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# Ultrastructural Features of the Intrinsic Lingual Muscles in the Frog, *Rana Ridibunda*

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**Abstract:** The ultrastructural features of the intrinsic lingual muscles in the frog were investigated. The pieces obtained from the anterior part of the tongue were fixed in 3% gluteraldehyde and postfixed in 0.1% osmium tetroxide. Ultrathin sections stained with uranyl acetate and lead citrate were examined in a JEOL-100SX electron microscope. The muscle fibers were irregular in shape. The sarcoplasm was occupied largely by the myofibrils. In the same muscle fiber, longitudinal, transverse and oblique sections of the myofibrils were observed. Thus, regular transverse striation was prominent in some areas. In these areas, Z lines, I bands and A bands were distinguishable. H zone was hardly visible. M line was not prominent. Sarcosomes were not arranged in linear chains between the myofibrils. Triads including terminal cisternae and T-tubules were located at Z disks. The caveolae were observed as subsurface vesicles or as small groups within the cytoplasm.

**Key Words:** striated muscle, tongue, frog, Rana ridibunda

## *Rana Ridibunda*'da Intrensik Dil Kaslarının Elektron Mikroskopik Özellikleri

**Özet:** Bu çalışmada kurbağanın intrensik dil kaslarının elektron mikroskopik özellikleri incelendi. Dilin 1/3 ön bölümünden alınan parçalar % 3'lük gluteraldehitle ve daha sonra % 0.1'lik osmium tetroksitle fikse edilidi. Uranil asetat ve kurşun sitratla boyanan ince kesitler, JEOL-100SX elektron mikroskopta incelendi. Kas lifleri düzensiz şekilliydi. Sarkoplazma büyük oranda miyofibrillerle işgal edilmişti. Aynı kas lifinde miyofibrillerin boyuna, meyilli ve enine kesitleri izlendi. Bu nedenle düzenli enine çizgilenme bazı alanlarda belirgindi. Bu alanlarda, I ve A bantları izlenebiliyordu. H zonu zorlukla görülüyordu. M çizgisi belirgin değildi. Sarkozomlar miyofibriller arasında linear zincirler şeklinde düzenlenmemişlerdi. Terminal sisternaları ve T-tubullerini içeren triadlar Z diskleri hizasında yer almaktaydı. Kaveolalar yüzeye yakın veya sitoplazma içinde küçük vezikül grupları şeklinde izlendi.

**Anahtar Sözcükler:** Çizgili kas, dil, kurbağa, Rana ridibunda

## Introduction

The primary function of muscle cells is contraction. The striated skeletal muscle fibers, which are cylindrical and multinucleated, show regular transverse bands along their lengths. The sarcoplasm is occupied largely by myofibrils. The muscle fiber in longitudinal section shows alternating dark (A) and light (I) bands. The A band shows a central H band that stains less intensely. A dark M line may be visible in the center of the H band. Each I band is bisected by a distinct Z line. The perinuclear area contains numerous sarcosomes, Golgi apparatuses, glycogen particles and some lipid droplets (1-4).

The bulk of the tongue consists of bundles of striated muscle that provide it with the mobility required for chewing and swallowing. In addition to chewing and swallowing, amphibians use their tongues for catching their food. The anterior part of their well-developed tongues has special muscles (5,6). In the present study we investigated the ultrastuctural features of these special intrinsic lingual muscles of the frog, *Rana ridibunda*. To the best of our knowledge, this is the first electron microscopic study about the intrinsic lingual muscles of the frog.

## Materials and Methods

Six frogs, (*Rana ridibunda*) were used. The animals were sacrificed by decapitation and the anterior parts of

their tongues were removed and cut into pieces. These were fixed in 3% gluteraldehyde buffered with 0.2 M $NaH_2PO_4$+$NaHPO_4$ (pH=7.2-7.3), and postfixed in 0.1% osmium tetroxide buffered with $NaH_2PO_4$+$NaHPO_4$ (pH=7.2-7.3). Specimens were dehydrated in acetone and embedded in Araldite CY 212. Semithin sections were studied with toluidin blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEOL-100SX electron microscope.

