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Research Article

Morphological Study of the Intraorbital Muscles *(Musculi bulbi)* in Rat and Guinea Pig

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ABSTRACT

Intraorbital muscles in the human are similar to those in the mammals as regards morphology and function, except for the absence of the retractor bulbi muscle in the human. The aim of this study is to determine the morphological and morphometric features of the eye muscles in the rat and guinea pig used as a model for various studies. Twenty-four bulbus oculi of 12 rats and 32 bulbus oculi of 16 guinea pig were investigated by subgross and histological methods. Certain morphometric data of musculi bulbi were obtained by stereo-microscope. Samples were fixed in 10% formalin for subgross procedures and preserved in Bouin solution for histological methods. The muscular part of the intraorbital muscles in the rat was examined and each muscle was determined to be originated from near the optic foramen and to be attached to sclera close to corneal limbus by tendinous ends. The intraorbital muscles of the guinea pig were relatively weak compared to the body size. The functional part of the intraorbital muscles located around the optic nerve was considerably short and attached to the sclera by the long tendinous ends. Retractor bulbi muscle was small muscle batch located ventrolateral of the optic nerve in rat and substantially weak muscle bundles that envelop the optic nerve in the guinea pig.

Keywords*: Ocular muscles, rat, Guinea pig.*

Rat ve Kobay Ekstraoküler Göz Kaslarının *(Musculi bulbi)* Morfolojik Çalışması

ÖZET

İnsanlarda intraorbital göz kasları, m. retractor bulbi kasının yokluğu dışında memelilere benzer. Bu çalışmanın amacı çeşitli araştırmalara model olarak kullanılan rat ve kobaya ait göz kaslarının morfolojik ve morfometrik özelliklerini ortaya koymaktır. Çalışmada 12 adet rata ait 24 ve 16 adet kobaya ait 32 bulbus oculi subgros ve histolojik metotlarla incelendi. Ekstraoküler göz kaslarına ait morfometrik veriler stereo mikroskop ile elde edildi. Örnekler subgros incelemeler için %10 formalin solüsyonunda tespit edildi ve histolojik metotlar için Bouin solüsyonu kullanıldı. Ratta intraorbital göz kaslarının iyi geliştiği, her kasın foramen (for.) opticum yakınından başlangıç aldığı ve limbus cornea'ya yakın olarak sclera'da tendinöz olarak sonlandığı belirlendi. Kobay göz kaslarının vücut büyüklüğüne oranla oldukça zayıf şekillendiği gözlendi. Nervus (n.) opticus etrafında yer alan intraorbital kasların fonksiyonel kısımları oldukça kısa şekillendiği ve uzun tendo ile sclera'ya tutunduğu belirlendi. *Musculus retractor bulbi* kasının ratta *n. opticus*'un ventrolateral'inde küçük bir kas ve kobay'da *n. opticus*'un etrafında zayıf kas iplikleri halinde belirlendi.

Anahtar Kelimeler: *Göz kasları, kobay, sıçan.*

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Introduction

Eye is responsible for transmitting the image from the environment to the brain. Position of orbita is different among the species. Orbita is located at rostral in the carnivore and omnivore and located at lateral in the herbivore (König and Liebich, 2007). In guinea pig and rats, orbita is located at lateral as in the herbivore. Due to the position of orbita, angle of sight is 340º in guinea pig and 360º in the rat. In contrast to well-developed extraocular muscles (EOMs) in the rat, they do not use their EOMs (O'Malley, 2005).

Intraorbital muscles are derived from prechordal mesoderm of the early embryo. A number of craniofacial muscles are different from trunk muscle as phenotypic properties such as myosin isoforms (Hyttel et al., 2010). Andrade et al. (2005), manifest that EOMs are more contractible than an extremity muscle by having more mitochondria and different transcription programme organization during aerobic exercise.

