Morphological Study of Myoneural Interaction between the Levator Labii Superioris Muscle and its Motor Nerve in Rats

Estudio Morfológico de la Interacción Mioneural entre el Músculo Elevador del Labio Superior y su Nervio Motor en Ratas

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SUMMARY: The progress of science in search of new techniques of the nerve regeneration and the functional repair in reinnervated muscle has been the target of many researchers around the world. Consequently, nerves and muscles in different body segments asked for more enlightenment of their morphology, their interrelation with other anatomic structures and their peculiarities. One of the most significant areas that need deeper studies is the region of the head and neck, since they are often affected by important pathologies. In order to offer the researcher's community a morphological myoneural interaction model, this study elected the levator labii superioris muscle and its motor nerve, the buccal branch of the facial nerve (VII pair) not only for its special characteristics, but also its value on the facial expression. The rat was chosen for this investigation for being easy to obtain, to keep, to manipulate and to compare this experiment with many others studies previously published. The techniques used were Mesoscopic (dissection), histoenzymologic and morphometric ones. In the results the muscle proved to have a predominance of fast twitch fibers (FG and FOG) and superficial location, with a proximal bone and a distal cutaneous insertion. Its motor nerve, the buccal branch of the facial nerve (VII pair), breaks through the muscle belly into its deep face, and comprised a heterogeneous group of myelinic nerve fibers disposed in a regular form in all fascicle. Near the motor point, the nerve showed to be composed of two fascicles with different sizes. Due to the small nerve dimensions, the nerve fibers have a smaller diameter if compared to the motor nerve of pectineus muscle of the cat. Further studies with neural tracers have already had a start in order to provide more information about the distribution and the architecture of these fibers.

KEY WORDS: Nerve; Morphology; Morphometry; Levator labii superioris muscle; Rat.

INTRODUCTION

Peripheral nerves and skeletal striated muscles are often the target of traumatic lesions (Millesi, 2000). According to a study performed in USA, each year, 50 thousand people suffer traumatic lesions in peripheral nerves (Evans, 2001). The severity of the nerve damage may vary, to the extent of the traumatic lesion. The nerve injuries can be classified in five categories (Sunderland, 1978), from a simple compression to a total nerve transection.

In the last 25 years, surgical techniques to recover nerves and muscles have disclosed significant advances, and have reached an optimal technical refinement (Rodrigues & Schmalbruch, 1995; Lundborg, 2000; Millesi; Strauch, 2000; Meek & Coert, 2002; Dvali & Mackinnon, 2003). This fact

led to many researchers to detail factors, mechanisms and techniques, that allow the restoration of these structures (Crisci & Ferreira, 2002).

In spite of many improvements on this area of investigation, a complete recovery of all sensory functions in an adult patient is still rare after nerve repair (Lundborg). This fact motivated us to offer the scientific community, a morphological myoneural interaction model, with morphological aspects, like the formation, the estimated number of nerve and muscle fibers, the area of these fibers, the types of muscle fibers, and other aspects. This model intends to offer the researchers a paradigm of muscle-nerve interaction, with specific patterns, accessible to professionals,

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which can be used in many different experimental procedures on this research line.

The region of the head and neck is one of the most significant areas that need deeper studies because it is often affected by important pathologies. Studies had investigated the structure of the facial nerve and its injuries, which can be caused by traumatic lesions, iatrogenic conditions or oncology principles. The complications, which are extremely unpleasant, and have serious consequences, not rarely, come to interfere in the patient's facial identity. Therefore, attempts to reestablish the function of the facial nerve, have long been published in the literature (Raldi *et al.*, 2002). Thus, we decided to choose as the aim of this study the levator labii superioris muscle and its motor nerve, a branch of the facial nerve (VII pair of cranial nerves), not only for its special characteristics, but also for its value on the facial expression. The embryological origin of this muscle makes this study a challenge, considering that in branchiomic muscles it is more difficult to standardize the technique to identify the different types of muscle fibers, than to do it in somitic muscles.