**Results**

Multinucleated fibers of the intrinsic lingual muscle in the frog were irregular in shape because of the branching protoplasmic projections. Nuclei were generally localized directly beneath the sarcolemma, sometimes in the deeper sarcoplasm. Euchromatic irregular nuclei sometimes showed indentations (Figure 1). The sarcoplasm was occupied largely by the myofibrils. In the same muscle fiber, longitudinal, transverse and oblique sections of the myofibrils were observed. Thus, regular transverse striation was prominent in some areas (Figures 2,3). In the areas that show regular transverse striation, Z lines, I and A bands were distinguishable. The H zone was hardly visible but the M line was not prominent (Figures 4,5). Perinuclear sarcoplasm was generally devoid of myofibrils. Clear cytoplasm adjacent to nuclei contained numerous sarcosomes with many criastae, prominent Golgi apparatus, large amounts of glycogen, some lipid droplets, rare lysosomes, ribosomes and tubular and vesicular elements of the sarcoplasmic reticulum (Figure 6). Sarcosomes, glycogen particles, ribosomes and elements of the sarcoplasmic reticulum were also observed between the myofibrils. Sarcosomes were not arranged in linear chains between the myofibrils (Figures 2,4,5,7). The plasma membrane contained many flask-shaped invaginations (Figures 2,6). Transverse tubules or T-tubules were extending inward from the surface of the fiber (Figure 7). Triads including T-tubule and terminal cisternae were located at Z discs (Figure 5). The lumen of the terminal cisternae contained an amorphous and granular material. There were clusters of particles next to the membrane of the terminal cisternae that adjoins the T-tubule, T tubule also contained a fine granular material. Electron-dense particles between the membranes of terminal cisternae and T-tubule were observed. The membrane of the terminal cisternae adjacent to the T-tubule showed indentations (Figure 8).

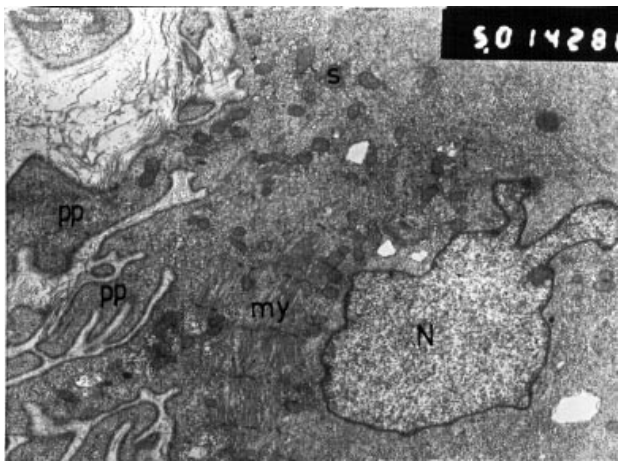


Figure 1. The intrinsic lingual muscle of *Rana ridibunda*. The muscle fibers are irregular in shape because of the branching protoplasmic projections (pp). The euchromatic, irregular nucleus (N) showing indentations is located in the deeper sarcoplasm. Myofibrils (my) showing transverse striation are observed near the nucleus. There are many sarcosomes (s) within the sarcoplasm. Uranyl acetate and lead citrate X5,000.

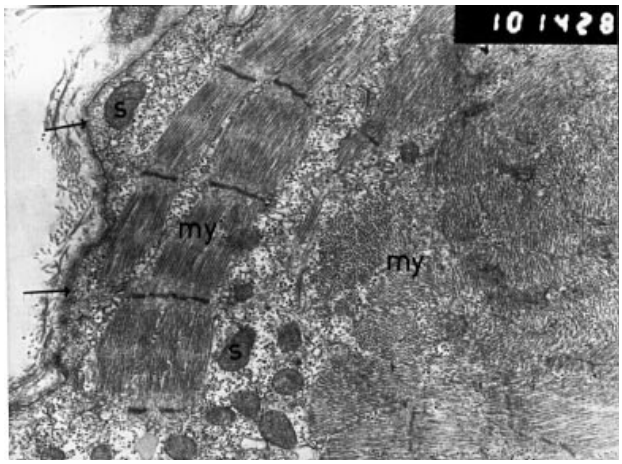


Figure 2. The intrinsic lingual muscle of *Rana ridibunda*. The sarcoplasm is occupied largely by the myofibrils (my). In the same muscle fiber, longitudinal, transverse and oblique sections of the myofibrils are observed. Regular transverse striation is prominent in some areas. There are many sarcosomes (s) around the myofibrils. Caveolae and pinocytotic vesicles are observed beneath the sarcolemma (arrows). Uranyl acetate and lead citrate X10,000.

