining: Sections were deparaffinized in xylene, rehydrated through graded ethanol series, and stained with Hematoxylin and Eosin (H&E) following routine protocols [11]. H&E staining provides sufficient contrast, which is necessary for morphological assessment of the muscle's cross-section.

Microscopic Examination: Stained sections were examined under a light microscope (Olympus BX-52) with a digital camera. Images were captured at 2x magnification and digitally reconstructed to show the entire muscles cross-section.

Data Analysis: Qualitative assessments were made for the organization of intramuscular nerves' sub-branches distribution among muscle fiber bundles and the general arrangement of intramuscular nerves at the level of the terminal plexus (at the level of each muscle's mid-length).

This methodology ensured consistent and systematic examination of the histological characteristics of extraocular rectus muscles using H&E staining, providing foundational data for further anatomical studies.

3. Results

The rectus extraocular muscles, comprising the superior rectus, inferior rectus, medial rectus, and lateral rectus, are innervated by the cranial nerves, specifically the oculomotor nerve (cranial nerve III) and the abducens nerve (cranial nerve VI). Intramuscular innervation of these muscles forms a complex and highly specialized system, ensuring the fine control necessary for accurate eyeball movement.

3.1. Superior rectus muscle

The superior rectus muscle elevates the eyeball and is innervated by the superior division of the oculomotor nerve. The nerve fibers enter the muscle at its proximal end (Fig. 1a), near the origin, and branch out gradually into smaller and smaller subdivisions, which are distributed evenly between the muscle bundles halfway along the muscle (Fig. 1b and c). The terminal plexus with endplates innervate individual muscle fibers, providing precise control over its contraction.

3.2. Inferior rectus muscle

The inferior rectus muscle, responsible for depressing the eyeball, is also innervated by the inferior division of the oculomotor nerve. Like the superior rectus, the nerve fibers penetrate the muscle near its origin and distribute evenly in its mid-length, ensuring uniform innervation of each muscle's part (Fig. 2).

3.3. Medial rectus muscle

The medial rectus muscle, which adducts the eyeball (moves it medially), receives its innervation from the inferior division of the oculomotor nerve. The innervation pattern involves multiple muscular branches entering the medial rectus muscle at numerous points of its lateral surface. Muscular sub-branches in the medial rectus muscle are also distributed evenly within its mid-length cross-section (Fig. 3), facilitating fine-tuned muscle action adjustments.

3.4. Lateral rectus muscle

The abducens nerve innervates the lateral rectus muscle, which abducts the eyeball. The abducens nerve enters the muscle at its medial aspect (Fig. 4a). It branches out gradually into subsequent muscular sub-branches to cover the entire muscle's cross-section, ensuring effective activation of each part (Fig. 4b and c).

