Characteristics of Group III Afferent Fibers Identified In Muscle Spindle

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KAS İĞCİKLERİNDE BULUNAN GR UP III LİFLERİN ÖZELLİKLERİ

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SUMMARY

In cats under anesthesia lateral and medial gastronecmius nerves were isolated. Dorsal roots of Lg, L7, and Si were prepared using etching the fibers until only one axon remained functional. Medial gastrocnemius nerve was then electrically stimulated and action potentials were recorded from the isolated single dorsal root fiber.

In 124 muscle spindles and related single afferent axons so prepared, latency, conduction velocity, and the amplitude (voltage) of the action potentials were studied. The results fell into three separate groups, significantly different from each other ($p < 0.001$) corresponding to the three known nerve fiber groups.

It was observed that the group III fibers of the muscle spindles are not sensitive to vibration. They respond however, to the alterations of muscle length, 88% of the group III fibers were sensitive to muscle tonus, they discharged especially during dynamic phase of contraction and continued doing so during the phase of relaxation.

Key words: Muscle spinds, group III afferent fibers, action potentials, conduction velocity, latency, resistance

ÖZET

*Nembutal ile anestezi edilmiş kedilerin lateral ve medial **gastroknemius** sinirleri **izole** edildi. Lg, Lf ve Sı **sinirlerinin** arka kökleri tek bir sinir lifi kalıncaya kadar inceltilerek **kesildi** (etching). Sonra medial **gastroknemius** kası elektrikle uyarıldı ve tek bırakılan **arka** kök sinirinden aksiyon potansiyelleri kaydedildi.*

*Böylece izole edilerek hazırlanan 124 kas iğciği ve afferent sinirinde latans, iletim sürati ve aksiyon potansiyellerinin voltajı **incelendi.** Sonuçlar birbirinden bariz farklar gösteren ($p<.0.001$) üç ayrı grupta toplandı. Bunlar bilinen üç sinir lifine tekabül ediyorlardı.*

Kas iğciklerinin gr III liflerinin titreşimlere hassas olmadıkları fakat kas uzunluğundaki değişikliklere duyarlı oldukları bulundu. Bu liflerin (gr III) % 88'i has tonusuna karşı hassas idiler ve bilhassa kasılmanın dinamik safhasında ve gevşeme döneminde deşarj yapıyorlardı.

Anahtar kelimeler: Kas iğciği, grup III afferent lifler, aksiyon potansiyelleri, latans, resistance

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Sherrington (1894) has shown that afferent nerve fibers terminate at the equatorial area of the muscle spindle (23). These fibers were later called the primary nerve endings. Flower spray endings which terminate at the polar areas of the muscle spindle on the other hand are named the secondary nerves endings (2, 6). No differences between these two kinds of nerve fibers could be found at the beginning except for their morphology. It was later uncovered, however, that the characteristics of the primary nerve fibers of the muscle spindle correspond to group la fibers of the Lloyd classification (17). The secondary nerves on the other hand belong to the group II of the Bradley and Eccles nomenclature (5).

The purpose of this communication is to present the results of a new study in which we identified group III fibers (Lloyd classification) in the muscle spindle (17).

MATERIAL AND METHODS

Experiments were carried on cats (Fellis domes-

tica) weighting 1.5-2.5 kg. They were anesthesized by a primary dose of nembutal (35 mg/kg) given intraperitoneally. Supplemental doses of 5 mg/kg were also injected intravenously as indicated. A Y shaped cannula was inserted into the trachea for artificial respiration. An appropriate polyethylene catheter was placed into the external jugular vein and was used for injection of drugs. A mercury manometer was connected to the left arteria carotis communis by means of a polyethylen catheter and the arterial blood pressure of the animal was monitored throughout the experiment. Rheomacrodex was infused when it dropped to below 70 mm mercury.

Medulla spinalis from L5 to Si was exposed by laminectomy. All the muscles of the left hind leg were denervated except for the medial and lateral gastrocnemius nerves. They were covered with saline wetted sponges to prevent drying. After these preparations medulla spinalis and the gastrocnemius nerve were covered with neutral liquid paraffin at 37-38°C. Durameter was now opened and Lg, L7 and dorsal roots were excized at the point of entrance to the cord. Their distal parts were placed on extracellular recording electrodes. One or both gastrocnemius nerves were placed on the stimulating electrode. One of the posterior roots, generally L7 was etched leaving only one afferent axon and was placed on the bipolar electrode allowing 0.8 mm distance between its two arms. Action potentials were now recorded from these extracellular electrodes.