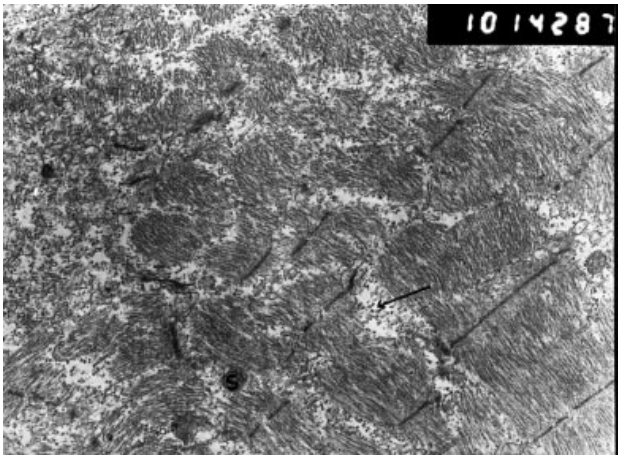


Figure 3. The intrinsic lingual muscle of *Rana ridibunda*. In the same muscle fiber, longitudinal, transverse and oblique sections of the myofibrils are observed. Sarcosomes (s) and glycogen particles (arrow) are observed between the myofibrils. Uranyl acetate and lead citrate X10,000.

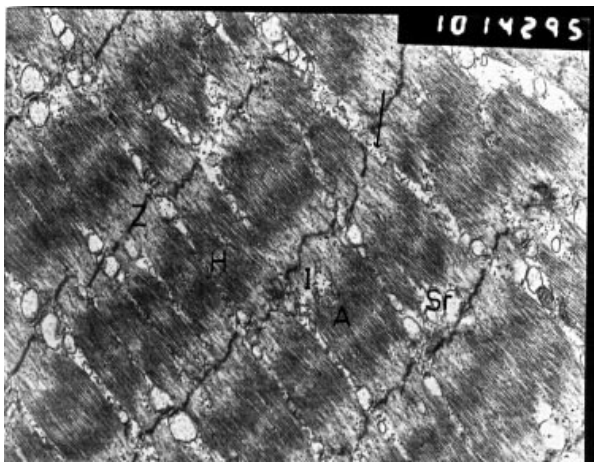


Figure 4. The intrinsic lingual muscle of *Rana ridibunda*. In the areas that show regular transverse striation, Z lines (Z), I bands (I) and A bands (A) are distinguishable. H zone (H) is hardly visible but M line is not prominent. The elements of sarcoplasmic reticulum (Sr) and glycogen particles (arrow) are observed between the myofibrils. Uranyl acetate and lead citrate X10,000.

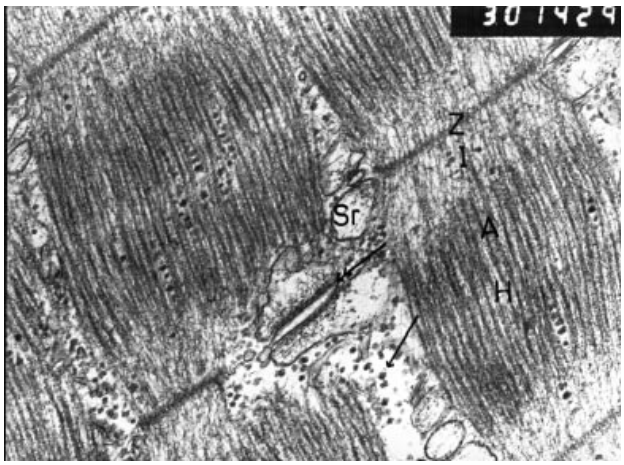


Figure 5. The intrinsic lingual muscle of *Rana ridibunda*. In the areas that show regular transverse striation, Z lines (Z), I bands (I) and A bands (A) are distinguishable. H zone (H) is hardly visible but M line is not prominent. The elements of sarcoplasmic reticulum (Sr) and glycogen particles (arrow) are observed between the myofibrils. Triad located at the level of Z line is clearly observed (double-arrow). Uranyl acetate and lead citrate X30,000.