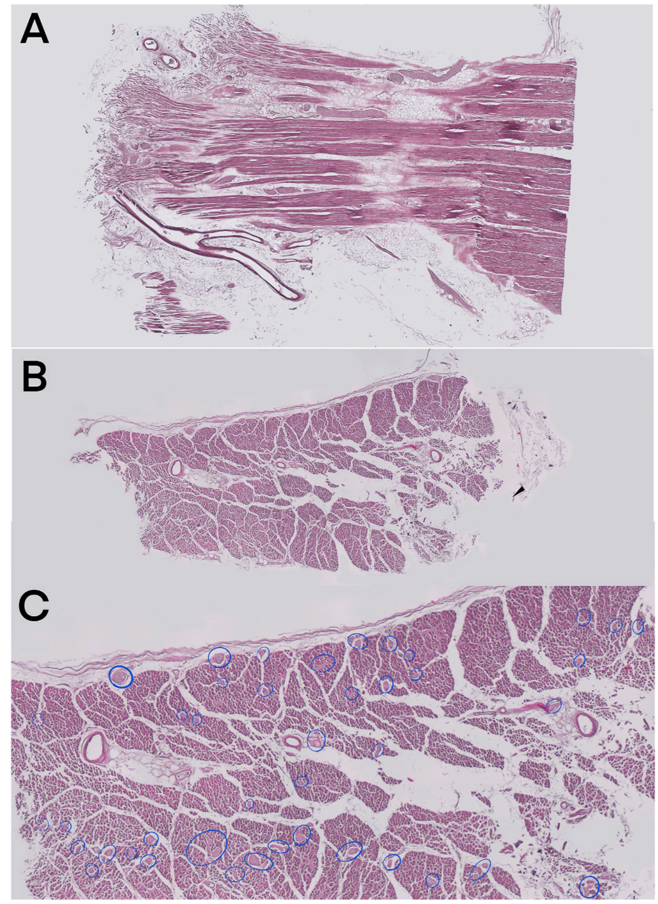

Fig. 1. Histological specimens of the superior rectus muscle. H&E staining. A. Section perpendicular to the inferior surface of the muscle. B. Cross-section halfway along the muscle. C. Magnification of Figure B. Blue circles indicate clusters of muscular sub-branches.

3.5. Intramuscular nerve endings summary

Within each of the REOMs, the intramuscular innervation is characterized by a dense network of motor sub-branches. The dense end even distribution of these motor sub-branches seems to be critical for the smooth and precise movements of the eyeball. The terminal sub-branches form the terminal plexus, where endplates are located, and nerve impulses are transmitted to the muscle fibers, triggering contraction.

3.6. Additional observations

On histological preparations, small blood vessels in the mid-length cross-section formed a less dense system.

4. Discussion

4.1. Anatomical overview

The REOMs, encompassing the superior, inferior, medial, and lateral rectus muscles, play a pivotal role in eyeball movement. Specific cranial nerves meticulously innervate each muscle: the oculomotor nerve (cranial nerve III) innervates the superior, inferior, and medial rectus muscles, while the abducens nerve (cranial nerve VI) innervates the lateral rectus muscle. Understanding the anatomical distribution of