Extraocular muscles are responsible for voluntary and reflexive movement of eye (McMullen et al., 2009) and these movements are significant for anchoring the image on the retina (Jampolsky, 1953). EOMs are the fastest of the skeletal muscles (Andrade et al., 2005; McMullen et al., 2009). The muscles providing movement for three spatial-axis around the eye are *musculus (m.) rectus dorsalis* and *m. rectus ventralis*; *m. rectus lateralis* and *m. rectus medialis; m. obliquus dorsalis, m. obliquus ventralis* and *m. retractor bulbi* in domestic mammals (King and McLelland, 1985; Von Noorden and Campos, 2002; Felder et al., 2005; Dursun, 2007; König and Liebich, 2007). Therefore, retractor bulbi muscle is absent in humans (Dursun, 2002). Rectus muscles envelop the optic nerve and insert into orbita (Von Noorden and Campos, 2002; König and Liebich, 2007). EOMs must be used concurrent for binocular vision. Because of this, the muscles work as synergetic and antagonist. Medial rectus muscle and lateral rectus muscle work synergic for left or right movement of eye (Von Noorden and Campos, 2002).

Distance to limbus of EOMs' insertion is significant for surgical treatment (Von Noorden and Campos, 2002). Intraocular muscles have studied morphologically in the human (Kocabıyık et al., 2004), rabbit (Gultiken et al., 2006), roe deer (Gültiken et al., 2010), common buzzard (Gültıken et al., 2011), horse, donkey, mule, buffalo, camel, sheep, goat, pig (Hifny and Misk, 1982), dog (Hifny and Misk, 1982; Klećkowska et al., 2003; 2006) and cat (Hifny and Misk, 1982; Klec´kowska and Pospieszny, 2005) so far. Rat and guinea pigs are frequently used for clinical and surgical practice. This study could shed light on clinical and surgical application by morphological data in the rat and the guinea pig.

Materials and Methods

Twelve rats' and sixteen guinea pigs' heads were used in this study. The material used in the study consisted of sacrificed animals which were Ondokuz Mayıs University Experimental Animal and Research Center for various reasons and preserved in the laboratory of the Department of Veterinary Anatomy. No rat and guinea pig were killed for this study. All the eyes were clinically normal and there were no evidence of ocular trauma or infection in any. Thirty-two bulbus oculi from 8 rats and 8 guinea pigs were fixed in 10% formalin solution for subgross examination. Extraocular muscles were dissected after fixation. The width, length, thickness, distance to limbus and optic nerve of each muscle were measured using a Mitutoyo Digimatic Vernier Scale (15 mm) (code no: 500-311, Model CD-15D, serial no: 7175731, Mitutoyo Corporation, Japan), except for retractor bulbi muscle. Insertion tendons of muscles were observed with Olympus SZ-61 TRC stereo-microscope. Other eyes of 4 rats and 8 guinea pigs were fixed in Bouin solution for histological examination. Subsequently, the specimens were subjected to standard histological tissue sequencing procedures and blocked in paraffin. After cutting 5-µm sections from paraffin blocks, Crossmon's triple staining method was