The levator labii superioris muscle is reported in many studies of macroscopic dissection, especially its intimate relation with the other facial muscles (Ferreira *et al.*, 1997; Spiegel & De Rosa, 2005), and with adjacent structures, like the ductal of parotid gland. Likewise, the branches of facial nerve are anatomic correlated, making it essential to know its real location, distinction of the different branches, and the verification of anastomosis between them (Freitas *et al.*, 1994). The course and the distribution of these branches are relevant to the treatment of facial palsy, and to the protection of facial nerve during surgery (Liu *et al.*, 2007). The buccal branch of facial nerve is described as the responsible for the innervation of other facial muscles, such as the procerus muscle (Hwang *et al.*, 2006) and corrugator supercilii muscle (Caminer *et al.*, 2006).

The reported muscle is so integrated into the facial expression that its electromyographic activity can be measured when a person is induced to imagine and to act according to some situations, or to perform some physical common activities (Cacioppo *et al.*, 1984). This means that there is a relationship between self-report of emotion and physiological expression of emotion (Vrana, 1993).

The importance of this muscle is undeniable in facial reconstructive surgeries, where a flap of a mimic muscle can be used to repair the loss of facial tissue (Ferreira *et al.*). The region of the mid-face represents a challenging area for surgeons who work in reconstructive and aesthetic procedures, due to its details, patterns and anatomic variations

of the muscular anatomy, specifically in the nasolabial area (Pessa *et al.*, 1998). This area also presents many correlated structures with an important connection to the soft tissue.

Finally, based on the information observed in the respective literature, the aim of this study is to evaluate morphologically the constitution of levator labii superioris muscle, especially the morphometry of its fibers and the frequency of occurrence of the different types of fibers. It also aims at evaluating by using morphological criteria, the nerve to the mentioned muscle, its origin and course from de facial nerve and the morphometry of its myelinic fibers. In summary, to offer to the scientific community a model of myoneural interaction, with specific patterns for the development of other studies involving nerve regeneration and functional repair in branchiomic muscles.

MATERIAL AND METHOD

Our work was previously approved by the Research Ethics Committee Involving Animals (FOB/USP/Bauru).

Animals used. Fifteen male Wistar rats, with an average weight of 300g and 90 days old, coming from the Central Vivarium of FOB /USP / Bauru, were utilized. The animals were kept in the Bioterium, in groups of 5 per cage, having access to water and food ad libitum, for three months. The lighting schedule in the housing room was 12 hours light and 12 hours dark, per day.

This study was divided into four phases: dissection, histochemistry, histoenzimology and morphometry.

Dissection. In the first phase five animals were sacrificed with an overdose (70 mg/Kg) of the anesthetic pentobarbital (hypnol), intraperitoneally. Next, tricotomy of the right hemiface was performed with a razor blade. The dissections were carried out with the help of the surgical microscope DF Vasconcelos, and microsurgery instruments, which fostered a better tissue manipulation, since the facial structures of the rat presented small dimensions. The dissection sessions were performed with the purpose to evaluate the dimension and the distribution of the levator labii superioris muscle and its motor branch in the facial composition, and the correlated anatomic structures. At this point, we noted that the buccal branch of facial nerve was responsible for the innervation in the mentioned muscle.

Histochemistry. In the second phase, five other animals were taken, weighted, and sacrificed. The tricotomy of the right hemiface was performed. The surgical microscope was used

to collect the muscle and the motor nerve (a branch of VII pair) in each animal. The muscles were weighed with an electronic scale (Table I). Samples were obtained from the medial portion of the muscles, and from distal segments of the nerves. The harvested slices were left in room temperature for 15 minutes, as recommended by Khan (1977). Then, the samples were fixed in a formol solution (10%). After the fixation time, the samples were washed in tap water for 12 hours and transferred to a container with alcohol 70%. After that, a number of sections of 8 μm thickness were obtained from the muscular samples and submitted to hematoxylin-eosin (HE) staining, in order to analyse the general morphology of the fibers in the muscle and the nerve.

Table I. The average body weight (BW) and the weight of the levator labii superioris muscle (MW).