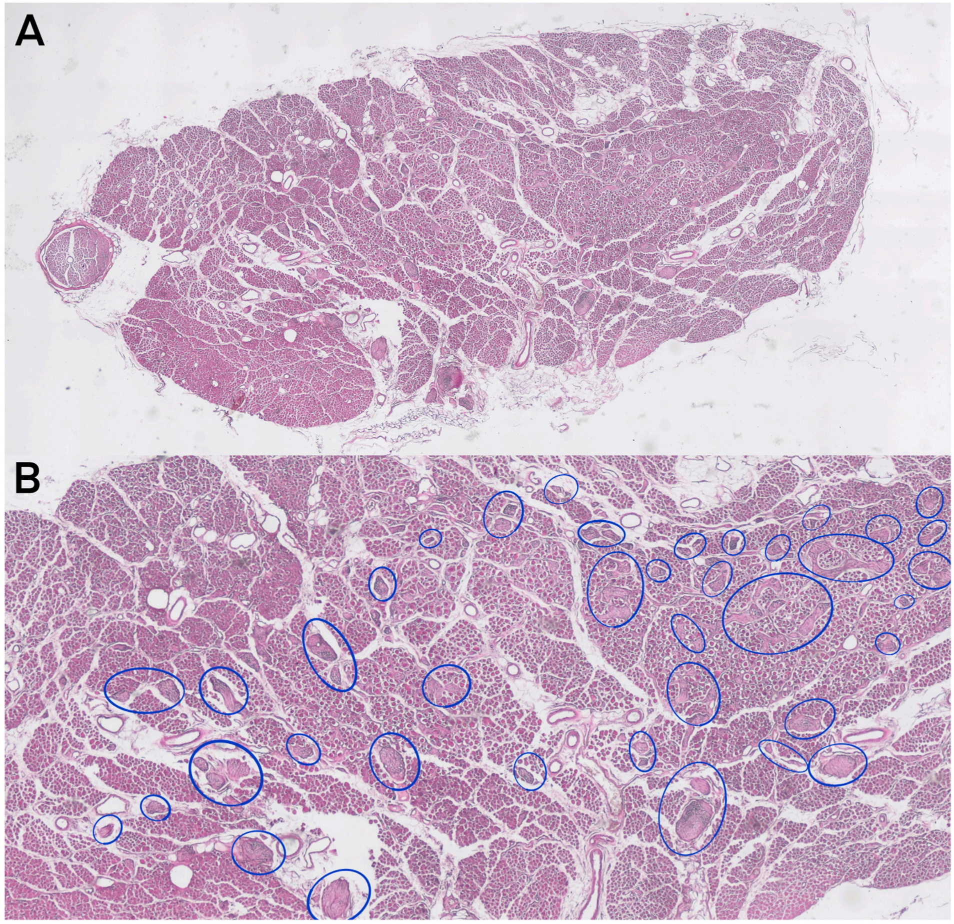

Fig. 2. Histological specimens of the inferior rectus muscle. H&E staining. A. Cross-section halfway along the muscle. B. Magnification of Figure A. Blue circles indicate clusters of muscular sub-branches.

these nerves within the muscles is critical for both physiological comprehension and clinical applications.

4.2. Intramuscular innervation

Intramuscular innervation refers to how nerve fibers penetrate and distribute within the muscle tissue, forming a network that enables precise muscle control. Recent studies, such as those by Adade and Das [12], have delved into the selective innervation patterns within extraocular muscle compartments. Their findings highlight the complexity and specificity of these patterns, suggesting a highly organized and compartmentalized structure that facilitates refined motor control. A key focus in these investigations has been the lateral rectus muscle. Some recent research [13] utilized Sihler's staining technique to map the intramuscular innervation of this muscle. This technique, which renders tissues transparent while staining nerve fibers, has provided unprecedented clarity in visualizing the intricate branching of the abducens nerve within the lateral rectus. The staining results revealed multiple nerve entry points and an extensive distribution network, underscoring the sophisticated control mechanisms governing this muscle. One of the most recent studies [14] comprehensively analyzes the intramuscular innervation of the medial rectus muscle, revealing significant variations and detailed morphology. The oculomotor nerve fibers enter the medial rectus muscle at multiple points, typically near the proximal third. Once inside, these nerve fibers branch extensively, forming a dense network that innervates the muscle fibers. The branching patterns observed in the cited study suggest that the medial rectus muscle's innervation is designed to ensure uniform activation across the muscle, preventing uneven contractions that could disrupt coordinated eyeball movements [14].

4.3. Sihler's stain results

Sihler's stain has proven invaluable in studying the intramuscular innervation of extraocular muscles. By applying this technique, researchers have observed the detailed nerve branching patterns previously obscured in traditional dissection methods. For instance, Haladaj et al. [13] demonstrated that the lateral rectus muscle receives nerve inputs at several locations along its length, with fibers spreading fan-like. This distribution is crucial for the muscle's ability to execute precise lateral eyeball movements.

Using Sihler's staining technique, which makes the tissue transparent while selectively staining the nerves, the researchers were able to visualize the complex innervation patterns within the medial rectus muscle [14]. The staining revealed that the nerve fibers spread out in a fan-like distribution after entering the muscle, covering a wide area and ensuring comprehensive innervation. The detailed mapping provided by Sihler's stain highlights the presence of both primary and secondary branches, with secondary branches further dividing to innervate smaller muscle fiber groups. This arrangement allows precise control over the muscle's contractions, essential for the fine movements required during tasks such as reading or tracking moving objects.

The superior and inferior rectus muscles exhibit similarly intricate innervation patterns, as da Silva Costa et al. [15] reported. Their research indicated that these muscles also have multiple nerve entry points, with a complex network of fibers ensuring precise control. Superior and inferior rectus muscles share a common pattern of intramuscular nerve subbranches' distribution, where characteristic