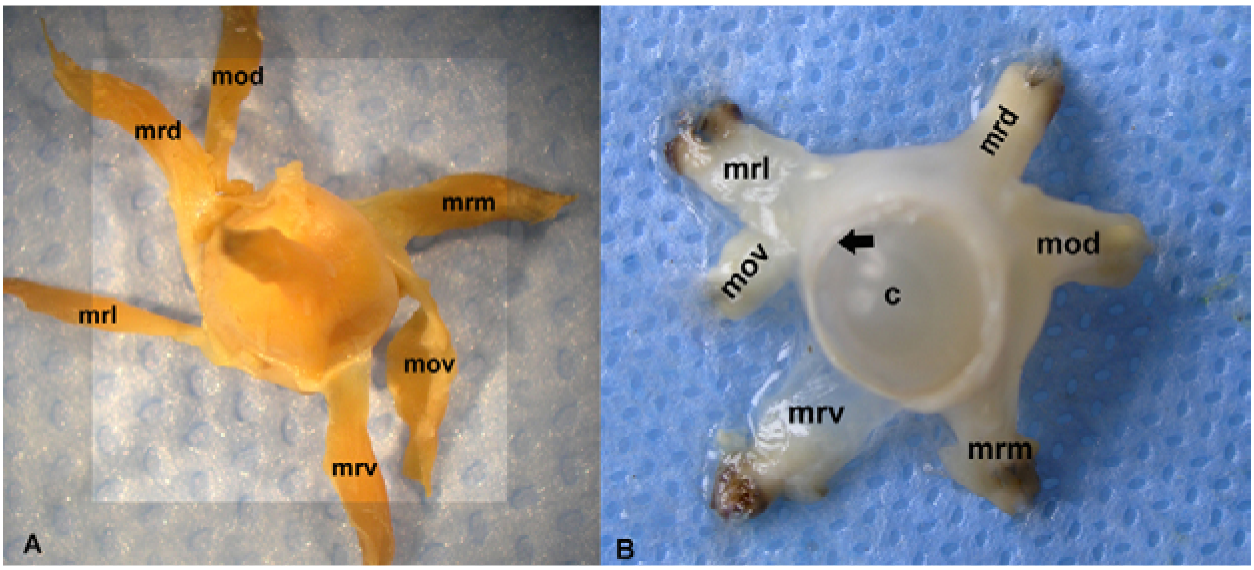

Figure 1. A: caudal aspect of extraocular muscle of the rat, B: rostral aspect of extraocular muscle of the guinea pig, mod: *m. obliquus dorsalis*, mov: *m. obliquus ventralis*, mrd: *m. rectus dorsalis*, mrl: *m. rectus lateralis*, mrv: *m. rectus ventralis*, mrm: *m. rectus medialis*, arrow: *limbus cornea*.

used to examine the histological structure.

Results

Morphologic Examinations

Intraorbital muscles were observed as *m. obliquus dorsalis* (dorsal oblique muscle), *m. obliquus ventralis* (ventral oblique muscle), *m. rectus dorsalis* (dorsal rectus muscle), *m. rectus ventralis* (ventral rectus muscle), *m. rectus lateralis* (lateral rectus muscle), *m. rectus medialis* (medial rectus muscle) and *m. retractor bulbi* (retractor bulbi muscle). Muscular part of rat EOMs were observed relatively well developed. Each muscle inserted near to limbus cornea on sclera. The functional part of the eye muscles that envelops the optic nerve of guinea pigs was relatively short. This part was observed to be originated from the surround of optic foramen. Nevertheless, muscular part terminated and inserted to the sclera with a long tendinous part (Figure 1). The morphometric data of the *musculi bulbi* of the rat and the guinea pig are given in "Table 1 and Table 2" respectively. Eye muscles of the rat determine to be thinner than in the guinea pig. It included numerous lymph follicles in guinea pigs (Figure 2). The Harderian gland of the guinea pig was relatively bigger than in rat. Localization of muscles resembled to each other, but dorsal oblique muscle and medial rectus muscle were thicker and distinct in guinea pig. The rectus muscles were virtually identical in the rat and the guinea pig. The rectus muscles run longitudinally, but the ventral and dorsal oblique muscles located transversely and then vertically.

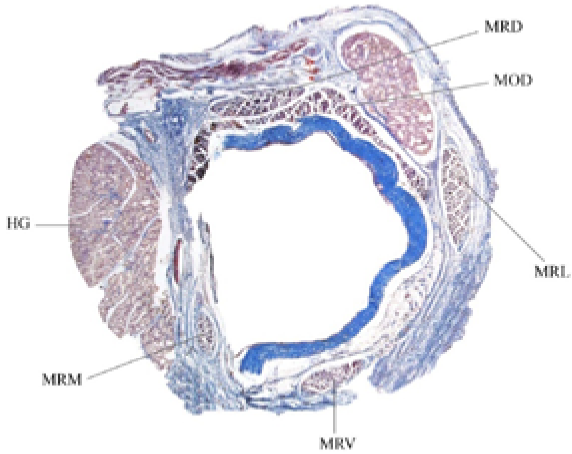

Figure 2. Histological aspect of extraocular muscles of the guinea pig. MOD: *m. obliquus dorsalis*, MRD: *m. rectus dorsalis*, MRL: *m. rectus lateralis*, MRV: *m. rectus ventralis*, MRM*: m. rectus medialis*, HG: *glandula palpebra tertia* (harderian gland).