Animal	BW (g)	MW (g)
1	346	0.026
2	415	0.042
3	324	0.038
4	331	0.032
5	300	0.036
$\bar{X}$	343.2	0.035
S	43.44	0.005

Histoenzymology. In the third phase, five animals were sacrificed, and the entire muscle was removed. After that, a sample of the harvested muscle was collected using a scalpel blade that help perform a cut without lacerate the fibers. These samples were covered with neutral talcum and frozen in n-hexane, cooled to -70°C , in liquid nitrogen for 2 minutes using the method of Chayen *et al.* (1969). The samples were kept in a container filled with liquid nitrogen until the moment of being submitted to histochemical reactions. These frozen samples were cut into 10 μm in thickness in serial cross-sections in microtome cryostat at -20°C . The sections were affixed in preheated slides at 36°C , and maintained in room temperature for 45 minutes, following the method of Eversole & Standish (1970). The sections were submitted to m-ATPase (with acid pre-incubation) and for NADH-TR reactions. Based on these enzymatics activities, the myofibers were classified as FG, FOG and SO, according to Peter *et al.* (1972).

Morphometry. Using the virtual resources of a digital image system coupled to an OLYMPUS B202 microscope, the images of the slides were stained by HE and by the enzymatic activity of NADH-TR (metabolic characteristics) and m-ATPase (contractile characteristics), and then captured to perform the morphometrical analysis.

RESULTS

The average body weight of the rats and the weight of the levator labii superioris muscle were 343.2 g and 0.035 g, respectively (Table I). The estimated total number of muscle fibers in the right hemiface was 244.6 fibers (Table II).

Table II. The estimated total number of the muscle fibers (TF), the average value of muscle fiber area (n=100) (MFA) and the total area of the muscle (TA).

Animal	TF	MFA (μm^2)	TA (mm^2)
1	269	9.29	2.25
2	305	8.24	2.23
3	154	9.16	1.62
4	238	9.07	2.42
5	257	9.85	2.76
$\bar{X}$	244.6	9.12	2.26
S	56.23	0.58	0.41

Fig. 1. Draw illustrating the levator labii superioris muscle and its motor nerve. 1. Skin; 2. Levator labii superioris muscle; 3. Buccal branch of facial nerve; 4. Marginal mandibular branch of the facial nerve; 5. Masseter muscle.

The nerve branch responsible for innervation of the levator labii superioris muscle, is unique in most of its extension, measuring approximately 0.5 cm in length and 0.5 mm in diameter. This branch starts from the buccal branch of facial nerve, and penetrates the mentioned muscle in its distal third, on the deep face, similar to humans. The branch origin can be noticed after the buccal branch of facial nerve crosses the anterior margin of the masseter muscle (Fig. 1). Throughout its course, the nerve is involved by the loose connective tissue, with many adipocytes. The histological cuts of the studied nerve were obtained from anterior region just before its penetration into the muscle, showing the presence of two branches, one of larger diameter. Each nerve fascicle is formed by a large number of myelinic fibers with heterogeneous diameters.

Among the nerve fibers, a moderate concentration of collagen fiber was observed. Externally, all fascicle were wrapped by a connective tissue, the perineurium (Fig. 2). In every animal, 200 myelinic fibers were evaluated in the nerve branch; the diameters of the fibers and thickness of myelin sheath were measured (Table III).

Table III. The diameter of myelinic fibers (DMF) and the thickness of myelin sheath (TMS) (n=100).

Animal	DMF (μm)	TMS (μm)
1	0.09	0.024
2	0.11	0.031
3	0.08	0.028
4	0.10	0.026
5	0.16	0.032
$\bar{X}$	0.108	0.032
S	0.031	0.003

Fig. 2. Photomicrography: the motor nerve to levator labii superioris muscle in rat. Two fascicles can be noted, and myelinic nerve fibers sectioned transversely. Perineurium (arrow head) e myelinated fiber (arrow), HE. 200X.

Using the routine coloration techniques (HE), the muscle presented a normal pattern, with a preserved morphology. The muscle fibers presented a polygonal profile, with a fascicle arrangement, and dyed nucleus eccentrically located (Fig. 3A).