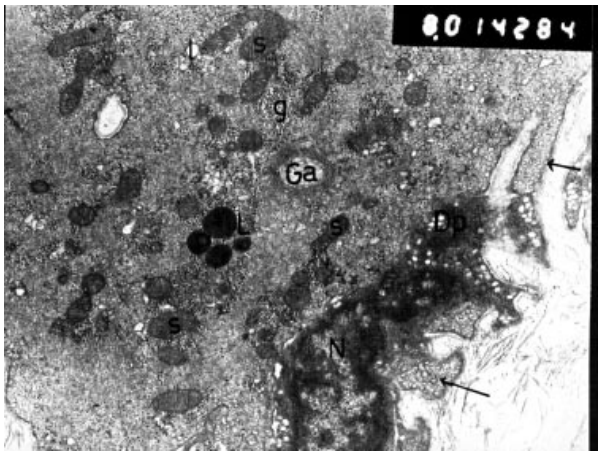


Figure 6. The intrinsic lingual muscle of *Rana ridibunda*. Nucleus (N) is located beneath the sarcolemma. Perinuclear sarcoplasm is generally devoid of the myofibrils. Clear cytoplasm adjacent to nuclei contains numerous sarcosomes with many criastae (s), prominent Golgi apparatus (Ga), large amounts of glycogen (g), some lipid droplets (l), rare lysosomes (L), ribosomes and tubular and vesicular elements of the sarcoplasmic reticulum. The caveolae occur as subsurface vesicles or as small groups within the cytoplasm (arrows). Dense plaques (Dp) are seen beneath the sarcolemma. Uranyl acetate and lead citrate X8,000.

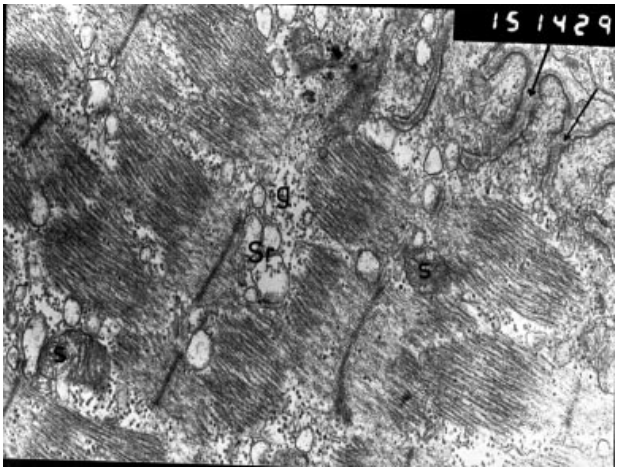


Figure 7. The intrinsic lingual muscle of *Rana ridibunda*. Transverse tubules or T-tubules are extending inward from the surface of the fiber (arrows). Sarcosomes (S), glycogen particles (g), ribosomes and elements of the sarcoplasmic reticulum (Sr) are observed between the myofibrils. Sarcosomes are not arranged in linear chains between the myofibrils. Uranyl acetate and lead citrate X15,000.

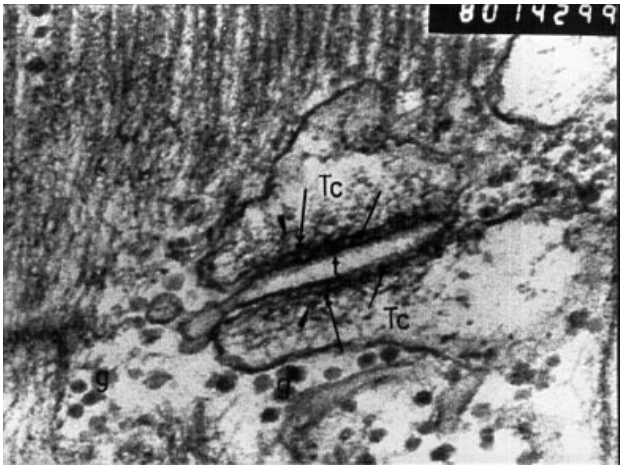


Figure 8. The intrinsic lingual muscle of *Rana ridibunda*. The lumen of the terminal cisternae (Tc) contains an amorphous and granular material. In the portion of the membrane of the terminal cisternae that adjoins the T-tubule, there are clusters of particles (arrowheads). T-tubule (t) also contains a fine granular material. The space between the membranes of terminal cisternae and T-tubules contains particles also (arrows). The membrane of the terminal cisternae adjacent to T-tubule shows indentations (double arrows). Glycogen particles are seen (g). Uranyl acetate and lead citrate X80,000.

