Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 2802-2810. OPEN ACCESS

# Innervation and structure of Extra ocular Muscles an experimental study of Goats

**Ali Hassan A. Ali[1,2], Saif Fahad Alkathiri[3], Eyad A A Hijan[3], Alhaytham M Z Almuaddi[3], Faisal S A Binowwais[3], Abdulaziz M M Alshamrani[3], Abdallah S A Alayyaf[3], Abdulmajeed Al Husain[3] and Raghad Muteb Alruwaili[4]**

[1]Anatomy Department, College of Medicine, Prince Sattam Bin Abdulaziz University, Alkharj 11942, **Kingdom of Saudi Arabia**

[2]Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, **Egypt**

[3]College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, **Kingdom of Saudi Arabia**

[4]Faculty of Medicine, Northern Border University, Arar, **Kingdom of Saudi Arabia**

***Correspondence:** alihassan3750@yahoo.com **Received 26-06-2021, Revised: 29-10-2021, Accepted: 05-11-2021 e-Published: 16-11-2021**

Extraocular muscles are unique in both their cellular organization and constituent muscle fiber types. Aim of the work is to study the structure of the extraocular muscles in goat to recognize the subtypes of muscle fibers and their pattern of motor innervation. This study was carried on the extraocular muscles of goat (the recti and oblique muscles). Ten orbits taken from 5 goats immediately after slaughtering. The stem of oculomotor nerves III, IV and VI were studied for ranges of fiber-size distribution and the sensory pathway. Silver block impregnation to technique was used to study the structure and innervation of muscles. Semithin sections of the nerves III, IV and VI and specimens of muscles were blocked and stained by Toludine blue. By silver impregnation technique, each of the recti and oblique extraocular muscles are distinguished to two zones of muscle fibers: orbital zone (outer facing the bony orbit) and inner global zone (inner facing the globe). The orbital fibers are smaller than the global and they all stain black. The global fibers are larger and show grades of stain from red, intermediate to pale fibers. Within these zones different subtypes of muscle fibers have been identified varying in color, pattern of motor innervation, in structure, metabolism and contractile properties. We studied different types of muscle fiber found in the extraocular muscles. Control of eyeball movements depends on the complex structure and function of extraocular muscles.

**Keywords: Extraocular Muscles, Innervation, Structure, Goat**

## INTRODUCTION

The extraocular muscles control the external movement of the eye. The four recti muscles originate from the Annulus of Zinn and are approximately 40mm in length. They insert onto the sclera a few millimeters from the limbus. The inferior oblique originates from the orbital floor and inserts on the sclera in the inferotemporal globe. The superior oblique originates in the posterior orbit and travels medially before inserting on the sclera in the superotemporal globe (Haładaj, 2019 Haładaj et al. 2020; Kels. et al. 2015)

The major classification schemes agree on the existence of three to four fiber types in typical skeletal muscle: type (I) slow twitch, fatigue resistant; type (IIA), fast twitch fatigue resistant; type (IIB), fast twitch fatigable and type (IIC),fast twitch intermediate,(Santocildes 2021). Extraocular muscles are unique in both their cellular organization and constituent muscle fiber types, and do not fit the traditional classification of skeletal muscles described above. Early studies identified two fundamental types of muscle fibers in the extra ocular muscles on the basis of their histological appearance (Lee, 2019). Fibers of larger diameter are rapid twitch fibers with a " fibrillenstruktur" having a regular distribution of myofibrils and abundant sarcoplasm. Innervation is by single 'en-plaque' endings (i.e. motor end plates). Other fibers are slow or tonic in action and called ' felderstruktur' with ill-defined myofibrillar arrangement and little sarcoplasm. Their respiratory metabolism is chiefly aerobic and are innervated by diffuse 'en-grappe' endings. More recent and most popular classification of the recti and oblique extraocular muscle fibers was demonstrated by Cunha et al, 2016 and porter et al, 1995 in monkey. This classification can be identified by light microscopic techniques and depends on the localization of muscle fibers - being orbital or global, on the colour of fibers- being dark, pale, red or intermediate; and on the forms of motor innervation -being singly innervated or multiply innervated. These authors classified the subtypes of extraocular fibers in the recti and oblique muscles in monkey to: 1- orbital singly innervated; 2- orbital multiply innervated; 3- global red singly innervated; 4- global intermediate singly innervated; 5- global pale singly innervated and 6- global multiply innervated. The levator palpebrae superioris does not show such zonal layers, and is formed of singly innervated fibers.

