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THE UNIVERSITY OF ALBERTA

MOTOR UNITS OF PARTIALLY DENERVATED TRICEPS SURAE MUSCLES OF THE CAT

BY

ROBERTO ELFIDIO OROZCO-FLORIAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommended to the Faculty of Graduate studies and Research for acceptance, a thesis entitled:

MOTOR UNITS OF PARTIALLY DENERVATED TRICEPS SURAE MUSCLES
OF THE CAT

©

submitted by: ROBERTO ELFIDIO OROZCO-FLORIAN

in partial fulfillment of the requirements for the degree of: MASTER OF SCIENCE.

....
(Supervisor)

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William F. Dryden

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Date: April 16, 1987

TO MY FAMILY.

ABSTRACT.

The process of reinnervation of partially denervated muscles was studied in the triceps surae muscles of the cat. Partial denervation was achieved by section of one of the two spinal roots (L7 or S1) contributing to the innervation of these muscles. At the time of denervation, animals were either kittens or young adult cats. After survival times of between 2 and 20 months, motor units were characterised by isolation and stimulation of dissected ventral root filaments containing the axon of only one functional alpha-motoneuron to the muscle of interest, and classified into motor unit types on the basis of twitch contraction time and fatigue index: S (slow-contracting, fatigue resistant), FR (fast-contracting, fatigue-resistant), FI (fast-contracting, fatigue-intermediate), or FF (fast-contracting, highly fatiguable).

The size of the residual motor units in the medial and lateral gastrocnemius muscles, as measured by their tetanic force, increased in proportion to the extent of muscle denervation and compensated completely for partial denervation. Small extents of denervation were compensated by a selective enlargement of slow motor units, while large extents of denervation were compensated by enlargement of slow and fast motor units. For any extent of denervation, slow motor units enlarged their original size more than fast motor units, but the final motor unit force was still governed by the size of its motoneuron.

Residual motor units of the soleus muscle often failed to adequately enlarge and compensate for partial denervation. Most motor units did not enlarge beyond a 4-6 fold increase. These findings suggest that the limitation of these motor units to enlarge is likely to be extrinsic rather intrinsic to the motoneuron itself.

Reinnervation of the muscle by axons regenerating from the sectioned spinal root took place in a number of cases. The extent to which this process contributed to the total reinnervation of the muscle varied from cat to cat; it was greater in kittens than in adults, greater in severely denervated than in mildly denervated muscles, and greater in lateral and medial gastrocnemius than in soleus muscle. Regenerated slow motor units also enlarged relatively more than fast motor units.

The decline in force produced by unfused tetani (Burke et al., 1971) is known as the "sag" phenomenon and has been used to separate motor units into slow (non-sagging) and fast (sagging). The general agreement between this criterion and the twith contraction time criterion to separate fast from slow MU's found in medial gastrocnemius muscle was found neither in normal nor in experimental lateral gastrocnemius motor units.

Axon diameter, as measured by axon potential amplitude, did not increase with muscle unit enlargement in the adult cat. However, it did increase in the kitten.

Other properties also changed in the experimental motor units, especially in the lateral and medial gastrocnemius muscles. In general the very slow and very fast contracting motor units acquired intermediate values. Similar changes were observed for motor unit fatiguability. As a result of these changes there was a relative increase in the number of FI motor units. But the normal relationships among axon diameter, muscle unit speed of contraction, and muscle unit fatiguability were preserved in sprouted and re-established in regenerated motor units.

ACKNOWLEDGMENT

I grateful to my supervisor, Dr. Tessa Gordon, for her guidance, enthusiasm, and dedication to work.

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CHAPTER ONE: INTRODUCTION

Sherrington (1929) was not only the first to introduce the concept of the motor unit (MU), but also the first to recognize its importance in the control of movement. The motor unit (MU) refers to the combination of an alpha motoneuron and the set of skeletal muscle fibers innervated by it. Thus, a motor unit has a neural component, the alpha motoneuron, and a muscular component, the set of muscle fibers supplied by the motoneuron. The latter has been called the "muscle unit" (Burke, 1967).

Muscles are complex structures, generally made up of a constellation of muscle units of different sizes and properties. In general, every muscle fiber in a mature mammalian muscle is innervated by only one motoneuron (Feindel et al., 1952); and every motoneuron innervates muscle fibers belonging to only one anatomically defined muscle. The number of muscle fibers innervated by an alpha motoneuron (innervation ratio) varies considerably, depending on the muscle size and function (reviewed by Stuart and Enoka, 1984) and on motor unit type.

1.1 CLASSIFICATION OF MOTOR UNITS

MU's may be divided into two groups on the basis of their twitch contraction time (Burke, 1967). In the medial gastrocnemius muscle of the cat, for example, units with twitch contraction times of 40 milliseconds or less are classified as fast (F), while those with twitch contraction times greater than 40 milliseconds are classified as slow (S). Fast MU's in turn are subdivided into three types on the basis of their susceptibility to fatigue: FF (fast contracting and highly fatiguable units; fatigue index <0.25), F.Int. (fast contracting units of intermediate fatiguability; fatigue index >0.25 but <0.75) and FR (fast contracting but resistant to fatigue; fatigue index >0.75). (Burke et al., 1971).

1.2 MOTOR UNIT ANATOMY AND PHYSIOLOGY

1.2.1. Alpha Motoneuron. Cell bodies of alpha motoneurons are located in the ventrolateral horn of the spinal cord, are among the largest in the central nervous system, and have abundant dendritic trees (Aitken and Bridger, 1954). Their axons can be as long as a meter in mammals and give off a few collaterals before exiting the spinal cord; these collaterals make contact with Renshaw interneurons, producing the recurrent inhibition of motoneurons (Eccles et al., 1954), or they make contact with cell bodies of motoneurons in a motor nucleus. When an axon potential is conducted, all the muscle fibers innervated by that axon will be activated (Krnjevic and Miledi, 1958). That axon potential is also known to propagate more slowly in motor axons innervating type S units than in those innervating type F units. Since conduction velocity is indicative of axon diameter (Hursh et al., 1939), the axons of S units are inferred to be smaller than those of F units. There is no clear difference between axonal conduction velocities of type FF and FR units, indicating that their motor axons are similar in diameter (Burke et al., 1976).