Table 1. The morphometric data of the bulbus oculi of the rats except for the retractor bulbi muscle (mean ± standard deviation).

Rat (n^*= 16)	Length (mm)	Width (mm)	Thickness (mm)	Distance to optic nerve (mm)
Dorsal oblique	9.32 ± 1.22	1.89 ± 0.33	0.54 ± 0.14	3.91 ± 0.38
Ventral oblique	7.33 ± 1.52	2.23 ± 0.39	0.48 ± 0.18	3.61 ± 0.51
Dorsal rectus	7.96 ± 1.01	2.31 ± 0.41	0.57 ± 0.13	3.59 ± 0.61
Ventral rectus	8.15 ± 1.81	2.23 ± 0.65	0.52 ± 0.09	3.63 ± 0.55
Lateral rectus	8.23 ± 1.22	2.58 ± 0.37	0.59 ± 0.10	3.73 ± 0.54
Medial rectus	8.18 ± 1.18	2.52 ± 0.41	0.57 ± 0.13	3.68 ± 0.59

* gives the total number of left and right bulbus oculi

Table 2. The morphometric data of the bulbus oculi of the guinea pigs except for the retractor bulbi muscle (mean ± standard deviation).

Guinea pig (n^*= 16)	Length (mm)	Width (mm)	Thickness (mm)	Distance to optic nerve (mm)
Dorsal oblique	6.11 ± 1.23	3.04 ± 0.56	1.10 ± 0.16	7.10 ± 0.46
Ventral oblique	5.58 ± 1.00	2.86 ± 0.56	1.03 ± 0.12	6.46 ± 0.68
Dorsal rectus	6.45 ± 0.94	3.02 ± 0.50	1.15 ± 0.19	7.16 ± 0.44
Ventral rectus	6.60 ± 0.73	3.80 ± 0.67	1.18 ± 0.12	6.83 ± 0.62
Lateral rectus	6.44 ± 0.93	3.36 ± 0.55	1.04 ± 0.06	7.24 ± 0.63
Medial rectus	6.16 ± 0.80	3.18 ± 0.58	1.19 ± 0.08	6.21 ± 0.55

* gives the total number of left and right b

M. obliquus dorsalis

M. obliquus dorsalis twisted to under the dorsal rectus muscle over the trochlea in the rat. Additionally, it inserted obliquely to dorsomedial of the dorsal rectus muscle in the guinea pig (Figure 3).