The delicate and simultaneous bilateral performance of some muscles in the territory of the cranial nerves has been a matter of argument for long time. Two views have been concerned as regards the fine adjustments of such muscles as those of the larynx and the extraocular muscles:-

The first view (Porter et al. 1995) suggested that -like other skeletal muscles- there must be a feedback mechanism from proprioceptive endings in these muscles:-typical as muscle spindle and Golgi tendon organs- or atypical such as spiral endings or palisade endings. (Turvey and Golden, 2012 Bohlen et al. 2019)

The aim of the present work is to study the structure of the extraocular muscles in goat to recognize the subtypes of muscle fibers and their pattern of motor innervation. The forms of proprioceptive endings in goat - typical and atypical forms and the pathway of their afferent fibers need further study to clarify their role in the feedback mechanism in the performance of these muscles.

## MATERIALS AND METHODS

This study of the structure and innervation of extra ocular muscles was carried on ten orbits taken from 5 goats just after slaughtering. The orbits were exposed and the eye globes with the orbital muscles were extracted in one mass. The whole specimens were put in 10 % formalin for fixation for 10 days. The four recti and the two oblique muscles and supplying nerves were identified and dissected out in due time for further processing.

For silver impregnation by Ranson block technique, each muscle was cleaned from fat and connective tissue and divided to 3 parts, proximal, middle and distal thirds. The latter was taken with the distal tendon of insertion. The parts of the muscle were impregnated with Ranson silver,blocked in paraffin and longitudinal and transverse serial sections 7u thick were cut.

Pieces of some muscles were taken and fixed in gluteraldehyde and osmic acid and blocked in epon for semithin sections stained by Toludine blue.

The stems of the cranial nerves 3, 4 and 6 of the ten orbits were extracted from the fresh specimens, fixed in gluteraldehyde and osmic acid, blocked for semithin sections and stained by Toludine biue.

In modified silver block impregnation technique, we fixed the pieces of muscles in absolute alcohol plus 1 % of ammonia for forty-eight hours and rinse in distilled water. After that, we transferred it to pyridine for twenty-four hours. Then, we washed it in repeated changes of distilled water until the smell of pyridine has gone (at least twenty-four hours). The muscle pieces transferred into 2 % AgNo3 in the dark at 35 for one to three days and rinsed in distilled water. Then washed, dehydrated, cleared and embedded in paraffin. Cut at 10u. Dewax and mount in synthetic resin. (Femi-Akinlosotu, 2019)

## RESULTS

We found the recti and oblique muscles arised from the annulus ring of Zin and were inserted to the globe by membranous tendons. The fibers of these muscles were arranged in discrete fasicles and the connective tissue between them was scanty. Rich blood vessels and nerves found between muscle fibers (Fig.1A).

In **s**ilver impregnated preparations each of the four recti and the two oblique extraocular muscles was divided to two zones. The orbital zone; includes the muscle fibers towards the bony orbit, almost all the muscle fibers of the orbital surface stain black by Ranson silver and are of different thickness. The other zone called global zone. It includes the fibers of the surface of the muscle towards the globe. In silver preparation the fibers were graded in stain from pale, intermediate to red colours. They also show different thickness.

In the superior oblique muscle, the outer orbital surface in more rounded rather than flat and the global zone fibers dips more to the core of the muscle (fig.1B). The nerve supply to each muscle is relatively rich (fig. 2A). In the orbital zone, the muscle fibers (dark fibers) were supplied by thick nerve fibers (4-8u) (fig.2B). In the global zone all the types of muscle fibers (pale, intermediate and red) were supplied by thin nerve fibers (2-4u).

In the orbital fibers, the thick nerve fibers supplied motor endings of the diffuse (en-grappe type) relatively long and with rich arborizations at the neuro-musculer junction (figs.2AandB).

In the global fibers, the thin nerve fiber supplied more localized motor ending of en-plate (en-plaque) type with almost no terminal arborization at the neuro-musculer junction.