1.2.2. **Muscle Unit.** The set of skeletal muscle fibers innervated by one alpha motoneuron is called a muscle unit (Burke, 1967). Since the experiments performed by Edstrom and Kugelberg (1968) in the rat, in which they depleted the muscle fibers of single MU's of their glycogen by prolonged activation of their motoneurons, it has been clear that muscle fibers of a given MU are scattered throughout an extensive territory. This large territory occupied by an apparently random distribution of muscle fibers of a muscle unit, leaves room for many other muscle units to share the same cross-sectional area. Burke and Tsairis (1973) estimated that the area occupied by a muscle unit can be shared by up to fifty other muscle units. Muscle fibers of a MU show similar histochemical and physiological properties (Edstrom and Kugelberg, 1968), however their diameter can vary from 2 to 8 fold (Bodine, et al., 1986).

1.2.3. **Muscle Unit Territory.** It is defined as the percentage of the cross-sectional area of a muscle occupied by one muscle unit. The glycogen-depletion technique allows a direct measurement of the muscle unit territory. In most muscles studied, the territory varies from 10 to 30 percent of the total muscle territory. In general, the muscle unit territory is relatively large and most fibers have no contact with other fibers of the same muscle unit. This scattering of muscle fibers of a MU is probably significant to the process of reinnervation of partially denervated muscles, where motor axons of the residual MU's can readily sprout and reinnervate denervated muscle fibers. If this scattering did not exist, axotomy of motoneurons which supplied contiguous muscle units would result in large denervated areas with no intact motor axons nearby. In other words, there would be an increased distance and intramuscular connective tissue between sprouting motor axons and denervated muscle fibers.

1.2.4. Innervation Ratio. It is defined as the number of muscle fibers innervated by an alpha motoneuron. Early indirect estimations made by dividing the total number of muscle fibers by the number of alpha motoneurons did not take into account the variability of ratios among different MU types. However, general conclusions were made for instance, that innervation ratio declines with muscle mass. Ratios can be as large as 600 fibers per muscle unit for large limb muscles such as the medial gastrocnemius, smaller for intrinsic muscles of the feet and hands such as the first lumbrical, whose value is about 100 fibers per muscle unit (Feinstein et al., 1955), and very small for the tiny extraocular muscles, whose values are between 13 and 20 fibers per muscle unit (Burke, 1981).

1.2.5. Twitch Contraction Time (time to peak twitch force). It gives information about the intrinsic speed of activation of the contractile machinery of a MU (Buchthal and Schmalbruch, 1980). Muscle units of a given muscle can exhibit a 3-5 fold range of contraction times (McDonagh et al., 1980).

1.2.6. **"Sag" Phenomenon**. It is defined as the decline in force produced by unfused tetani during a one second repetitive-stimulus train with intershock intervals set at 125 percent of the contraction time (Burke et al., 1971). Such a stimulation regime causes neither neuromuscular-transmission failure, nor contractile failure. Sag is exhibited only by fast units and has been used in preference to contraction time to distinguish fast from slow MU's (Burke et al., 1971). However, the mechanism of sag is not known and the usefulness of this test is controversial (Fleshman et al, 1981). After reinnervation of denervated muscles this criterion is less predictable, there are MU's that do not sag and therefore could be classified as slow despite obviously fast contraction times (Thomas., 1986).

1.2.7. **Fatigue Index**. It is a measure of the MU susceptibility to fatigue and is defined as the ratio of force developed after two minutes of tetanic stimulation to that developed at the beginning of the the test (Burke, 1973).

1.2.7. **Force**. MU's produce forces whose amplitude increases in the following order: S < FR < FF, with considerable overlap between S and FR units. The force produced by a MU depends on the following factors: innervation ratio, the average cross-sectional area of the muscle fibers, and specific tension (Burke et al., 1974). In a mixed muscle like the MG of the cat, the force can vary from 10 millinewtons (mN) for the smallest S units to 1000 mN for the largest FF units.

This structural and physiological organization allows the muscle to perform functions requiring different degrees of precision and force.

1.3 SIZE PRINCIPLE

Henneman's "size principle" refers to the orderly recruitment of MU's, starting from the smallest to progressively larger ones. This principle establishes an inverse relationship between size and excitability of a MU (Henneman et al., 1965; Henneman, 1980).

In a muscle, the smaller MU's are the first to be activated, they have axons with smaller diameters, conduct action potentials more slowly and produce smaller forces. The larger motoneurons are the last to be recruited, they have axons with faster conduction velocities, innervate muscle units that contract faster and produce bigger forces. An inverse relationship between contraction time and tetanic force is always demonstrable (reviewed by Stuart and Enoka, 1984). The linear correlation between axonal conduction velocity and force is, in general, stronger in small muscles than in large ones (Stuart and Enoka, 1984).

1.3 CORRESPONDENCE BETWEEN HISTOCHEMICAL AND PHYSIOLOGICAL PROPERTIES OF A MOTOR UNIT.

The hypothesis that the muscle fibers show histochemical uniformity, as well as physiological homogeneity within a given MU, initially proposed by Henneman et al. (1965), has proven to be correct in rat muscles (Kugelberg, 1973) and in cat muscles (Burke et al., 1973). Myosin isoenzymes can be recognized, and the preferred aerobic and/or anaerobic pathways utilized by different muscle fibers can be distinguished by histochemical identification of glycolytic and oxidative enzymes (Peter et al., 1972; Brooke and Kaiser, 1970).

Muscle fibers belonging to FF motor units use the anaerobic, glycolytic pathway and correspond to the FG (also known as IIB or FF) muscle fiber type. This type of MU is suitable for brief, high intensity activities. Muscle fibers belonging to slow motor units use the oxidative, aerobic pathway and correspond to the SO (also known as I or S) muscle fiber type. This type of MU is resistant to fatigue, and suitable for long lasting, low intensity activities. Muscle fibers belonging to FR motor units use both pathways and correspond to the FOG (also known as IIA or FR) muscle fiber type. This type of MU exhibits intermediate fatiquability and force (Burke et al., 1973).

1.4 MOTOR UNIT ADAPTABILITY

1.4.1. Adaptability to usage, exercise, electrical stimulation and cross-reinnervation. There is abundant evidence supporting the idea of MU interconversion (transformation of one type of MU into another). This evidence comes from histological, biochemical, and physiological studies of whole muscles that have been subjected to different experimental manipulations.

Kugelberg (1973) observed in the rat soleus muscle that type II fibers were replaced by type I fibers with age, suggesting a continuous redistribution of soleus MU's during the rat's life span.

