VISUALIZATION OF NERVE DISTRIBUTION OF THE TONGUE IN RABBITS: A PRELIMINARY STUDY

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SUMMARY

Purpose: *This study was designed to investigate the distribution of the nerve supply in the tongue of adult rabbits.* ***Methods:*** *Twelve tongues were carefully dissected and stained by modified Sihler's staining technique.* ***Results:*** *The nerve fibers were observed lying straight at the root of the tongue while they became gradually convoluted towards the tip of the tongue. No significant symmetry was observed between the two sides of the tongue from the view point of the distribution of the nerve fibers. In addition, no anastomoses were observed between the nerve branches of the two sides of the tongue.* ***Conclusion:*** *Such a staining technique is useful not only to determine the anatomical structure in details but also to evaluate the post-operative nerve recovery in surgical researches as well.*

Key Words: *Tongue, Sihler's Staining Technique, Neuromuscular Compartment, Rabbit.*

INTRODUCTION

The tongue is a complex muscular organ with a rich sensory and motor innervation. It moves in a finely coordinated fashion, and provides sensory information for mastication, articulation and swallowing. Information on the distribution of intramuscular nerve fibers within the tongue muscles will enhance the understanding of their anatomy and functions. However, little is known about the innervation pattern of the tongue. Especially for complicated surgical approaches, such as allotransplantation of the hemitongue and associated neurovascular apparatus, detailed knowledge about the innervation pattern of the tongue is necessary (1, 2).

The innervation pattern of an organ can be analyzed by gross or microscopic dissections. However, this has been limited by the inability to trace the nerve fibers from the extra muscular branches to intramuscular terminal branches, as the latter are very fine and invisible even under the dissecting microscope (3-7). Besides, direct dissection would damage muscle fibers and disrupt the normal anatomical relationship between the muscle and the nerve fibers (8,9). For this reason, in order to demonstrate the distribution of the nerve fibers in the tongue, one of the following methods should be used. The first is the three dimensional reconstruction of the innervation pattern of the organ by tracing images of that organ from serial histological sections. The other possibility is in toto staining of the organ with subsequent clearing of the muscles (10).

Thirdly, in the present study, a largely forgotten technique, initially designed by Charles Sihler in the last century (1895) and recently modified by Liem and Van Willigen (1988), was used to investigate the distribution of the intramuscular nerve supply of the tongue muscles in adult rabbits (10,11). In this study the modified Sihler's technique was used to visualize the innervation pattern of the tongue and the results were compared with those of other studies.

MATERIAL AND METHODS

Twelve tongues from adult New Zealand white rabbits weighing 2.5 - 3.5 kg were used for the study. The animals were deeply anesthetized using intravenous sodium pentobarbital (0.4 ml/kg body weight) injection. Tongues were carefully dissected with their primary nerve branches from the lingual nerve as well as with the extra muscular nerve branches. On completion of the surgical procedure, the rabbits were sacrified by overdose sodium pentobarbital injection. The surgical procedure was performed in accordance with international guiding principles for biomedical research involving animals (12).

Modified Sihler Staining Technique: All specimens were stained by modified Sihler's staining technique (7,11,13, 14).

Briefly the tongues were fixed in 10% unneutralized formalin for at least four weeks and then macerated in 3% potassium hydroxide solution for three weeks. During this period, the specimens gradually became white. The well-macerated specimens were then transferred into Sihler's solution I (1 part glacial acetic acid, 1 part glycerin and 6 parts 1% aqueous chloral hydrate) for decalcification for a period from three weeks until they became transparent. The specimens were then taken into Sihler's solution II (1 part stock Ehrlich's acid haematoxylin, 1 part glycerin and 6 parts 1% aqueous chloral hydrate) for staining, for a period not exceeding three weeks. When the specimens were stained deep purple, they were transferred again into Sihler's I solution in order to be purified from the excess stain. This procedure was stopped when the muscles were light purple and the nerve fibers were dark blue. The specimens were put into a weak aqueous solution of lithium carbonate for an hour to darken the colour of the nerves. Then, they were transferred into increasingly graded glycerine (40%, 60%, 80% 24 hours in each grade) with a few thymol crystals for clearing the excess of stain. Succesfully stained specimens had dark blue nerves and translucent non-nervous tissues.