The motor band was broad and lied in the middle third of the muscle. According to location, colour of staining by Ranson silver, and according to form of motor innervation, the muscle fibers of the recti and oblique extraocular muscles of goat are divided to six types:

1. Orbital singly innervated muscle fibers (fig.3A).
2. Orbital multiply innervated muscle fibers (fig.3B).
3. Global red singly innervated muscle fibers (fig.4A).
4. Global intermediate singly innervated muscle fibers (fig.4B).
5. Global pale singly innervated fibers (fig.5A).
6. Global multiply innervated fibers (fig.5B) the fibers of this type can be red, intermediate or pale fibers.

Pathway of proprioceptive afferent fibers from extraocular muscles: The segments taken from the trunks of the nerves III, IV and VI close to brain stem contained mylinated fibers ranging from 2 u to 22 u. This range covers the y- efferent motor fibers to the intrafusal dbail muscle fibers (2-4u), the a motor efferent fibers to extrafusal fibers pobrar of extraocular muscles (4-8u) and group II afferents from the muscle spindles (8-12u) and the group I afferents from the muscle spindles and tendon organs(12-22u) (Figs. 7AandB).

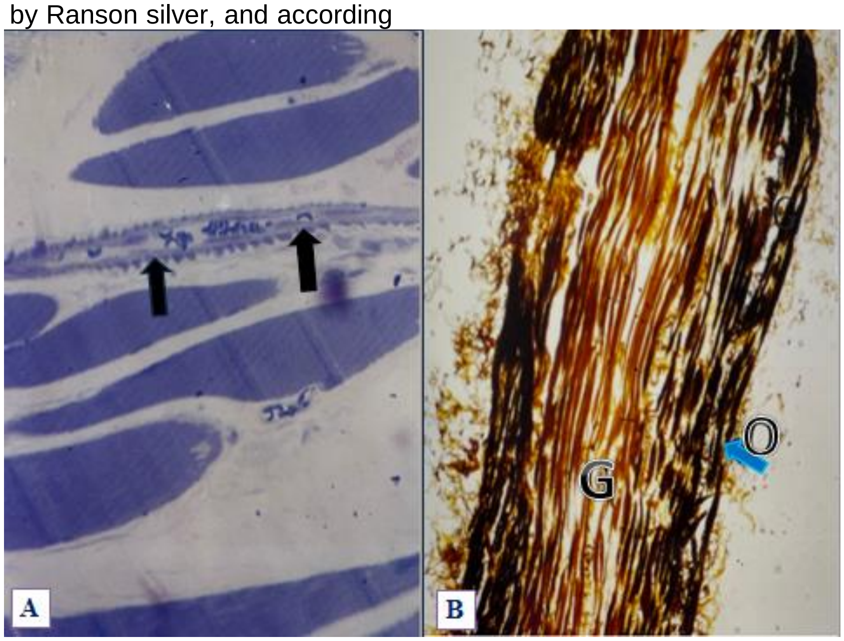


**Figure1: (A) Semithin section of the superior rectus muscle showing blood vessel between the muscle fibers (black arrows). (Toludine blue x 400). (B) Longitudinal section in superior oblique showing the global zone fibers (G) red in the middle and the orbital fibers black (O) on each side. The global fibers dip more to the core of the muscle and are covered by the orbital fibers to greater extent. (Ranson silver x 100)**