Physical exercise increases the force and resistance of the muscle fibers to fatigue (Bernard et al., 1970). It also causes an increase in the staining intensity for oxidative enzymes of the muscle fibers (Bernard, 1970), which is consistent with increased resistance to fatigue. In animals on endurance training programs (treadmill running) there is an increase in FOG and a decrease in FG fibers of the plantaris and gastrocnemius muscles. FOG muscle fibers of chronically exercised animals have a greater number of capillaries and, therefore, greater aerobic capacity than FOG from control muscles. Hypertrophy of muscle fibers of the FOG fibers occurs in endurance trained muscles of the guinea pig (Edgerton et al., 1973). In man, hypertrophy of SO fibers occurs after a six month endurance training program, but no changes occur in FOG fibers (Gollnick et.al., 1973). Weight lifting selectively enlarge FG fibers (Gollnick et al., 1972). These results suggest that pattern as well as type of exercise training influences the adaptive response of each MU type.

Guth and Yellin (1971) showed in the young rat that an increase in postural load upon soleus muscles by removal of its synergists results in a relative increase of type I fibers. Watt and Goldspink (1984) studied the effects of dynamic exercise (jumping) and static overload (synergist tenotomy) on soleus muscle of juvenile rats. They found that synergist tenotomy enhanced the normal transformation of type II into type I; and that dynamic exercise delayed such transformation.

Conversion of FG and FOG muscle fibers into SO fibers has been achieved through chronic electrical stimulation (Salmons and Vrbova, 1969; Pette el al., 1973). Electrical stimulation of a motor nerve to a fast-twitch muscle at a frequency similar to that of a slow MU (10 Hz), transforms the mechanical, biochemical and histological properties of that muscle into those of a slow-twitch muscle (Al-Amood et al., 1973; Brown, et al., 1976). Transformation of the slow-twitch soleus muscle into a fast-twitch muscle has also been achieved through direct electrical stimulation of denervated soleus muscle at a 100 Hz frequency (Lomo et al., 1980).

When the nerves to a fast and to a slow muscle are sectioned, and the proximal stump of the nerve to the fast-twitch muscle is surgically connected to the distal stump of the nerve to the slow-twitch muscle or vice-versa (cross-reinnervation), the cross-reinnervated fast-twitch muscle acquires the properties of a slow-twitch muscle and the cross-reinnervated slow-twitch muscle acquires those of a fast-twitch muscle (Buller et al., 1960; Close and Hoh, 1969), supporting the idea that motoneurons control the properties of the muscle fibers they innervate.

1.4.2. **Adaptability to injury and disease.** When all the innervation to a muscle is removed by accident or under experimental conditions, the proximal segments of the severed axons can regenerate and reinnervate the muscle. In such cases, the scattered distribution of muscle unit seen in normal muscles is replaced by a more localized group of fibers (Brooke and Engel, 1966). Kugelberg and coworkers (1970) found that MU territory is smaller for a reinnervated muscle unit than for a normal one, and observed that intramuscular connective tissue was, to some extent, determining the boundaries of the reinnervated muscle unit. Evidently, the regenerating axon reinnervates muscle fibers that formerly belonged to many different MU's. After section of a nerve followed by nerve-to-nerve suture, the regenerated MU's recover their size so that the peripheral reorganization of the reinnervated muscle is still appropiate for MU recruitment during movement (Gordon and Stein, 1982).

When part of the innervation to muscle is removed, denervated muscles can be reinnervated either by sprouting of intact motoneurons ("collateral sprouting") or by regenerating axons ("axonal regeneration"). It has been known for a long time that muscles are able to recover their force after partial denervation; however, it was not until the 1940's when scientists (van Harreveld and Weiss and Edds, 1945) investigated the question of whether hypertrophy of normally innervated muscle fibers or reinnervation of denervated muscle fibers was responsible for such compensation. Weiss and Edds in 1945 reported restoration of force after partial denervation of rat soleus muscle. They sectioned one of the two contributing spinal roots to the innervation of the soleus muscle and capped its proximal end to prevent axonal regeneration. Stimulation of the remaining neurons, contained in the intact spinal root, showed a progressive increase in muscle force. They concluded that force recuperation in partially denervated muscles was mainly due to reinnervation of denervated muscle fibers by collateral sprouting. A later compensatory process may take place as the cut axons regenerate and reinnervate the denervated muscle fibers. Karpati and Engel (1968) demonstrated in experimental animals that the grouping of muscle fibers of the same histological type ("type grouping") observed in biopsies from patients with chronic peripheral neuropathies, was due to reinnervation of denervated muscle fibers by regenerating axons. These processes of reinnervation (collateral sprouting and axonal regeneration) constitute important adaptive responses to compensate for partial denervation, the understanding of which has relevant implications in the clinical field where accidents and peripheral neuropathies often result in partially denervated muscles.

Partial or total denervation of a muscle can also result from diseases affecting the motoneurons (cell body or axon). Muscle biopsies from patients suffering myopathies of neurogenic origin show grouping of atrophic fibers. Changes observed in muscle biopsies are not specific to any disease, in patients with motoneuron diseases findings such as muscle fiber atrophy and muscle fiber type grouping suggest denervation, reinervation or both. The severity of the changes varies with the degree of weakness, duration of the disease, and rate of progression of the disease (Mokri, 1977). Dubowitz and Brooke (1973) report that patients with amyotrophic lateral sclerosis suffer a more pronounced atrophy of the type IIB muscle fibers. However, this or any other histological finding suggesting a selective participation of certain MU types has to be interpreted with caution, since they may be reflecting adaptive changes rather than selective susceptibility of a MU type to the disease. The histochemical study of cat muscles with different extents of denervation and different periods of time after partial denervation may reflect some similarities in changes with those exhibited by patients with motoneuron diseases and may help clarify the origin of these changes.

1.5. PURPOSE OF THE STUDY

The purpose of this work was to investigate the process of reinnervation of partially denervated triceps surae muscles of the cat with respect to:

1) The capabililty of residual motor units to enlarge.

2) The possible differences among motor unit types in their capabilities to enlarge.

3) The efficacy of axons regenerating for long distances to reinnervate denervated muscle fibers, and to displace collateral sprouts from reinnervated muscle fibers.

4) The effect of muscle unit enlargement on motor axon size.

5) The changes in muscle unit properties of residual and regenerated motor units. 6) The possible influence of muscle on the processes of collateral sprouting and axonal regeneration.