According to histochemical tests reactivity, the levator labii superioris muscle is consisted of a mosaic distribution

of three types of muscle fibers: 1- SO (slow-oxidative) intense reaction and smaller area ; 2- FOG (fast-oxidative-glycolitic): moderate reaction and intermediate area, and 3- FG (fast-glycolitic): weak reaction and larger area (Fig. 3).

The average value of a muscle fiber area was $9.12 \mu\text{m}^2$, while the average value of the total muscle area was 2.26 mm^2 (Table II).

DISCUSSION

The goal of this descriptive study was to evaluate the levator labii superioris muscle and its motor nerve, and to offer the cientific community a model of myoneural interaction, based on specific morphologic parameters.

The nerve to levator labii superioris muscle of the rat is unique; it starts from the buccal branch of facial nerve (VII pair of cranial nerve), and it is correlated with the ductal of parotid gland, as it occurs in humans (Liu *et al.*), and with the zygomatic branch of the facial nerve (Erbil *et al.*, 2007; Saylam *et al.*, 2006). The knowledge of these data provides surgeons with anatomic basis for future surgical strategies (Woltmann *et al.*, 2006), avoiding lesions in the facial nerve region, wich is often prone to injuries that require interventions (Guntinas-Lichius *et al.*, 2007). Important data can also be obtained through clinical examination, using electromyography method (Wolf *et al.*, 2006; Armstrong *et al.*, 2007), as many facial muscles present more than one motor unity. Thus, each modality of physiotherapy used in the treatment of facial palsy (Shafshak, 2006), or even the manual stimulation (Guntinas-Lichius *et al.*), can be better applied, by using the information about the quantity and location of the motor points. It was possible to observe that the motor branch penetrated the muscle rat in its deep face, obeying the frequent disposition of most myoneural interaction.

Although not addressed into depth, we observed that the branch nerve presented two fascicles close to the muscle, one having a larger area if compared to the other one. The arrangement of myelinic fibers inside the nerve fascicles was homogeneous, with no concentration in different regions. Similar results were observed by Rodrigues *et al.* (1994) about the nerve branch to the pectineus muscle of the cat (*Felis domestica*). To analyse the diameters in the myelinic fibers, measurements were taken from their smaller diameter, so as to avoid errors with overestimated values (Mayhew & Sharma, 1984; Dubowitz, 1985). The diameter measurements of the buccal branch of facial nerve, in 200 fibers of each animal, presented values varying between 0.007mm and 0.041mm,

Fig. 3. Photomicrography of fibers in levator labii superioris muscle in rat. A – region near to the fascicle margin, polygonal fibers (*), perimysium (p) and adipocytes (+), HE 100X. B - muscle submitted to NADH-TR reaction, the different types of fibers (SO, FOG e FG), based on metabolic characteristics, 100X. C – muscle submitted to m-ATPase reaction, the different types of fibers (SO, FOG e FG) based on contractile characteristics, 100X.

different from the results found by Schmalbruch (1986), in which the values were between 0.42 mm and 7.65 mm in the tibial and femoral nerve in the rat. This observation is probably related with the frequency of the different fiber types in the levator labii superioris muscle, that has a predominance of white fibers, that is fast twitch fibers (Table IV).

The nerve fibers with larger diameter are classified as alpha fibers, and they innervate the motor units of fast twitch fibers (Asbury & Johnson, 1968; Mastaglia & Walton, 1984). Our estimate results, of the total number of fibers present in the levator labii superioris muscle, 60.2% were FG, 26.6% were FOG and 13.2% were SO. The final results were similar to the values found for Rodrigues *et al.*, in the pectineus muscle of the cat.

Table IV. Percentage of the different types of muscle fibers in levator labii superioris muscle in rats.