Dense bodies (plaques) were observed beneath the sarcolemma (Figure 6). There were many mast cells within the connective tissue (Figure 9).

**Discussion**

We observed the muscle fibers of the intrinsic lingual muscle in the frog, *Rana ridibunda*, to be irregular in shape because of the branching projections. It is known that sarcolemma becomes irregular during contraction (2). But, we think that irregularity that we observed was not a result of contraction. Because these protoplasmic projections were long and showed a branching pattern. There is no reported study about the intrinsic lingual muscle of the frog but it is reported that the fiber ends in the fourth extensor digitorum longus and extraocular muscle of the frog are characterized by the presence of small pit-like and short slit-like invaginations. These findings confirm that muscle fiber ends in the frog consist of sarcolemmal invaginations to increase the surface area where collagen fibrils attach to the basal laminae of muscle fibers (7). We suggest that the difference in the surface specializations may intimately reflect the functional properties of the muscle.

The sarcoplasm was occupied largely by the myofibrils. In the same muscle fiber, longitudinal, transverse and oblique sections of the myofibrils were observed. Thus, regular transverse striation was prominent in some areas. This unusual type of myofibril organization and orientation may be specific for the intrinsic lingual muscle of the frog because the anterior part of the tongue for catching insects can move and curl freely (5,6). We suggest that movement through all directions simultaneously may be the result of the longitudinal, oblique and transverse orientation of the myofibrils in the same muscle fiber. As a result of this organization we could observe the transverse striation only if the plane was suitable. In many other striated muscles as frog sartorius muscle, the myofibrils show a typical branding pattern of the sarcomere (8). In the present study, in the areas that showed regular transverse striation, the M line was not prominent. In vertebrates, the purpose of the M line is to anchor the myosin thick filaments and align them as contraction occurs (9). It is reported that the M line is not prominent in the invertebrate striated muscle. It has been speculated that the alignment of these filaments in the sarcomeres have the ability to move in relation to each other since

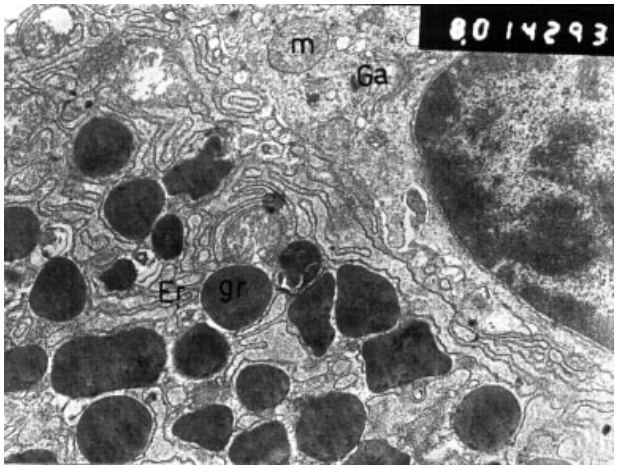


Figure 9. A mast cell, including electron-dense granules (gr), endoplasmic reticulum (Er), a prominent Golgi apparatus (Ga) and mitochondria, (m) located within the connective tissue is observed. Uranyl acetate and lead citrate X8,000.

they are not held by the M line. As an effect, the thick filaments stretch, allowing interaction between the thick and thin filaments, which is the root of muscle contraction. The possible problem of thick filament misalignment during contraction is solved by the interaction with the thin filaments, which are held in place by the Z lines and A bands. As the thin filaments slide toward the center of the sarcomere, those thick filaments that have become misaligned are repositioned as the sarcomere shortens and contraction continues (10). It is reported that there are actomyosin cross-bridges in the A-I overlap region in the amphibian fast twitch muscle (11).