CHAPTER TWO: MATERIALS AND METHODS

Experiments were conducted on 21 cats, 3 kittens and 18 young adults. Control MU's were studied from 5 normal cats, while experimental MU's were studied in 16 animals.

Partial denervation of the muscle under study (medial gastrocnemius, lateral gastrocnemius and/or soleus) was achieved through rhizotomy of one of the two ventral roots (S1 or L7) innervating the muscle; the right side of the animal was always experimental while the left side served as control for measurement of the total muscle forces and determination of the electrical charges in the nerves.

All surgical operations were conducted under sodium pentobarbital anesthesia. An initial dose of 40 mg/Kg of body weight was administered intraperitoneally and supplemental doses were given intravenously to keep satisfactory levels of anesthesia.

2.1 PARTIAL DENERVATION. Under general anesthesia and aseptic conditions, the experimental animal underwent a laminectomy from S1 to L7, and either S1 or L7 right spinal root was cut extradurally. After surgery, the animals were maintained freely moving in the animal room during day time, and in large cages during night time, for periods of time ranging from 2 to 18 months.

2.2 ACUTE EXPERIMENTS. Under general anesthesia, a wide laminectomy from S2 to L5 was performed. All muscles of the hindlimbs and hips were denervated, except the muscle(s) under study. The nerve to the medial gastrocnemius muscle

(MG) or the nerve to the lateral gastrocnemius and soleus muscles (LGS) was dissected free for a length of about 20-25 mm proximal to the muscle. An electromyogram (EMG) probe containing 2 surface electrodes was sutured onto the surface of the muscle(s) of interest. The muscle was also dissected free, except for its proximal attachments. Care was taken to minimize damage to tissue and blood supply. A small piece of the calcaneous bone was left attached to the tendon for a secure tie. The tendon was attached to a force transducer, preserving the anatomical position of the muscle. A tracheostomy was performed in all animals, only three cats needed artificial ventilation . A vein was also dissected for administration of maintainance doses of anesthesia.

The cats were mounted in a stereotaxic frame and immobilized with clamps at the levels of the head, hip, knees and ankles. The skin flaps surrounding the laminectomy and the incisions on the legs were pulled up to form 3 pools, which were filled up with mineral oil at 37 degrees Celsius. Temperature probes were placed into the pools as well as into the rectum. Temperature was kept at about 35 degrees Celsius by means of a heating pad and a radiant lamp. Once nerve and muscle were immersed in oil, the L6, L7 and S1 dorsal and ventral roots were successively dissected free and cut as proximally as possible. The nerve to the muscle was mounted over five silver electrodes spaced 1-2 mm apart, this array served for stimulation of the nerve and recording of action potential of single motor axons.

2.3 ELECTRICAL CHARGE. Electrical charge generated in spinal roots has been previously used as an indication of the average diameter of nerve fibers (Hoffer et al., 1979). We used electrical charge to obtain an estimate of the contribution of each root to the motor innervation of the muscle in the control side. The summation of S1 and L7 charges gave us the total charge, which was taken as 100% of the motor innervation to the muscle. The charge measured on any one of the two roots in response to stimulation of the muscle nerve could then be expressed as percentage of innervation. By comparison, the percentage of denervation originally achieved on the experimental side could be estimated.

Electrical charges of L6, L7 and S1 dorsal and ventral spinal roots were determined as described by Hoffer et al. (1979). Dorsal and ventral roots were bilaterally prepared and successively mounted on a array of five electrodes each spaced 2 mm apart. Electrical impedance of each root was determined by measuring the impedance at each electrode with respect to the cut end of the root, using a 10 Hz signal. Impedances were plotted as a function of interelectrode distance, from the regression line, the tissue impedance of the root was calculated by subtracting the contact impedance (given by Y-intercept of the regression line) from the total value between the recording electrodes. The common peroneal (CP), lateral gastrocnemius-soleus (LGS) and medial gastrocnemius (MG) nerves were each stimulated and their compound monophasic action potentials (CAP) were recorded in each root and digitized using a laboratory microcomputer (LSI 11/23). Electrical charge was obtained by dividing the area under the curve by the impedance of the root.

2.4: TOTAL MUSCLE FORCES. The tendon of the muscle was attached to a 10 Kg grass strain gauge, which was mounted on a manipulator to adjust muscle position and obtain the maximal length. Muscles forces were measured in the control and experimental muscles in response to stimulation of its muscular nerve and in response to stimulation of its contributing ventral roots. Extent of denervation originally achieved by root section in the experimental side was again estimated by comparison with the normal side. Summation of forces produced by the muscle in response to stimulation of S1 and L7 was taken as 100% of innervation and, the force produced by stimulation of any of the two roots could be expressed as percentage of innervation on the control side. The two methods of estimating the percentage of denervation (electrical charge and muscle forces) agreed very well (table 3.1).

2.5. MOTOR UNIT RECORDING. Single MU's were isolated by splitting the ventral roots until a single functional axon to the muscle of interest could be identified in a root filament. The criteria for identifying single MU's were all-or-none action potentials recorded on the muscle-nerve and the absence of changes in EMG configuration and force production. For each MU, the extracellular action potential recorded on the MG or LGS nerve and the EMG and force recorded on the corresponding muscle, were simultaneously displayed on a storage oscilloscope with variable persistance and digitized on the LSI microcomputer. Force and EMG were also displayed on a Gould pen recorder.

Once a single MU was isolated, the following measurements were made:

2.5.1. Twitch Force and Twitch Contraction time. These were obtained by averaging 30 responses to 1 Hz stimulation of the ventral root filament at 2-4 times the threshold voltage for the axon.

2.5.2. Tetanic Force. The tetanic force was averaged from 5-10 responses to trains of 20 pulses at intervals corresponding to 40% of the twitch contraction time.

2.5.3. Posttetanic Potentiation (PTP). In order to standardize the test, the PTP was obtained immediately after 5 trains of 20 pulses, by averaging 30 responses to 1 Hz stimulation.

2.5.4. Sag Test. Sag was evaluated in the unfused tetanic contraction in response to 800 ms of stimulation using an interstimulus interval of approximately 125% of the twitch contraction time.

2.5.5. Fatigue Index (FI). This was determined by the ratio of the force produced at the beginning and after 2 minutes of tetanic stimulation of 40 Hz for 330 ms at 1 second intervals.

2.5.6. EMG and axonal action potential. The EMG amplitude, the axon potential amplitude, and the axonal conduction velocity, were also studied and analyzed.