<i>Animal</i>	<i>FG (%)</i>	<i>FOG (%)</i>	<i>SO(%)</i>
1	59	23	18
2	61	26	13
3	65	24	11
4	57	34	9
5	59	26	15
$\overline{X}$	60.2	26.6	13.2
S	3.03	4.34	3.5

The diameter of nerve fibers is variable in the course of any nerve (Boyd & Davey, 1968). In the dorsal root, the

diameter is nearly three times greater than close to the motor point (Gilliat, 1966). Hence, we can expect that the nerve fibers near to the motor point in the muscle, have smaller diameters than close to the facial trunk. According to previous studies, the nerve fibers with larger diameter have thicker myelin sheath (Asbury & Johnson; Machado, 1983); and the thickness of nerve fiber is intimately related to nerve conduction speed, and making these measurements important. Considering all these information, we can infer that the levator labii superioris muscle has a predominance of motor units of fast twitch, indicating its intense activity related to the vibrissae movement and the tactile sensory in the rat (Faria *et al.*, 2006). In other animals, like the cat for example, each μm in thickness corresponds to a velocity of 6m/s in a nerve fiber conduction (McPhedran *et al.*, 1965). There is a certain disagreement about the number of myelinated nerve fibers and the function of the nerve, which can be afferent or efferent fibers. Thus, to Rexed & Therman (1948) 60% of myelinated nerve fibers in a determined nerve are motor fibers. On the contrary, to Boyd & Davey (1968) this value is approximately 50%. Roughly, we don't really believe that general values can be applied to all nerves, but each nerve answers to its functional demand, and even that there is a pattern of electrical activation in a muscle

(English, 1984). Further studies with neural tracers, using derived from peroxidase could be more enlightening.

The nerve to the levator labii superioris muscle presented its fibers intertwined with the fibers of the infraorbital nerve, which is a sensitive nerve related to vibrissae movement in the rat (Veronesi *et al.*, 2006) and prone to injuries during surgical procedures (Hu *et al.*, 2007).

In summary, the purpose of this study was to contribute to a better knowledge of the morphology of the levator labii superioris muscle and its motor nerve, along with other research lines that evaluate branchiomeric muscle and cranial nerve. Other projects are being done with the main objective of describing the nerve fibers in the studied muscle and correlate it to its functions. A detailed knowledge of microscopic anatomy of nerves in rats, helps to obtain data which are essential for experimental studies of induced neuropathies. Besides that it provides a better understanding of human pathologies, and the development of safer surgical techniques.

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MELO, C. G. S.; SHINOHARA, A. L.; ANDREO, J. C. & RODRIGUES, A. C. Estudio morfológico de la interacción mioneural entre el músculo elevador del labio superior y su nervio motor en ratas. *Int. J. Morphol.*, 27(3):627-634, 2009.

RESUMEN: El progreso de la ciencia en busca de nuevas técnicas para la regeneración neural y la recuperación funcional de los músculos reinervados, ha atraído el interés de muchos investigadores en todo el mundo. En consecuencia, los músculos y los nervios merecen más aclaraciones sobre su morfología, relaciones anatómicas y particularidades. Entre las áreas que merecen estudios más profundos y detallados, está la región de cabeza y cuello, que es a menudo afectada por enfermedades graves. Con el propósito de ofrecer a la comunidad científica un modelo morfológico de interacción mioneural, se eligió el músculo elevador del labio superior y su nervio motor, la rama bucal del nervio facial (VII par craneal), por sus especiales características y su importancia en la expresión facial. En esta investigación se optó por la rata, por las facilidades de obtención, de manejo y para comparar los datos obtenidos con estudios previos. Se utilizaron técnicas mesoscópicas de disección, histoenzimológicas y morfométricas. Los resultados mostraron un músculo con predominio de fibras de contracción rápida (FG y FOG), ubicación superficial, inserción proximal ósea e inserción distal en la piel. Su nervio motor, la rama bucal del nervio facial, ingresa en el vientre muscular en la cara profunda, y está compuesto por un grupo heterogéneo de fibras nerviosas mielínicas dispuestas de forma regular por todo el fascículo nervioso cerca del punto motor. El nervio es formado por dos fascículos de diferentes tamaños. Debido a las pequeñas dimensiones en la rata, el diámetro de las fibras nerviosas presenta valores reducidos, en comparación con el nervio motor del músculo pectíneo en el gato, por ejemplo. Los datos aportados podrán ser usados como referencia en estudios de regeneración en nervios y músculos. Otros estudios con marcadores neuronales se iniciaron para aclarar la distribución y la estructura de las fibras mencionadas.

PALABRAS CLAVE: Nervio; Morfología; Morfometría; Músculo elevador del labio superior; Ratas.

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