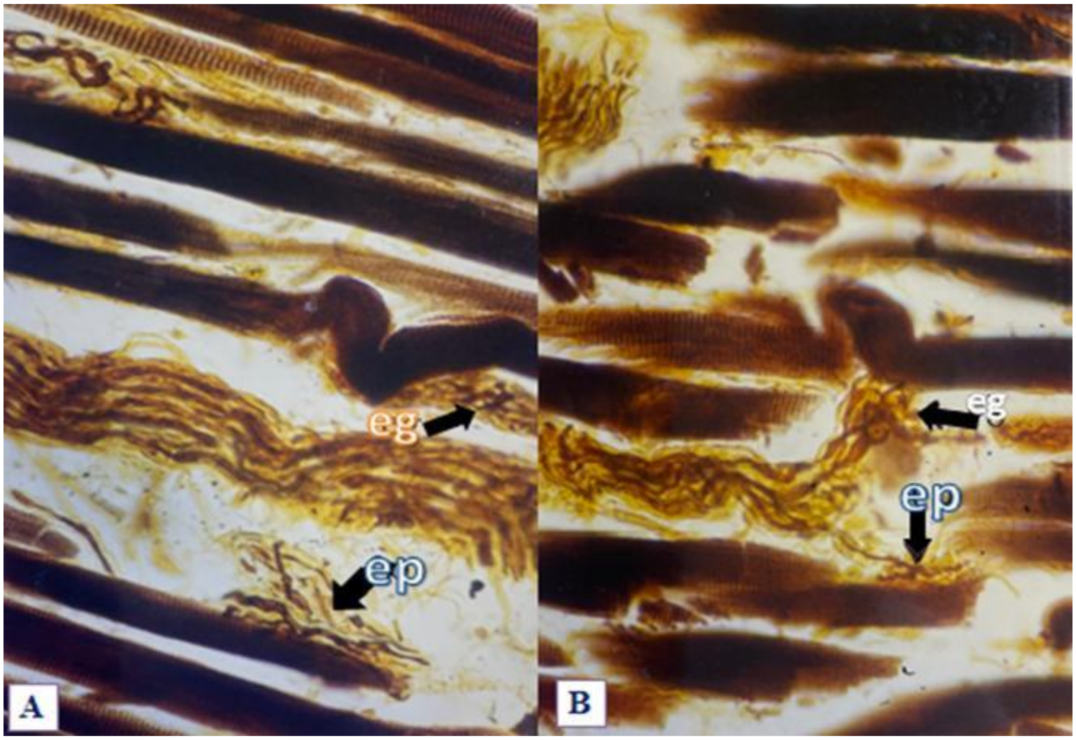


**Figure 2: (A) Section in medial rectus muscle orbital zone, showing dark - muscle fibers supplied by thick nerve fibers ending in en-grappe endings (eg). Another muscle fiber with en-plate ending (ep). (Ranson silver x 400). (B) Medial rectus muscle -orbital zone- showing dark muscle fiber supplied by thick nerve fibers terminating in en-grappe endings (eg →). Another muscle fiber with en-plate ending (ep ). (Ranson silver x 400)**

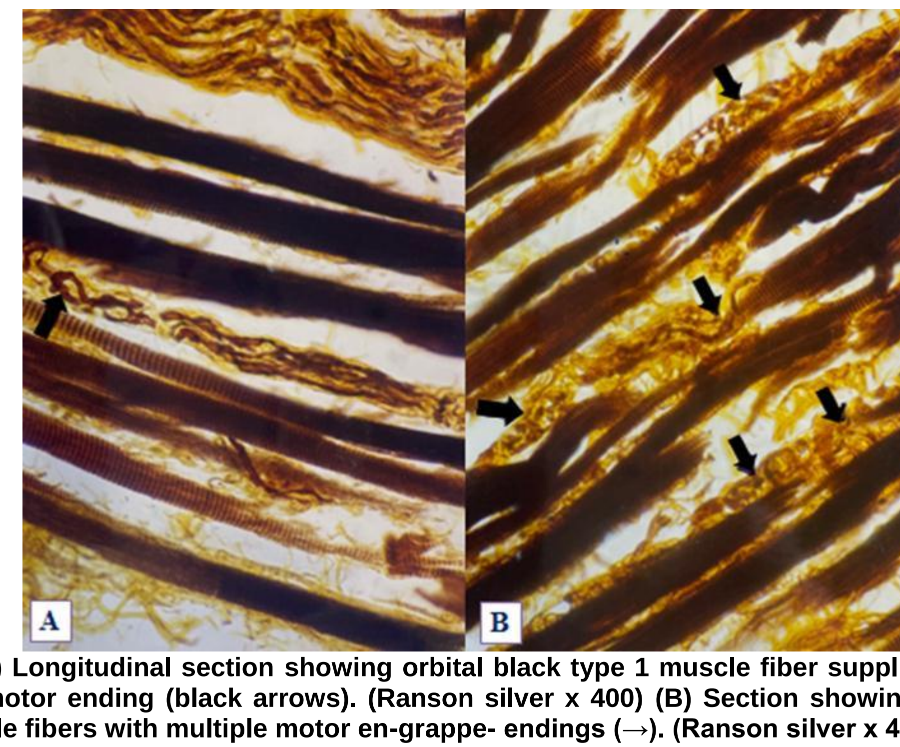


**Figure 3: (A) Longitudinal section showing orbital black type 1 muscle fiber supplied by a single en-grappe motor ending (black arrows). (Ranson silver x 400) (B) Section showing orbital black type 2 muscle fibers with multiple motor en-grappe- endings (→). (Ranson silver x 400)**