2.6. ANALYSIS OF DATA

Through this thesis motor units are represented as follows: control: squares, sprouted: triangles, and regenerated: diamonds. Symbols for slow motor units are filled, while those of fast motor units are empty.

Most of the MU data are plotted on scatter diagrams and straight lines are fitted according to a least mean squares criterion (Sokolnidoff and Redheffer, 1958). Statistical T tests were performed on the muscle fiber diameter data and the probability level $p<0.05$ was chosen for significance.

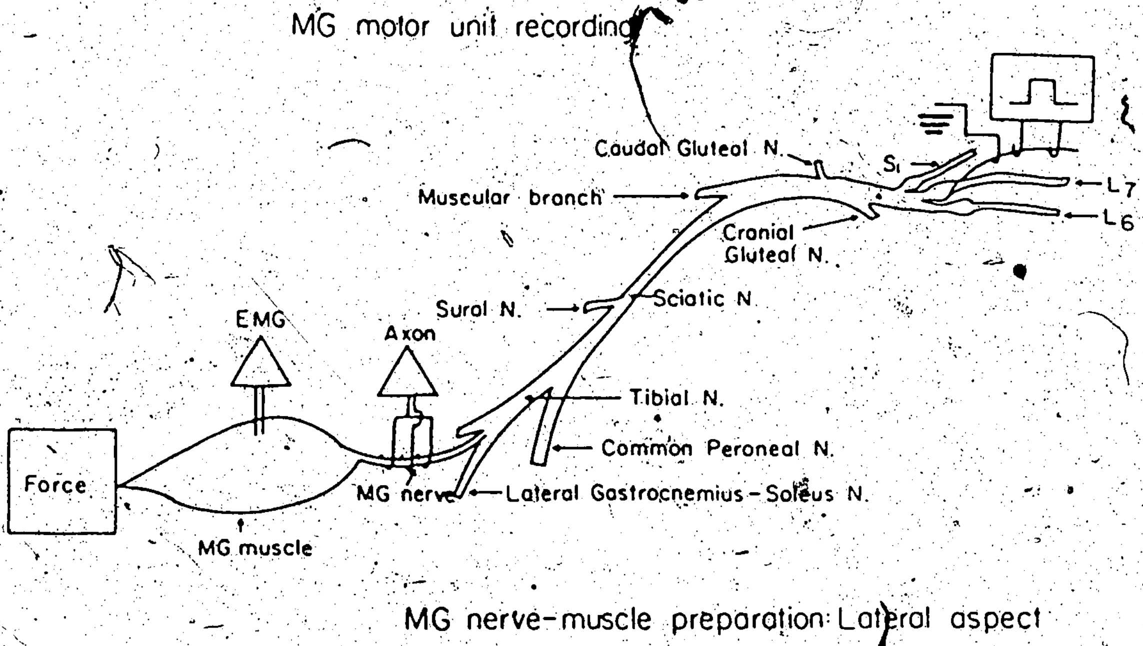


Figure 2.1. Motor unit recording. After denervation of all muscles of the leg but the one under study, single MU's were characterised by stimulation of ventral root filaments containing only one functional axon. Axon potential amplitude was recorded on the nerve and EMG amplitude on the muscle. Muscle unit force was measured through a force transducer.

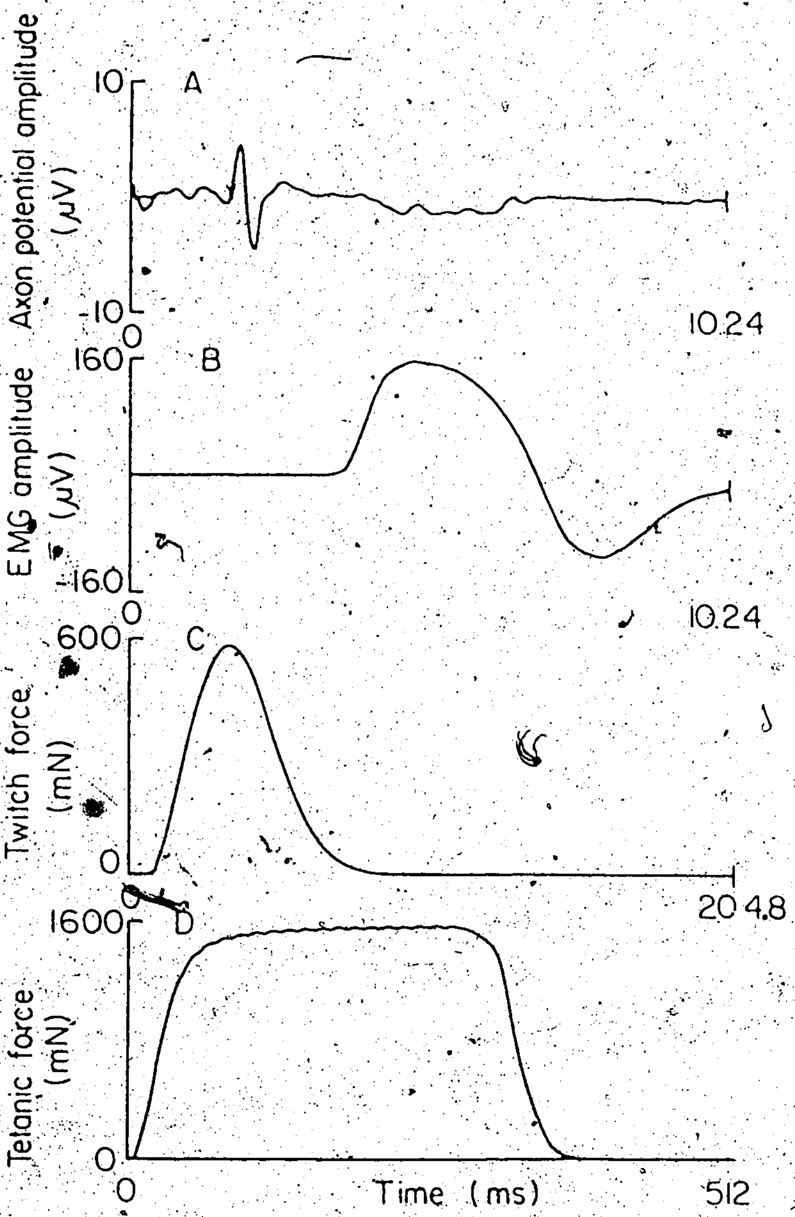


Figure 2.2. Motor unit properties. After isolation of a single MU, axon potential amplitude (graph A), EMG amplitude (graph B), and muscle unit force in response to single stimulus (graph C) and to a tetanic stimulus (graph D) are recorded.