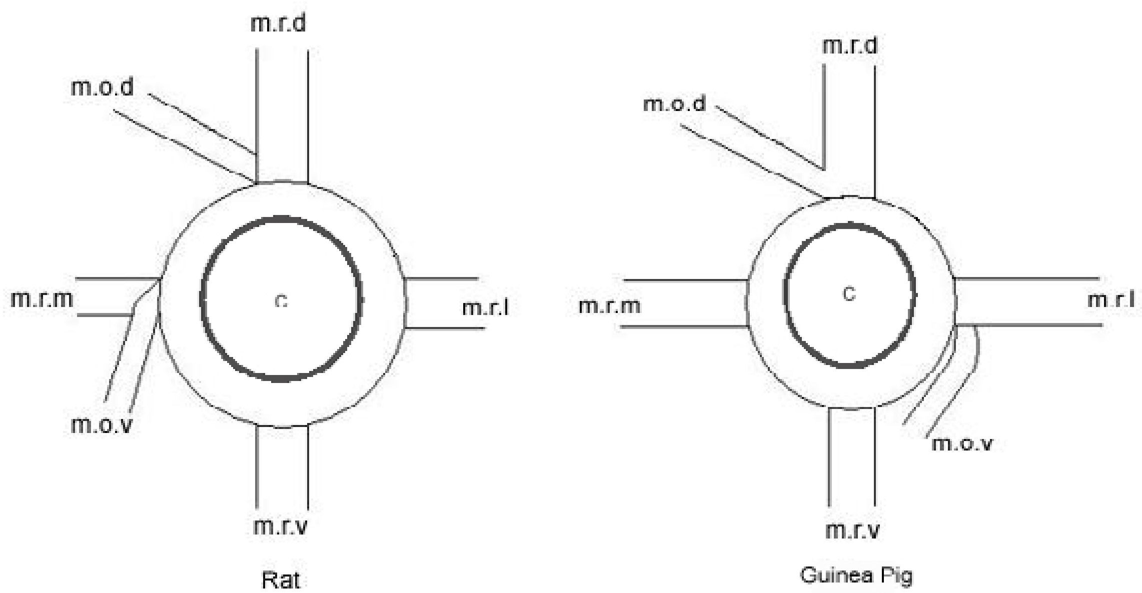

Figure 3. Insertion lines of eye muscles of the rat and guinea pig. c: cornea, m.r.d: *m.rectus dorsalis*, m.r.m: *m. rectus medialis*, m.r.v: *m. rectus ventralis*, m.r.l: *m.rectus lateralis*, m.o.d: *m. obliquus dorsalis*, m.o.v*: m.obliquus ventralis*.

M. obliquus ventralis

Ventral oblique muscle was embedded into fatty tissue in the rat and inserted to the sclera form dorsal of the medial rectus muscle. Insertion line of the ventral oblique muscle was different between the individuals. The muscle was inserted to the ventral of the lateral rectus muscle in the six guinea pigs, and inserted between the lateral rectus muscle and ventral rectus muscle in the one and inserted to ventral of the ventral rectus muscle in the another one. No differences have been detected between the right and left oculus.

M. rectus dorsalis

M. rectus dorsalis muscle inserted separately to sclera on the dorsal of dorsal oblique muscle in the rat and inserted to sclera joining the ventral of dorsal oblique muscle in guinea pig with tendinous part.

M. rectus ventralis

Ventral rectus muscle inserted to the ventral of the bulbus oculi in the rat. It inserted to the sclera on dorsal of ventral oblique muscle in only one guinea pig. In the other guinea pigs, it inserted as in the rat.

M. rectus lateralis

Tendon of the lateral rectus muscle inserted to sclera between the retractor bulbi muscle and ventral rectus muscle in the rat. It was attached to sclera on dorsal of the ventral oblique muscle in the six guinea pigs. The others were not involved in the ventral oblique muscle.

M. rectus medialis

Medial rectus muscle was connected to the sclera with tendinous insertion on the medial of bulbus oculi in both rats and guinea pigs.

M. retractor bulbi

Retractor bulbi muscle was seen as a small muscle on ventrolateral of the optic nerve in the rat. The retractor bulbi muscle has only a few muscle fibers around the optic nerve in the guinea pig.

Discussion

Extraocular muscles are different from the skeletal muscles and have multiple or focally innervated fiber (Pachter, 1983). These muscles are mentioned as having orbital and global layers before attaching to the sclera in rat (Khanna and Porter, 2001; Felder et al., 2005) Orbital and global layers have different nerve endings (Pachter, 1983). Orbital layer attaches to the orbita and global layer attaches to the sclera. They are of importance for visuomotor reflexes (Khanna and Porter, 2001). An external marginal zone was described for the first time in human eye muscles by Wasicky et al. (2000), except for orbital and global layers. Orbital and global layers were determined in dorsal rectus muscle in the present study but they were not seen in the ventral rectus muscle defined by Felder et al. (2005), and in the dorsal oblique muscle defined by Pachter and Colbjörnsen (1983).