Succinyl choiin was injected intravenously in order to prove that the axon is the afferent fiber of a muscle spindle. Whether the spindle under consideration is tonic or phasic was determined by extending the gastrocnemius muscle.

After each experiment was over length of the nerve between the stimulating and the recording electrodes was accurately measured and using the Ohms relation the resistance and diameter of the fiber were calculated.

RESULTS

All recordings from the muscle spindle afferents were collected and then classified according the physical characteristics of the action potentials. They were first compiled into one group (Table la) and later separated into two groups according to the tonic or phasic (discharge characteristics of the stimulated spindle. Each action potential was again studied in reference to its conduction velocity, latency, diameter, fiber resistance and amplitude and histograms were made in accordance with Lloyd's classification (17).

Conduction Velocity: It is seen in Table la that the results of the conduction velocity of the recorded action potentials formed three distincly different groups. They obviously correspond to gr I, II and III fibers of Lloyd nomenclature. The differences of the means and standard deviations were markedly significant between gr I and II ($t = 11.56$, $p < 0.001$) and between gr II and gr III ($t = 10.12$, $p < 0.001$).

There were no statistically important differences between the tonic and phasic discharges of the three groups (Table lb) with reference to the parameters investigated. Their means were almost identical for group I of tonic and phasic discharges as were for groups II and III (Table lb).

When the histograms of conduction velocity is studied the same observation is made. In all populations the results from three groups were characterized by distincly different conduction velocity (Table lb).

Further classification of the figures of the conduction velocity of Table la it is observed that gr I afferent fibers made 56% of the action potentials while 30.11% are in gr II and 13.82% are group III fibers.

Latency: The figures of this parameter also made three distinctly different groups (Table la, Fig. 2).

Table — la

Means Standard Deviations of Characterizing Parameters of Groups I, II and III Tonic and Phasic Fibers

Tonic + Phasic Fibers	Group I	Group II	Group HI
Conduction velocity (m/sec)	89,62 + 11.75	51,84 ± 13.48	22,73 ± 3,96
Latency (//sec)	1603,23 ± 217,26	29 45,44 ± 844,39	6023,35 ± 717,08
Amplitude of action potential (mm)	14.87 ± 7.11	9.18 ± 5.71	6.54+ 3.33
Diameter (Mm)	2,060 ± 0',70	1.3933 ± 538	1.2947 ± 0,33
Fiber resistance (ki"j)	407,98 ± 252,20	462,93 + 246,12	681,24+ 500,02

Table - lb

Between Groups Differences of Characterizing Parameters and Their Statistical Importance of Tonic Phasic Fibers

Tonic Phasic Fibers	Group I and Group II	n1	n2	Group II and Group III	n1	n2	Group I and Group III	n1	n2
Conduction velocity (m/sec)	$14.67 > t_{0,001} = 3,43$	69	38	$12,38 > t_{0,001} = 3,59$	38	17	$3,96 > t_{0,001} = 3,43$	69	17
Latency (μsec)	$9,75 > t_{0,001} = 3,43$	68	38	$14,21 > t_{0,001} = 3,59$	38	17	$25,88 > t_{0,001} = 3,43$	68	17
Amplitude of action potential (mm)	$4,54 > t_{0,001} = 3,43$	68	38	$2,19 < t_{0,001} = 2,03$	38	17	$7,18 > t_{0,001} = 3,43$	68	17
Diameter (μm)	$5,51 > t_{0,001} = 3,43$	68	38	$0,88 < t_{0,05} = 2,021$	38	15	$6,35 > t_{0,05} = 3,43$	68	17
Fiber resistance (kΩ)	$1,08 < 0,005 = 1,99$	68	36	$1,66 < t_{0,05} = 2,03$	36	15	$0,79 < t_{0,005} = 1,99$	68	2

The differences between group II and III were statistically very important ($p < 0.001$).

Amplitude: The amplitude of the spikes were accurately measured in the entire population and in tonic and phasic muscle spindle fibers (Table lb, Fig. 3). The results fall into three obviously different groups ($p < 0.001$).

Fiber Diameter: There was significant difference between the fiber diameters of gr I and gr II fibers ($t = 4,7$, $p < 0.001$), but not between groups gr II and III ($t = 1.75$, $p > 0.05$). Nevertheless these two groups are distinctly seen in histograms Fig, 4).