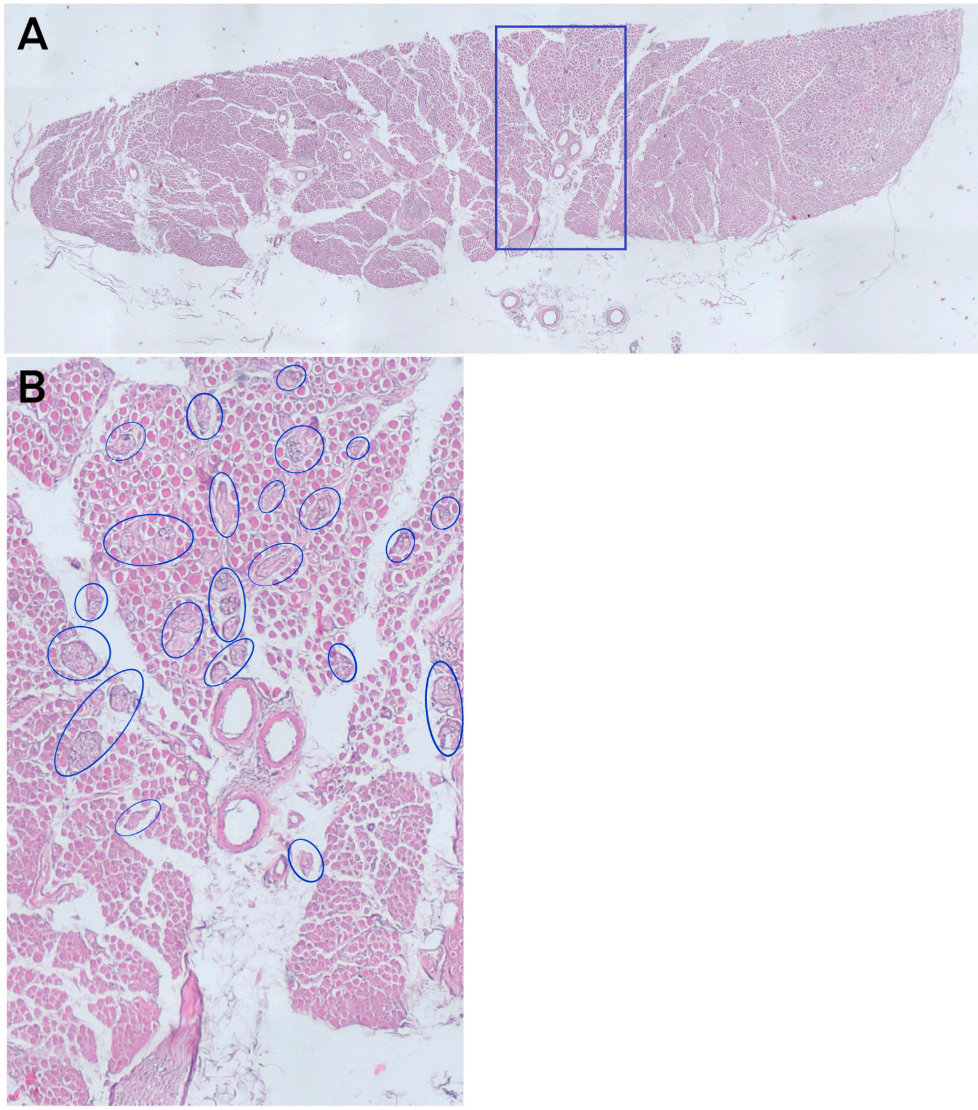

Fig. 3. Histological specimens of the medial rectus muscle. H&E staining. A. Cross-section halfway along the muscle. B. Magnification of Figure A. In Figure A, the magnified area is marked in a blue rectangle. In Figure B, blue circles indicate numerous muscular sub-branches scattered between bundles of muscle fibers.

Y-shaped ramifications form the terminal nerve plexus located near half of the muscles' length [16]. This nonclassical innervation pattern highlights the evolutionary adaptations that allow for the fine-tuned movements necessary for vision [15].

4.4. Clinical implications

Understanding the detailed intramuscular innervation patterns of the rectus muscles has significant clinical implications, particularly in strabismus surgery. Strabismus, characterized by the misalignment of the eyes, often requires surgical intervention to adjust the tension and position of the extraocular muscles. Insights gained from studies using Sihler's stain, such as those by Haladaj et al. [13,14,16] and Nam et al. [17], provide surgeons with critical information on where to target their interventions to achieve the desired outcomes without compromising the overall function of the muscles. Moreover, the compartmentalization of muscle function, as discussed by Demer [18], suggests that surgical techniques could be refined to address specific compartments within a muscle, leading to more precise corrections of ocular misalignments. This compartmental approach could potentially reduce postoperative complications and improve the effectiveness of strabismus surgeries.