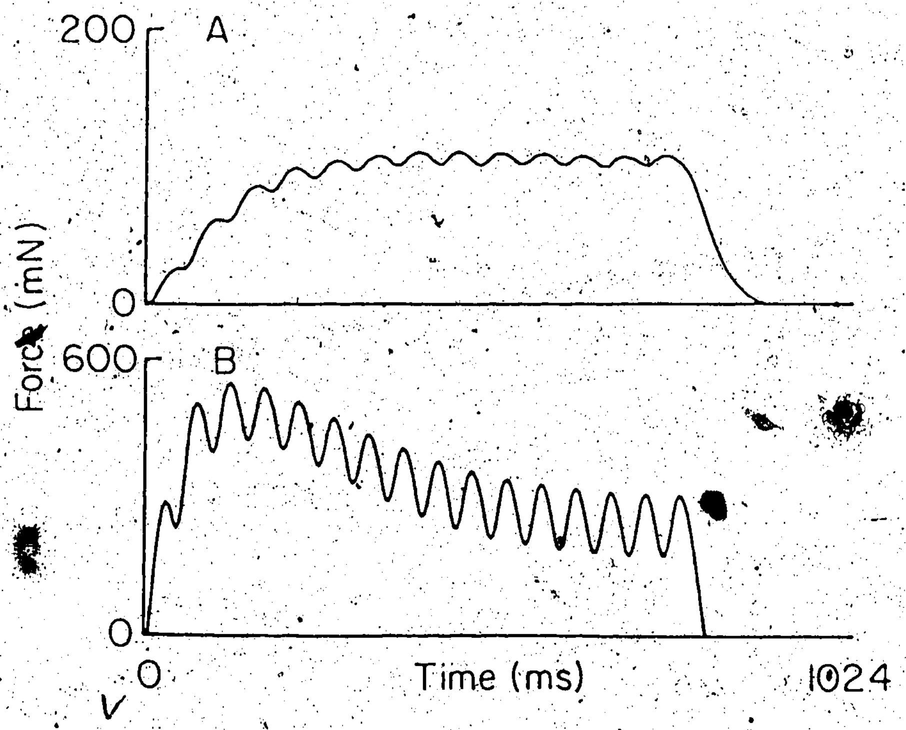


Figure 2.3. The sag phenomenon. The sag property is evaluated in the unfused tetanic contraction in response to 800 ms of stimulation using an interstimulus interval of approximately 125% of the twitch contraction time (Burke et al., 1971). A) Some MUs do not sag and ~~are~~ classified as slow and B) some sag and are classified as fast.

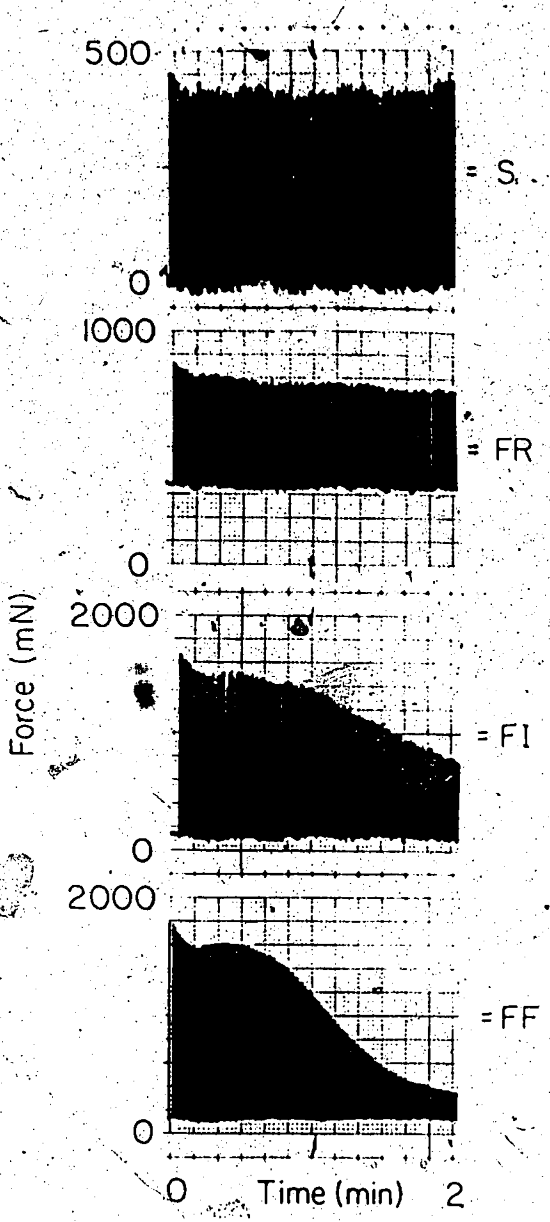


Figure 2.4. Motor unit fatiguability. It is determined by the ratio of the force produced at the beginning and after 2 minutes of tetanic stimulation of 40 Hz for 330 ms at 1 second intervals. On the basis of their susceptibility to fatigue MU's are subdivided into S (fatigue index > .75), FR (fatigue index > .75), FI (fatigue index > .25 and < .75), FF (fatigue index < .25).

CHAPTER THREE

MOTOR UNITS OF PARTIALLY DENERVATED MEDIAL GASTROCNEMIUS MUSCLE OF THE CAT

3.1. INTRODUCTION

When a muscle is partially denervated, the remaining motoneurons sprout and reinnervate neighboring denervated muscle fibers. This enlargement of the MU by collateral sprouting is an important compensatory mechanism for partially denervated muscles.

Histological and physiological studies of partially denervated rodent hindlimb muscles, such as rat soleus (Weiss and Edds, 1946; Thompson and Jansen, 1977; Gorio et al., 1983), rat anterior tibialis (Kugelberg, Edstrom and Abbruzzese, 1970), mouse peroneus tertius (Brown and Ironton, 1978) and others, have shown that MU's can enlarge by up to 3-7 times their original size. This consistency of results among studies has led to the generally accepted idea that MU's can only enlarge their original sizes by up to 5 times. This 5-fold increase appeared to be the upper limit beyond which motoneurons are unable to adequately compensate for any further denervation (Jansen and Thompson, 1977; Gorio et al 1983). However, a recent study of partially denervated flexor digitorum longus muscles of young cats indicates that the capacity of a motoneuron to extend its territory is very much greater than previously suggested (Hatcher et al.