Fiber resistance: The results of fiber resistance also make three different groups when tabulated (Table lb and Fig. 5). But because of wide variation of the figures the differences between gr I and gr II, and between gr II and gr III are not significant ($p<0.05$).

Continuous discharges of group III afferent fibers: It was observed that group III afferent fibers continually discharge even before applying any stimulation to the muscle spindle (Fig. 6A). When the gastrocnemius muscle is extended only 2 mm (C) the frequency of the discharge is increased. This became even more impressive when the muscle is extended 4, 6 and 8 mm (Fig. 6D, E and F respectively).

In Fig. 6F discharge characteristics of gr III afferent fibers are seen with respect to muscle contraction. It is clear that gr III fibers are resuming discharges during relaxation phase.

In other experiments the gr III afferent fibers are observed to begin discharging during the dynamic phase and to continue during the static phase.

Another important characteristic of the group III fibers is that they are not sensitive to vibration.

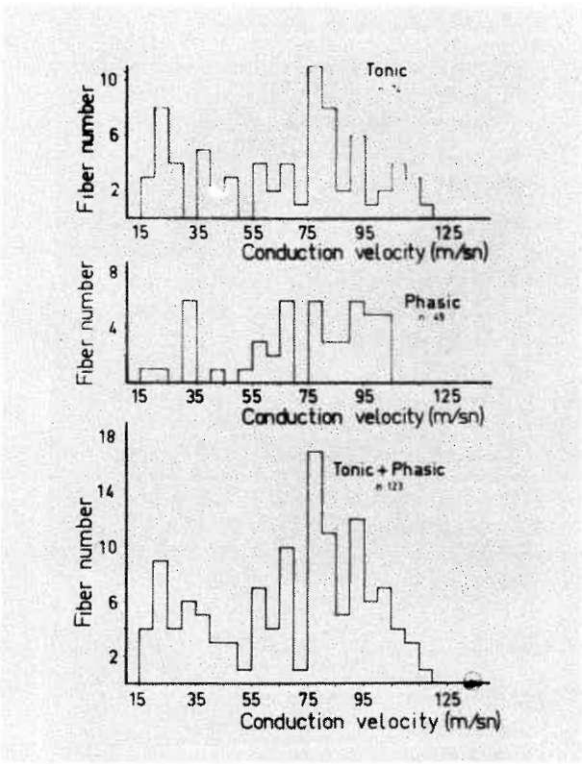

Figure-1. *Histograms of velocity of conduction of the isolated fibers*

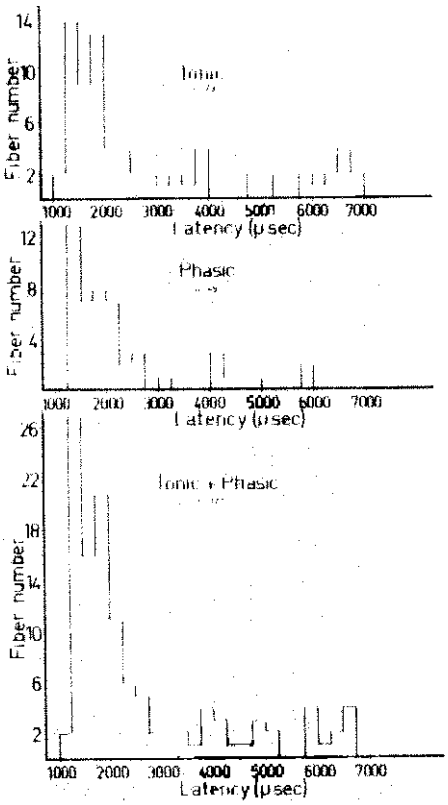

Figure-2. *Latencies of the isolated fibers*

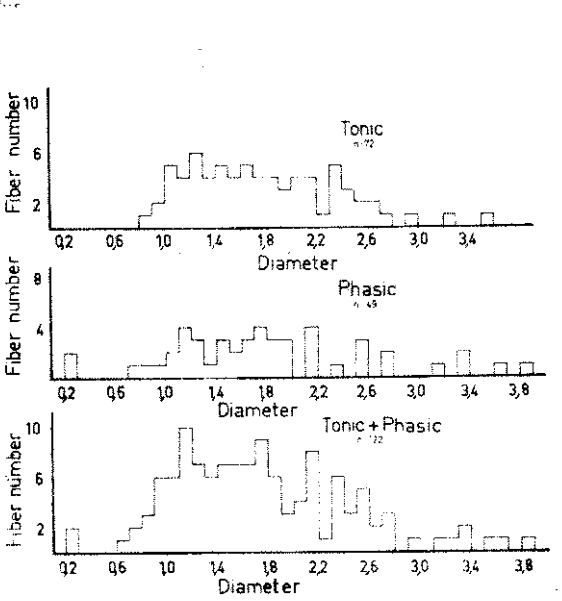

Figure-4. *Histograms of diameter of the afferent fibers*

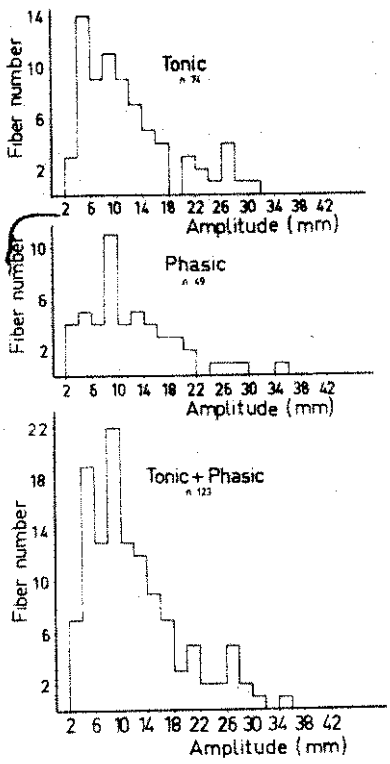

Figure-3. *Histograms of amplitude (mm) of action potentials*

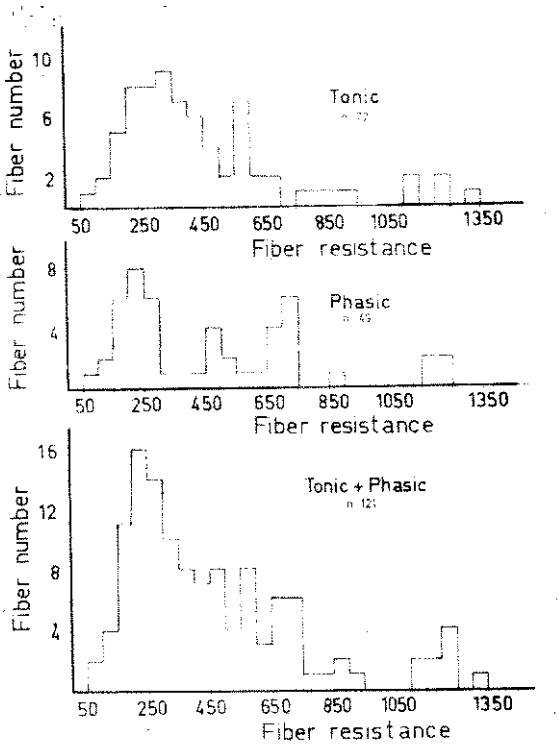

Figure-5. *Histograms of the resistance of the afferent fibers*

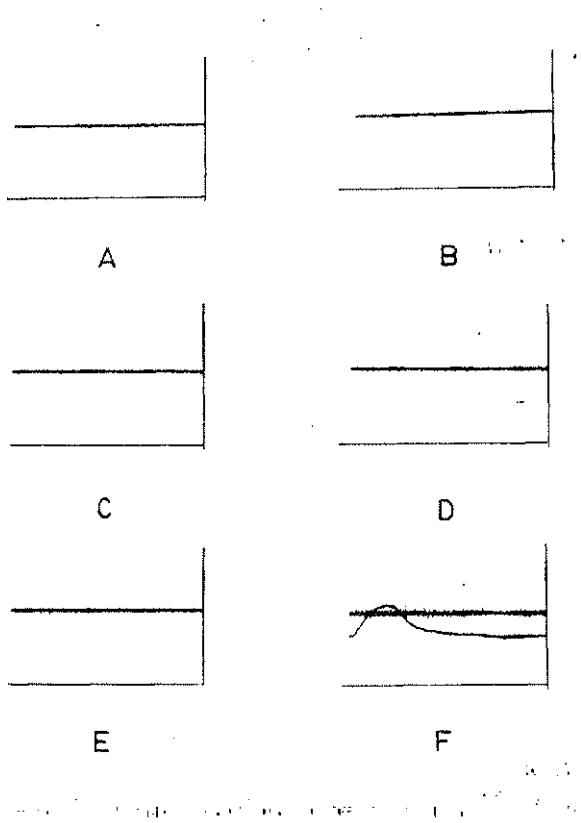

Figure-6. *Responses of Group III afferent fibers of the muscle 0 (A) spindle at the initial length, and after extending 2 (B), 4 (D), 6 (D), 8 (E) milimeters and the resulting contraction.*