4.5. Study limitations

The main limitation of this study is its qualitative nature. Future studies may include accurate determination of the distribution density of motor sub-branches in specific muscle regions and three-dimensional reconstructions. Comparing vessel and nerve densities in different areas of the muscle would also be a valuable contribution. Immunohistochemical techniques may also be used to map different types of nerve fibers in the abducens nerve muscular sub-branches. However, the present study summarizes the intramuscular distribution of muscular sub-branches of all four REOMs together, complementing observations from previous works using histological techniques for anatomical research.

5. Conclusions

In conclusion, the intramuscular innervation of the rectus extraocular muscles is a highly complex and organized system crucial for precisely controlling eyeball movements. Histological techniques used in this study provided more profound insights into REOMs innervation's anatomical aspects, revealing detailed nerve branching patterns. These findings enhance our understanding of ocular motor control and have

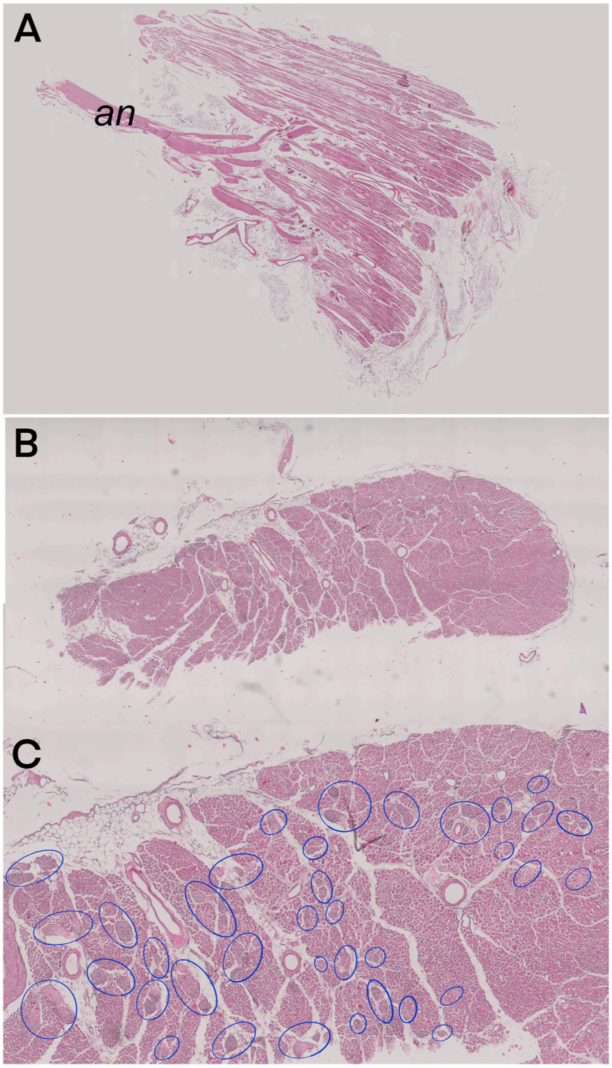

Fig. 4. Histological specimens of the lateral rectus muscle. H&E staining. A. Section perpendicular to the medial surface of the muscle. The bundles of abducens nerve (*an*) form a characteristic tufty branching before penetrating the muscle belly. B. Cross-section halfway along the muscle. C. Magnification of Figure B. Blue circles indicate clusters of muscular sub-branches.

potential implications for the surgical treatment of conditions like strabismus. The ongoing research in this field promises to elucidate the intricacies of extraocular muscle innervation further and improve clinical outcomes for patients with eyeball movement disorders.

Ethical statement

The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research [19].

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CRediT authorship contribution statement

Robert Haładaj: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ivan Varga:** Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tria.2024.100320.

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