**Figure 4: (A) Global red muscle fiber type 3 with single en- plate motor ending (black arrows). (Ranson silver x 400). (B) Global intermediate type 4 muscle fiber with single en-plate- motor ending supplied by thin nerve fibers (black arrows). (Ranson silver x 400)**

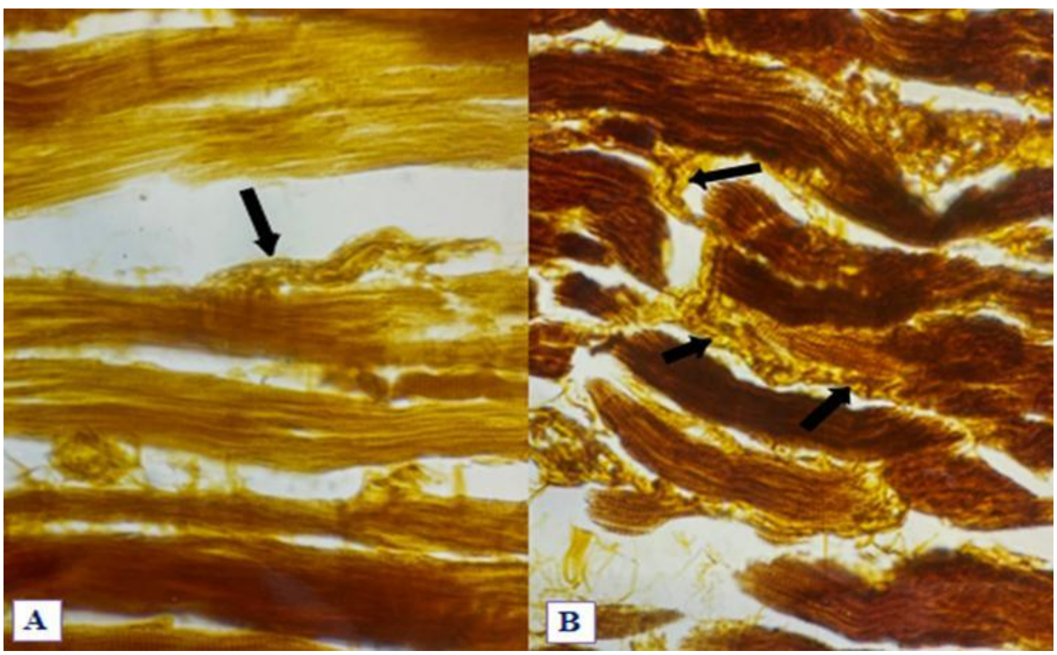


**Figure 5: (A) Global pale type 5 muscle fiber with single en- plate- motor ending supplied by thin nerve fiber (black arrow). (Ranson silver x 400) (B) Global muscle fibers type 6 with multiple en-grappe and en-plate motor endings (black arrows) (Ranson silver x 400)**