DISCUSSION

Pumprey and Young observed that the nerve fibers with large diameters conducted faster than those with smaller diameter (21). Other investigators later confirmed this relationship between conduction velocity and fiber diameter (9, 10, 15, 16, 22, 26). As a general rule the diameter multiplied by six gives the conduction velocity of a given nerve fiber (20). Depending on this knowledge investigators who study nerve fiber characteristics always regarded this important parameter in classification of nerve fibers. Barker (2) therefore concluded that the primary fibbers of the muscle spindle correspond to group IA of Lloyd nomenclature (17). Conduction velocity of these fibers is 70-120 m/sec while their diameter are between 9-12 /jm. The secondary fibers of the muscle spindle conduct the action potential with a speed (30-70 m/sec) corresponding to gr II fibers (17) and their diameter average 6-9 *pm.*

In our studies we observed that the mean conduction velocity of group I afferent fibers was 89.62 ± 11.72 m/sec and of the group II and III fibers 51.84 ± 13.48 and 22.73 ± 3.39 m/sec respectively. The latter figure is almost identical with the findings of Hunt (16). The difference between the diameters of the fibers in gr I and gr II of our series was found statistically very important ($p < 0.001$). Rusthon (22), Hodgkin and Huxley (13) and again Hodgkin (14) found that in group I and II fibers the diameter is linearly proportional to the conduction velocity. The conduction velocity of the unmyelinated nerve fibers (group III) however, was proportional to the square root of the fiber diameter. In our experiments the difference between the diameters of gr II and gr III fibers was not significant ($p < 0.05$) (Table lb). Our observations therefore is in agreement with their results.

Mountcastle uncovered an inverse relationship between the conduction velocity and the latency of a given fiber (20). In our study we found that the latent periods of the three groups observed are very significantly different from each other. This finding also confirms our conclusion that there are gr III fibers innervating the muscle spindle besides groups I and II.

In 1939 Gasser and Grundfest (9) and Hursh (15) found that there is a linear relationship between the magnitude of the spike potentials and the fiber diameter. This linearity becomes less obvious for the fibers with the diameter below 8 /jm (9). Stein and Pierson on the other hand observed a linear relationship between the square root of the fiber diameter and the amplitude of the action potential (24). Clamann and Henneman found a linear relationship between the square of the conduction velocity and the fiber diameter (7). The results of these authors suggest that the hight of the spike potential may not be statistically different between groups II and III. This conclusion is in agreement with our findins (Table lb).

It is interesting that the differences between the fiber resistance and diameter are not statistically important (Table lb). This is explained by the results of Barret and Crill who state that conduction velocity, fiber diameter and the resistance are not related linearly (1).

It is known that the primary afferent fibers conduct' information concerning extention velocity of the muscle as well as about its static length. The number of spikes conducted increases with the rate of change inthe muscle length and the amount of extention (19). The secondary afferent fibers on the other hand conduct information related to static length of the muscle (12, 18). The results presented here indicate that the gr III fibers innervating the muscle spindle discharge mostly in the dynamic phase of changing the length of the muscle and continue doing so during the static phase (Fig. 6). The discharges of gr II afferents stop instantly during the relaxation

period of the muscle while those of the secondary fibers are gradually reduced (25). The behavior of gr III fibers from this point of view are quite similar to that of gr II.

Granit and Henatsch have found out that vibration is a satisfactory stimulation for gr I fibers (11). The secondary fibers however, were not found sensitive to vibration (11). Biancony and Van DerMeulen stated that the sensitivity to vibration was a function of conduction velocity (3). We therefore investigated this characteristic and found that these gr III fibers are not sensitive to vibration.

In conclusion we presented evidence in this communication indicating existence of group III afferent fibers in the muscle spindle. They are characterized by different conduction velocity, latency, amplitude and resistance. They discharge continuously and are not sensitive to vibration.

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