1986). The limited compensation seen in small rodent muscles after partial denervation may not necessarily be a generalized phenomenon for partial nerve injuries. The question addressed in the present study is whether there really is a limit in the capability of MU's to enlarge: is the maximum size of sprouted MU's described in the literature a true indicator of an intrinsic limitation of the sprouting motor axon, or, are the size and type of the experimental muscles studied to date, and the extent of muscle denervation, artificial constraints imposed upon the capability of the remaining motor axons to sprout?

A second question that we addressed here is whether all MU's possess the same capability to enlarge. The possibilities are: a) that all MU types exhibit a similar capability to enlarge, where all MU's enlarge by the same amount regardless of their original size or extent of muscle denervation, b) that all MU's enlarge in proportion to their original size; in this case, MU's would increase by the same factor with respect to their original size, with large MU's compensating more for partial denervation due to their much bigger original size, which would be in accordance with the "size principle", and c) that MU types exhibit differences in their capability to enlarge, the best responding MU type will enlarge by a bigger factor than the rest in all extents of denervation if there is no limit to that capability. Because distance between denervated muscle fiber and responding motor axons will play a role regardless of MU type, especially where there is very little denervation, any preferential response of MU type that may exist, will become evident only under considerable muscle denervation.

3.2: MATERIALS AND METHODS

Experiments were conducted on 11 young adult cats of both sexes. Final acute experiments were carried out between 2 and 11 months after the initial operation (see table 3.1). At the time of the final acute experiments, the cats were between 8 and 21 months of age and weighed between 3.0 and 4.0 Kg (see general materials and methods for details). One hundred and eighty-six MU's were studied. Sixty-one MU's were studied in 3 control cats and 125 MU's were studied in 8 experimental cats.

3.2.1. Chronic Partial Denervation. Under general anesthesia and aseptic conditions, the experimental animals underwent a laminectomy from S1 to L7. On the right side, the S1 spinal root was cut extradurally. No attempt was made to prevent axonal regeneration after surgery. The animals were maintained freely moving in large cages.

3.2.2. Extent of Muscle Denervation. Because the relative contributions of the two roots to the innervation of the MG muscle varies among animals, section of S1 ventral root resulted in different degrees of partial denervation among the cats. The relative contribution of the cut root, indicative of the extent of denervation, was estimated by two methods.

1) Electrical Charge. Electrical charge (Q) generated in spinal roots has been previously used as an indication of the average diameter in a population of nerve fibers (Hoffer et al., 1979). The summation of charges generated in S1 and L7 ventral roots upon stimulation of the MG nerve is the total charge, which is taken as a measure of the total motor innervation of the muscle. The charge generated in each ventral root, S1 and L7, when expressed as percentage of the total charge, provides a reasonable estimate of the proportion of motor axons to the MG nerve contained in each root. Since animals are consistently symmetrical in their root contributions to a muscle (Hoffer et. al., 1979), estimations of the percentage of motor axons in S1 on the control side provide a measure of the percentage of motoneurons axotomized by section of the S1 on the contralateral side.

2) Muscle Force. Maximum indirect twitch and tetanic forces were recorded from the normally innervated MG muscles in response to stimulation of S1 and L7 ventral roots. Muscle force in response to stimulation of S1 was expressed as a percentage of total muscle force from the control side to provide an independent measure of the proportion of motor axons in each root and therefore the extent of partial denervation. Since the two methods provided consistently values which agreed (see table 3.1),

an average of the two was taken, in most cases, as the measure of percentage of denervation.

3.2.3. Acute Experiments. For details on MU recording see general methods.

3.3. RESULTS

The motor axons innervating the MG muscle exit the spinal cord through S1 and L7 ventral roots. Although the contribution of S1 ventral root to the innervation of the MG muscle varied considerably, from 14 to 88 %, there was a predominance of motor axons exiting through S1 ventral root as seen from the root contribution (table 3.1). Figure 3.1 shows a typical example of the contributions of the 2 roots to MG force on the control side (graph A), and the complete compensation by L7 just 2 months after section of S1 on the experimental side (graph B). The speed of contraction shown in graph B is also typical of partially denervated muscles, and reflects the increase in the number of FI units. Two to 11 months after root section all partially denervated MG muscles except one (E.1, table 3.1) recovered their original force.

One hundred and eighty-six MU's were studied in 11 cats, 3 control and 8 experimental. Control MU's: 61, "sprouted": 93, and regenerated: 32. No precautions were taken to prevent regeneration of the cut S1 root, which regenerated successfully in a number of cases, particularly for periods longer than 4 months. These animals and their MU data are

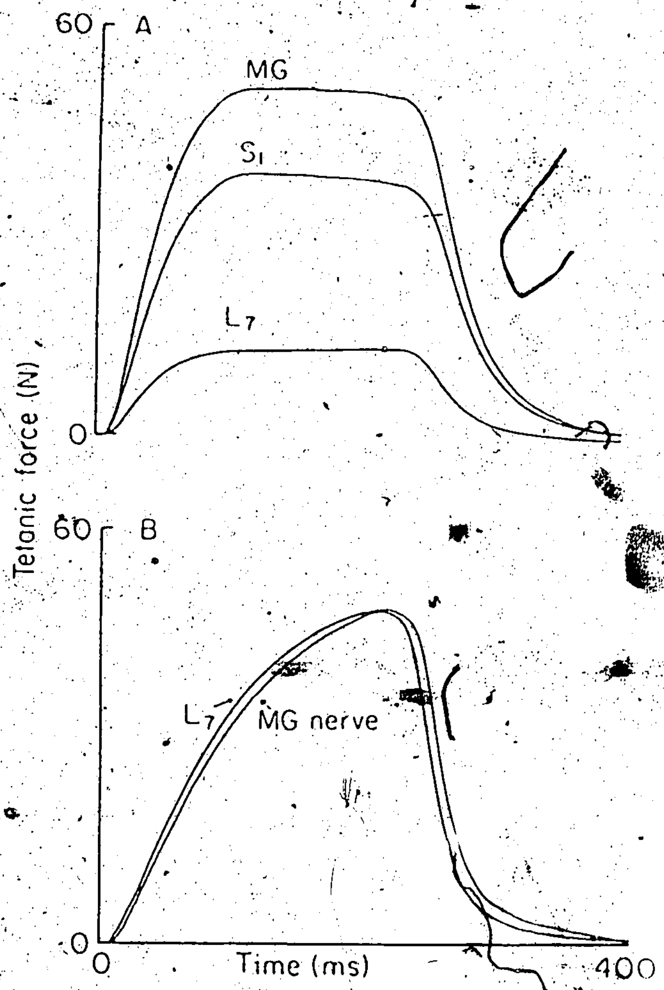


Figure 3.1. Sprouting of the intact ventral root. Two months after section of S1 spinal root on the right side, forces were recorded on the control (graph A) and on the experimental side (graph B). L7 fully compensated for the 75% denervation caused by S1 section.