Dissection

The specimens were microdissected in order to demonstrate the anatomical structures. No structures have been removed. All dissections were made with an Olympus SZ - 40 stereo microscope under 6.7 to 20 power magnification. Some tissues were removed in order to follow the nerve pathways into the muscular structures. Dissected specimens were photographed with a camera connected to Olympus SZ - 40 stereomicroscope.

RESULTS

In all specimens, the bodies and tips of the tongues were cleared adequately, while the clearing process was inadequate at the radix because of the large amount of muscular tissue mass in the region. At the end of the study, almost transparent specimens with all their extra- and intra- muscular nerve fibers, stained deep blue or purple, were obtained. Consequently, as the tongue muscles still remained intact, the normal anatomical relationship between the muscles and nerve fibres could be demonstrated clearly (Fig. 1).

When the nerve had been examined, nerve fibers were observed lying straight at the root of the tongue while they gradually became convoluted towards the tip of the tongue (Fig. 2). It was also observed in the cleared specimens that peripheric nerve fibers were branched and became thinner before they terminated. Myelin sheets surrounding the nerve fibers gradually disappeared while the fibers gave branches and became thinner. Branched, thinner nerve fibers endied on an effector cell (Fig. 3). Since the terminal fibers were extremely thin, they were hardly observed in the cleared specimens.

Within the apical part of the tongue some nerve fibers were observed leaving the main bundle lying at the lateral border of the tongue, and crossing the fibrous septum in the midline. However, it was concluded that the innervation of the two sides of the tongue were independent

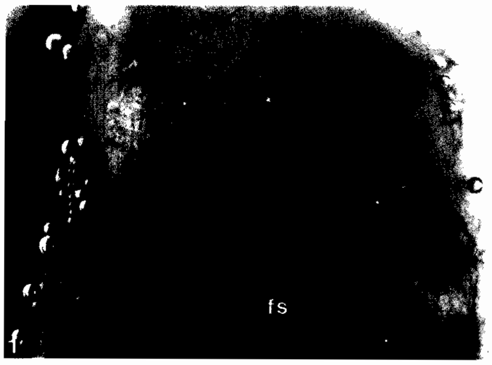

Fig. 1: General view of the base of the tongue; No significant symmetry was observed between the two sides regarding the nerve fibres (*) lying medially towards the fibrous septum (fs). X 6.7 Haematoxylen.

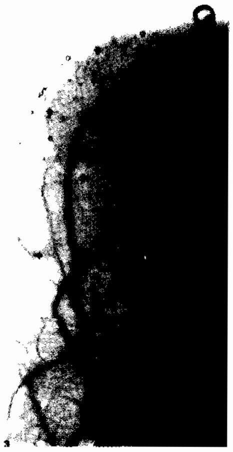

Fig. 3: General view of upper surface of the tongue; At the dorsal and lateral sides of the tongue nerve fibers were thicker related with the distribution of the taste buds (➤). X20 Haematoxylene.

Fig. 2: Lateral view of the tongue; Intramuscular nerve fibers were observed lying straight at the root of the tongue, but gradually becoming convoluted towards the tip of the tongue (➤). X10 Haematoxylene.

from each other and no nerve plexuses were observed between the two sides. No perceptible symmetry was observed between the two sides regarding the nerve fibers lying medially towards the fibrous septum (Fig. 1). Nerve fibers at the root of the tongue could not be observed clearly because of the large amount of the muscular tissue in the region (Fig.1), although the nerve distribution was observed clearly at the corpus and apex (Fig. 3).