There are manifold diseases regarding eye muscles such as Strabismus (Jampolsky, 1953; Lennerstrand, 2007; Schutte et al., 2009; Lai et al., 2012), Graves' disease (Maurits et al., 1990) and Sarcoidosis (Brooks et al., 1997). These muscles have substantial localization and morphometric properties for diagnosis (Maurits et al., 1990; Brooks et al., 1997; Wright, 2009; Lai et al., 2012). Lai et al. (2012) determined that the rectus muscles except for medial rectus muscle in Taiwan are shorter than in western Asian country. This situation might explain the diagnosis of strabismus types and surgical treatment as well as ethnical differentiation. In the present study, intraorbital muscles insert into the sclera closed to the limbus in the rat and in the guinea pig, except for the retractor bulbi muscle. As rectus ventralis muscle attached with muscular part to the sclera and the others attached with tendinous insertion in New Zeland Rabbit (Gultiken et al., 2006), all rectus muscles attached with tendinous insertion in the dog (Klećkowska et al., 2003; 2006), the rat and the guinea pig. Thickness, width and length of the extraorbital muscles are major criteria for the diagnosis like sarcoidosis characterized with granulomas. Sarcoidosis occurs idiopathically and is diagnosed by the imaging techniques such as MR (Brooks et al., 1997). Autoimmune eye diseases, associated with thyroid function, are frequently formed by the mediation of the receptors in orbital fibroblasts and preadipocytes. Most of the patients with opthalmopathy are associated with Graves' disease (Khanna and Porter, 2001; Wall and Lahooti, 2010). The most successful treatment for Graves is surgical (Maurits et al., 1990). Because there has been aggregate lymph follicle, the guinea pig could be more suitable for experimental studies on Graves than the rat. However, concentration and presence of the related receptor must be investigated in the periorbital connective tissue. In our study, the harderian gland was observed in the medial part of the eye, but Demircioğlu et al. (2016), stated that the harderian gland is located on the caudal part of the guinea pig eye.

Retractor bulbi muscle envelops the optic nerve in mammals (Dursun, 2002; Klećkowska et al., 2003; 2006; Gultiken et al., 2006), except in humans (Kocabıyık et al., 2004; Woodburne and Burkel, 1994) and birds (Klec´-kowska-Nawrot et al., 2018). This muscle must be notice in experimental study in the rat and the guinea pig. Also, the insertion line of the ventral oblique muscle varies among animal species (Klec'kowska et al., 2003; Klec'kowska and Pospieszny, 2005; Gultiken et al., 2006; 2010; 2011; Zhou et al., 2011; Klec'kowska-Nawrot et al., 2018). Zhou et al. (2011) determined that the dorsal oblique muscle does not have a trochlea. In our study, dorsal oblique muscle was observed similar to the Zhou et Zhou et al. (2011). However, it was different from the findings of Zhou et al. (2011) that described for guinea pig with the single medial rectus muscle and lateral rectus muscle. The placement of the muscles in the guinea pig was similar to that of the New Zealand rabbit's intraocular muscles (Gultiken et al., 2006). It is mentioned that the lateral rectus muscle is absent in the guinea pig, and the ventral oblique muscle inserts below the ventral rectus muscle (Zhou et al., 2011). In this study, the ventral oblique muscle terminated below the ventral rectus muscle in only one guinea pig. In our study, the ventral oblique muscle, which has different termination points, generally inserts under the lateral rectus muscle. It is important to note that this study emphasized that the eye muscles, especially the lateral rectus muscle, medial rectus muscle, and ventral oblique muscle, may have different insertion points in studies to be performed on guinea pig eye muscles. That should be considered, especially when planning studies with guinea pig eye muscles.

Conclusion

Consequently, the morphologic features and connections to the sclera of the insertion tendons in the rat and guinea pig were clarified by morphometric evaluation of the width, length and thickness of intraorbital muscles and the distance to the optic nerve from the point where they attach to the sclera. The results of this study contribute to clinical research since the intraorbital muscle of these species may be used as a model in evaluating new approaches in the treatment of diseases that affect intraorbital muscles.

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Conflict of interest

There is no conflict of interest.

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