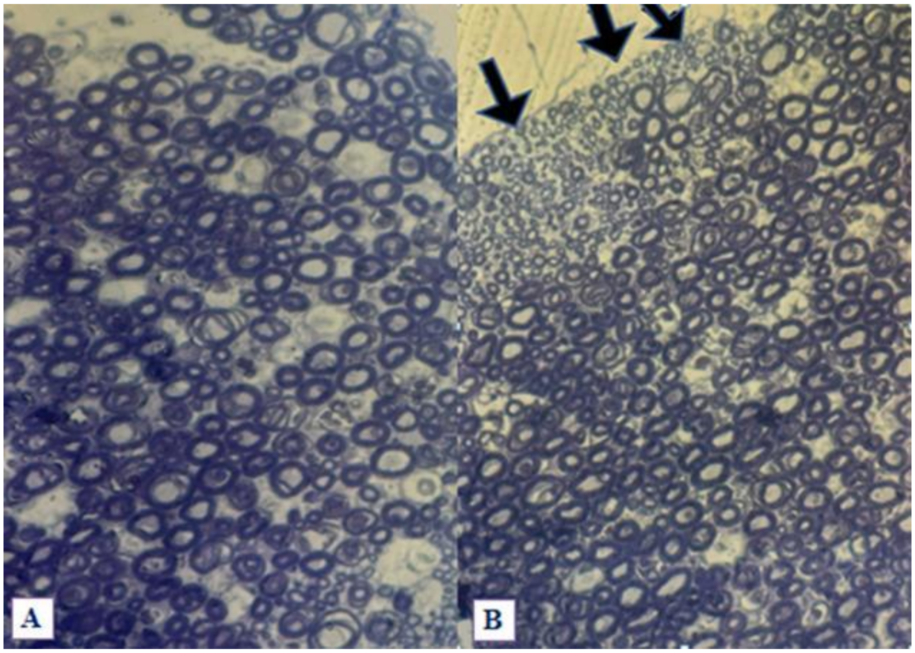


**Figure 6: A) Transverse Section of semi-thin section in the trunk of oculomotor nerve close to the brain stem shows fiber-size components in the nerve. (Toludine blue x 400) B) Semi-thin T.S. section in the trunk of oculomotor nerve showing the area of thinly mylinated parasympathetic fibers (black arrows). (Toludine blue x 400)**

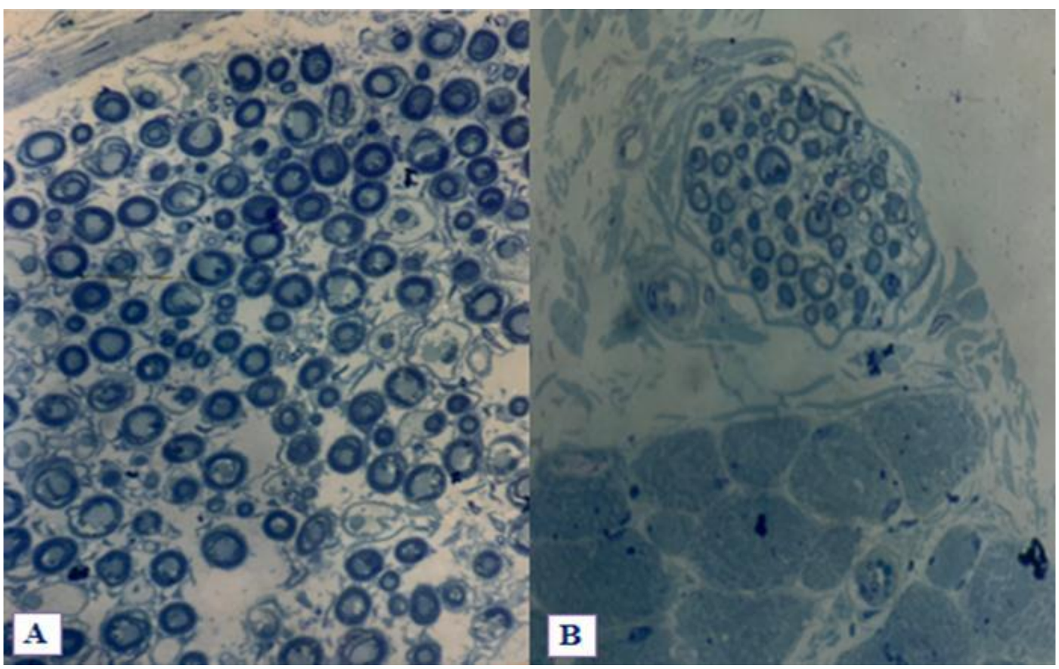


**Figure 7: A) Semi-thin T.S. section in abducent nerve showing the fiber-size distribution in the nerve. (Toludine blue x 400) B) Semi-thin section in the superior oblique muscle showing nerve fibers of all sizes, efferent and afferent in its nerve supply. (Toludine blue x 400)**

## DISCUSSION

In the present work we applied the most popular system of classification of extraocular muscles as described by (Spencer and Porter, 1981). This system can be applied by light microscopy and it depends on three items: location of fibers (orbital or global ), colour of fibers

( dark, intermediate or pale ), and the pattern of innervation ( single or multiple innervation).

We identified six types of fibers in extraocular muscles of goat. The first type is orbital singly innervated fibers. This is the predominant fiber type in the orbital layer of rectus and oblique muscles it stains dark neuromuscular contact is at a single site in form of en-grappe edgings. It is supplied by relatively thick nerve fiber and the ending is in the mid-region of the muscle fibers.