At the dorsal and lateral sides of the tongue, nerve fibers showed a thicker morphological structure apparently related to the distribution of the taste buds whose nerve fibers over the dorsum of the tongue were observed branching and terminating over the multiple taste buds, becoming thicker before ending (Fig. 3).

No anastomotic connection between the nerve fibers, and no looping back to reconnect were observed.

DISCUSSION

Since there is no detailed information about the anatomical structure of the intramuscular nerve distribution of the tongue apart from the dissections performed on dogs (15), in the present study complete innervation pattern of the tongue has been studied in rabbits. In order to visualise the intramuscular nerve fibers, the tongues were stained using modified

Sihler's technique (11). However of the studies done in the last 50 years, Sihler's technique had been reported in only a few (7,10, 16,17).

In several studies on different animals and various muscle groups, it has been reported that certain mammalian skeletal muscles were divided into distinct neuromuscular compartments, arranged in a parallel structure (18-23). In addition, it has been reported that fine branching was often convoluted, passing through different levels and looping back to reconnect with itself (13). In this study, intramuscular nerve fibers were lying parallel with each other, straight at the radix, convoluted at the apex, but no looping back was observed.

In text books, it was reported that nerve fibers are surrounded by a myelin sheath, give branches and become thinner before they terminate. The myelin surrounding the nerve fibers disappears gradually while they give branches and become thinner. Branching fibers separate from each other and at the end single nerve fibers remain surrounded with a little endoneural connective tissue. Nerve fibers terminate on another neuron or a non-neuronal receptor cell, losing the myelin sheath surrounding itself (24-26). In the present study nerve distribution of the rabbit tongues have been demonstrated as reported above.

In their study on dogs, Mu and Sanders reported that, within the rostral compartment of the geniohyoid muscle, individual terminal branches crossed the midline and joined with the nerves from the opposite side in order to form an extremely dense plexus. Such a plexus suggested that paired rostral compartments were innervated bilaterally. In contrast the caudal compartment of the tongue was innervated ipsilaterally (27). In another study on canine tongues by the same group, it has been reported that a branch of XII crossed the midline at the junction between the anterior and middle thirds of the tongue and innervating the muscle on the contralateral side. In addition, they also observed a branch of lingual nerve crossing the midline and innervating the mucosa of the contralateral side (15). In the present study terminal nerve branches were observed leaving the main bundle lying at the lateral margin of the tongue and crossing the fibrous septum as determined by Mu and Sanders, but at the proximal one third of the tongue. However, in contrast to the Mu and Sanders' results, innervation of both sides were completely independent from one another and no nerve plexuses were observed between the two sides.

On the other hand, when the relationship between the intramuscular nerve distribution and the taste buds was examined, taste buds were found as specialised microscopic arrangements around the taste nerves. Each nerve fiber may have many terminals to innervate widespread spreaded taste buds. They were innervated by the corresponding nerve fiber over each region (24-26). Also in the present study nerve fibers over the dorsum of the tongue were observed branching and terminating over more than one taste bud and becoming thicker before ending. Such an anatomical structure was related with the taste buds innervated by the nerve fibers.

In their studies on rabbits Liu et al. examined the intramuscular nerve distribution in the triceps muscle. They reported anastomoses between the branches of either a single nerve or different nerves. They also reported that the communicating nerve fibers were "Y", "I" or "U" shaped. No standard anastomosis pattern was observed in different animals and different muscle groups (7). In the present study no anastomotic relations were observed between the nerve fibers. Only a few fibers were observed crossing the fibrous septum.

CONCLUSION

Detailed information about the anatomical pattern of the intramuscular nerve distribution of the tongue without doubt, is very important for the complicated surgical approaches. Moreover, during the studies on the surgical models, recovery is mostly evaluated by examining the regeneration of the nerve fibers. Post-operative application of a technique such as Sihler's modified neural staining technique would be quite useful to evaluate the recovery and success of the surgical model during surgical researches.

Such a technique could be easily applied not only on the rabbits but on several other mammals.

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