The fibers that make up a muscle are not all identical. They vary in color, diameter and in cytochemical and physiological properties. Traditionally three types have been described: red fibers, white fibers and intermediate fibers. The red fibers have a dark color, attributable to their greater content of myoglobin and to the cytochromes in their large and abundant sarcosomes. Lipid droplets are common in their sarcoplasm and the Z bands are wider than in the other fiber types. Sarcosomes of the white fibers are smaller than those of red fibers, and sarcosomes between myofibrils are relatively few. Glycogen particles are abundant in white fibers and sparse in red fibers. But neutral fat content is all low in white fibers (1,2,12). The muscle fibers of the intrinsic lingual muscle of the frog, *Rana ridibunda* were white fibers. Because sarcosomes were sparse between the myofibrils and were not arranged in linear chains, sarcoplasm contained numerous glycogen granules and fewer lipid droplets. The three-dimensional structure of the sarcosomes in the red and white skeletal muscle fibers, of the vastus lateralis muscle of the Japanese meadow frog (*Rana nigromaculata Hallowell*), was examined in the study of Ogata et al. The small red fibers have numerous sarcosome columns of large diameter, while the large white fibers have small numbers of sarcosome columns of small diameter (13).

In the present study we observed that sarcolemma showed many flask-shaped invaginations. In some areas, these invaginations are irregular in shape and size and may be involved in pinocytosis. In other areas these are regular in shape and distribution and are called caveolae (14,15). The caveolae were observed as subsurface vesicles or as small groups within the cytoplasm in our study. The organization of caveolae is different from each other in fast and slow muscle fibers. For example, in the fast fibers in cruralis and iliofibularis muscle small groups of caveolae form linear patterns (16). The sarcolemma shows numerous apertures of caveolae in the frog sartorius muscle (8). The different patterns of folds may correspond to the different contractile properties of the fiber types (16).

Endoplasmic reticulum of the muscle fibers is the site of sequestration of calcium during muscle relaxation and release of calcium into the sarcoplasm to trigger muscle contraction. It consists of a network of membrane-bound tubules surrounding each myofibril. The two parallel terminal cisterna and the intervening T-tubule, a tubular invagination of the sarcolemma, form a complex referred to as the triad (2). The sarcolemma shows numerous T-system tubules in the frog sartorius muscle. Around the myofibrils the sarcoplasmic reticulum and T-system are clearly observed (8). In all three types of fibers of the frog the terminal cisternae and transverse tubules form triads at the level of each Z line (13). We observed triads including terminal cisternae and T-tubule at the level of Z line. At this level, a slender transverse tubule (T-tubule) runs transversely to the longitudinal axis of the myofibril. Small, spherical or ovoid terminal cisterna couple laterally with the T-tubule and form a "terminal cisterna-T tubule complex" on whose surface tiny indentations are occasionally seen (17).

Sarcoplasmic reticulum serves a central role in calcium uptake and release. The terminal cisternae contain two types of membranes, the calcium pump membrane and junctional face membrane. The terminal cisternae are filled with electron-opaque contents which serve as a Ca+2 reservoir. The longitudinal tubules consist mainly of the calcium pump membrane (18). A classification of the sarcoplasmic reticulum membrane into areas is described. The junctional sarcoplasmic reticulum is covered by feet, it faces towards the transverse tubules, and it is probably devoted to receiving a signal from them. The free sarcoplasmic reticulum contains the calcium pump. It is proposed that the feet join the junctional sarcoplasmic reticulum membrane to particles contained within the transverse tubules and that they play a direct role in exication-contraction coupling (19-22).

In the present study we observed that the lumen of the terminal cisternae contained amorphous and granular material. The amorphous material of low density in the lumen of the terminal cisternae consists mainly of calsequestrin (2). In the portion of the membrane of the terminal cisternae that adjoins the T-tubule, there were clusters of particles. T-tubule also contained a fine granular material. The membrane of the terminal cisternae adjacent to T-tubule showed indentations probably because of the "feet" junctional structures between the transverse tubule and terminal cisternae.

Dense bodies located beneath the sarcolemma are the attachment areas of contractile elements to the sarcolemma (15).

Mast cells are indigenous connective tissue cells that function in the process of inflammation and edema. These are observed in the connective tissue interstitium. They are most frequently seen adjacent to neurovascular elements within the muscle. The close proximity of mast cells to muscle spindles and nerve fascicles suggests that these cells may play a role in modulating their activities (23).

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