On the basis of oxidative capacity, this fiber type is fast twitch fatigue resistant and corresponds to skeletal type II fiber; (Anthony, et al.1997 and Poter et al. 1995; Paduca and Bruenech, 2018). The second type was orbital multiply innervated fiber. This fiber accounts for the remainder of the orbital layer (20%). This fiber type exhibits the multiple nerve terminals of the –en-grappe type along its length supplied by relatively thick nerve fibers as the orbital type I.

Although these fibers are multiply innervated, they slow a twitch capability near their center and slow contractility proximally and distally (Jacoby,1989; Büttner-Ennever, 2007) associated with structural variation of myocin isoforms along their length. According to Jacoby et al. (1990) the longitudinal differences in fiber type may reflect innervation by motoneurons with differing functional characteristic. However this theory of multineuronal innervation was disapproved by other workers (Anderson et al.2011) who found the multiple plates on muscle fibers were supplied by the same nerve fiber. The third type was global singly innervated fiber. This type forms about one third of the global fibers. The histochemical ultrastructure and myocin heavy chain resembles that of the singly innervated orbital fibers. It carries single en-plate endings at its middle and is supplied by thin nerve fibers. It expresses myocin is a form of type II A of skeletal muscles but its mitochondrial content is high. Functionally these features suggest that this singly innervated global red fiber are fast twitch and highly fatigue resistant (Moncman et al. 2011; Bruenech and Haugen 2015). The forth one was global intermediate singly innervated fibers. This type forms one quarter of the total global fibers. Ultrastructure and ATPase content suggest that it is fast twitch fibers and myocin reactivity suggest resemblance to skeletal type II B fibers (Moncman et al. 2011). The muscle fibers carries a single ending of en-plate type which is supplied by thin nerve fiber.Global pale singly innervated fibers was the fifth type. This fiber type comprises 30% of the global fiber. It resembles type II B skeletal with respect modest levels of oxidative enzymes, high anaerobic metabolic capacity and fast type myocin ATPase over all profile suggest a fast twitch fiber of low fatigue. It carries single en-plate ending supplied by thin nerve fiber. Finally, the last one was global multiply innervated fibers.

These fibers constitute the remaining 10% of the global layer. They carry multiple motor ending of the en-plate type (or small en-grappe endings) along the fibers. These also show staining of palisade endings at the myo-tindinous junctions. Mammalian skeletal muscles (other than the extraocular ) consist almost exclusively of singly innervated twitch fibers that undergo all-or none contraction. In contrast to this scheme for twitch fibers, some adult vertebrate skeletal muscle fiber types are multiply innervated ( i.e. multiple nerve contacts along their length ) and may not propagate action potential. Instead, these fibers undergo slow graded contraction at each synaptic site. The nerve supply of the extraocular muscle is relatively rich which indicates that the motor units (number of muscle fibers for each nerve) are relatively small ( up to 10 muscle fibers) (Lienbacher et al. 2011). The size of motor unit determines the fineness with which muscle force can be increased or decreased. Small motor units allow gradual incrimination of force and usually are associated with fine control; these are most suitable to adjust the force required in movement and fixation of eye ball. On the other hand, large motor units can alter force only in large increments and usually associated with postural and weight-bearing muscles (Brook and Kaiser, 1970 Eberhorn et al. 2006).

## CONCLUSION

We studied different types of muscle fiber found in the extraocular muscles. Control of eyeball movements depends on the complex structure and function of extraocular muscles.The role of proprioception on control of eye movement is still a matter of argument. In particular, proprioception may specify visual direction, modulate visual processing and participate in binocular function particularly during development of the visual sensory system.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEGEMENT

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Alkharj, Saudi Arabia. In addition, we thank those who participated and contributed to the study.

## AUTHOR CONTRIBUTIONS

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Abdallah S. A. Alayyaf and Saif Fahad Alkathiri (https://orcid.org/0000-0003-2785-8536) participated in the study design and wrote the first draft of the manuscript. Eyad A. A. Hijan (https://orcid.org/0000-0003-1056-8102), Alhaytham M. Z. Almuaddi, and Faisal S. A. Binowwais collected and processed the samples. Abdulaziz M. M. Alshamrani (https://orcid.org/0000-0003-1576-9974), Abdulmajeed Al Husain and Raghad Muteb Alruwaili participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.



## REFERENCES

Anderson BC, Daniel ML, Kendall JD, Christiansen SP, McLoon LK. Sustained release of bone morphogenetic protein-4 in adult rabbit extraocular muscle results in decreased force and muscle size: potential for strabismus treatment. Investigative ophthalmology and visual science. 2011 Jun 1;52(7):4021-9.

Anthony J, Ramesh C and Brenda J (1997): Wolff's Anatomy of the Eye and Orbit. Eighth edition, Chapman and Hall Mędical, London, New York, chapter 4, 107-135.

Bohlen MO, Bui K, Stahl JS, May PJ, Warren S. Mouse extraocular muscles and the musculotopic organization of their innervation. The Anatomical Record. 2019 Oct;302(10):1865-85.

Bruenech JR, Haugen IK. How does the structure of extraocular muscles and their nerves affect their function?. Eye. 2015 Feb;29(2):177-83.

Büttner-Ennever JA. Anatomy of the oculomotor system. Neuro-Ophthalmology. 2007;40:1-4.

Cunha CM, Oliveira LE, Kfoury Jr JR. Comparative anatomy of the extraocular muscles in four Myliobatoidei rays (Batoidea, Myliobatiformes). Journal of anatomy. 2016 May;228(5):877-86.

Eberhorn AC, Büttner-Ennever JA, Horn AK. Identification of motoneurons supplying multiply- or singly-innervated extraocular muscle fibers in the rat. Neuroscience. 2006; 137:891–903.

Femi-Akinlosotu OM, Shokunbi MT, Naicker T. Dendritic and synaptic degeneration in pyramidal neurons of the sensorimotor cortex in neonatal mice with kaolin-induced hydrocephalus. Frontiers in neuroanatomy. 2019 Apr 24;13:38.

Haładaj R, Polguj M, Tubbs RS. Comparison of the Superior and Inferior Rectus Muscles in Humans: An Anatomical Study with Notes on Morphology, Anatomical Variations, and Intramuscular Innervation Patterns. BioMed Research International. 2020 Apr 30;2020.

Haładaj R. Normal anatomy and anomalies of the rectus extraocular muscles in human: a review of the recent data and findings. BioMed research international. 2019 Dec 28;2019.

Jacoby J, Chiarandini DJ, Stefani E. Electrical properties and innervation of fibers in the orbital layer of rat extraocular muscles. Journal of neurophysiology. 1989 Jan 1;61(1):116-25.

Jacoby J, Kahn DN, Pavlica MR, Ko K, Breinin GM. Diltiazem reduces the contractility of extraocular muscles in vitro and in vivo. Investigative ophthalmology and visual science. 1990 Mar 1;31(3):569-76.

Kels BD, Grzybowski A, Grant-Kels JM. Human ocular anatomy. Clin Dermatol. 2015 Mar-Apr;33(2):140-6.

Lee Y il. Differences in the constituent fiber types contribute to the intermuscular variation in the timing of the developmental synapse elimination. Scientific reports. 2019 Jun

18;9(1):1-3.
Lienbacher K, Mustari M, Hess B, Büttner-Ennever JA, Horn AK. Is there any sense in the palisade endings of eye muscles? Ann N Y Acad Sci. 2011a; 1233:1–7.
Moncman CL, Andrade ME, Andrade FH. Postnatal changes in the developing rat extraocular muscles. Investigative ophthalmology and visual science. 2011 Jun 1;52(7):3962-9.
Paduca A, Bruenech JR. Neuroanatomical structures in human extraocular muscles and their potential implication in the development of oculomotor disorders. Journal of Pediatric Ophthalmology and Strabismus. 2018 Jan 1;55(1):14-22.
Porter JD, Baker RS, Ragusa RJ, Brueckner JK. Extraocular muscles: basic and clinical aspects of structure and function. Survey of ophthalmology. 1995 May 1;39(6):451-84.
Santocildes G, Viscor G, Pagès T, Ramos-Romero S, Torres JL, Torrella JR. Physiological effects of intermittent passive exposure to hypobaric hypoxia and cold in rats. Frontiers in Physiology. 2021;12.
Spencer RF, Porter JD. Innervation and structure of extraocular muscles in the monkey in comparison to those of the cat. Journal of Comparative Neurology. 1981 Jun 1;198(4):649-65.
Turvey TA, Golden BA. Orbital anatomy for the surgeon. Oral and maxillofacial surgery clinics of North America. 2012 Nov;24(4):525.