TABLE 3.1. MEDIAL GASTROCNEMIUS DATA.

Cat.	Age at time of: Dener. (---months---)	Age at time of: Exp. (---months---)	Time after dener.	Weight @ Exp. (Kg)	% of force recup.	Contribution of S1 root to MG nerve (%). mean	F	Q	Number of Motor units sampled. Spr.	Regen.	Group
C.1	-	9	-	3.2	-	64	64	-	132	0	I
C.2	-	10	-	3.5	-	75	75	-	23	0	I
C.3	-	8	-	3.3	-	59	59	-	6	0	I
E.1	13	20	7	3.0	80	14	22	7	18	0	II
E.2	12	16	4	3.0	105	47	47	47	31	0	III
E.3	10	19	9	3.4	100	51	55	16	18	0	III
E.4	10	12	2	3.0	100	75	75	-	16	0	IV
E.5	13	17	4	3.0	120	88	94	82	9	10	IV-V
E.6	1	11.5	10.5	3.5	104	88	77	99	-	5	V
E.7	14	16.2	4.1	4.0	66	87	87	87	0	11	V
E.8	12	20.2	8.2	3.6	102	43	-	43	-	6	V

TABLE 3.1. MEDIAL GASTROCNEMIUS DATA. Motor unit data were obtained in 3 control (C) and 8 experimental (E) cats varying in age from 9 to 20.2 months, and in weight from 3.0 to 4.0 Kg. S1 spinal root was cut at approximately 1 year of age (except E.6 which was 1 month old and E.7 in which S1 and L7 were cut), and motor units were studied from 2 to 10.2 months later. Extent of denervation was established by 1 or 2 of the following methods: a) recording tetanic force (F) of unoperated contralateral muscle in response to stimulation of L6, L7, and S1 ventral roots, and b) recording electrical charge (Q) on L6, L7, and S1 ventral roots in response to stimulation of the MG nerve. The contribution of S1, expressed as a percentage, obtained from these two procedures was averaged and the result was taken, for most cases, as percentage of denervation. In 3 animals (E.6-8) the cut root regenerated and reinnervated the muscles. MU's were grouped as follows: I=MU's from control muslces, II=MU's from muscles denervated < 25%, III=MU's from muscles denervated 50%, IV=MU's from muscles denervated >75%, and V=regenerated MU's.

considered later in this chapter. MU's were collated according to extent of muscle denervation in one the following groups: I) MU's from normal MG muscles, II) MU's from MG muscles with less than 25% denervation, III) MU's from MG muscles with about 50% denervation , IV) MU's from MG with more than 75% denervation, and V) regenerated MU's.

3.3.1. **Control Motor Units.** Although MU populations of cat MG muscle have been extensively described, MU's from 3 cats of comparable weight to that of experimental cats were used as normal data for direct comparison. While MU force, contractile speed and fatiguability compare well from study to study, direct comparisons of axon size from measurements of action potentials are best made with recording electrodes with the same configuration.

Normally motor axon size, as measured by the amplitude of the action potential or conduction velocity, determines axonal conduction velocity, and is directly correlated with tetanic force. Contraction speed of the muscle units is inversely correlated with its tetanic force (McPhedran et al., 1965; Gordon and Stein, 1982). These relationships are shown in figure 3.2.

The classification of MU's into 4 types on the basis of contraction time and susceptibility to fatigue is shown in figure 3.3. The MG units, shown as individual points, are distributed as follows:, 29 percent belonged to the slow type, 23 percent to the FR type, 5 percent to the FI type,

Figure 3.2. Motor unit size relationships. On logarithmic scales, axonal conduction velocity increases as a function of axon potential amplitude (graph A). Tetanic force is also proportional to axon size (graph B). Twitch contraction time is inversely proportional to tetanic force (graph C). (r: A=.68, B=.72, and C=-85).

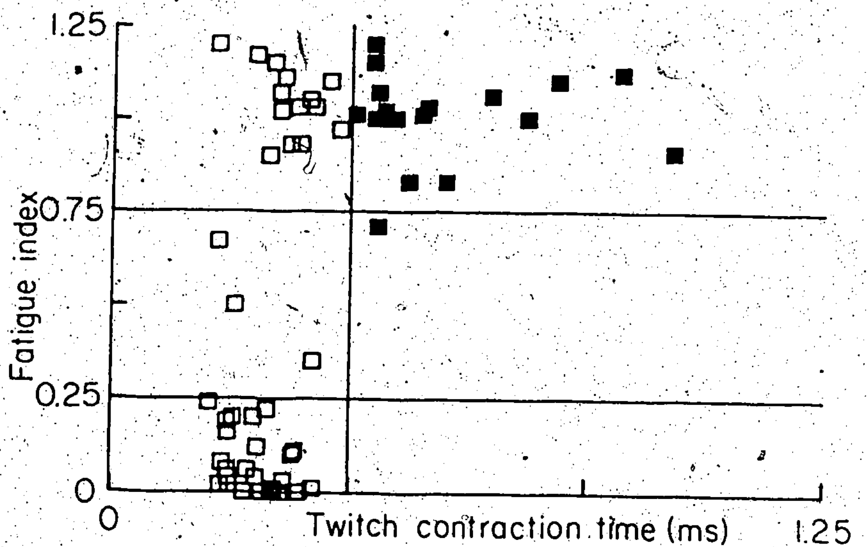


Figure 3.3. Classification of MG motor units. MU's are divided into two groups on the basis of their twitch contraction time. MU's with twitch contraction times of 40 ms or less were grouped as fast (empty squares). MU's with twitch contraction times greater than 40 ms were grouped as slow (filled squares). Fast MU's in turn were subdivided into three types on the basis of their susceptibility to fatigue: FF (fast contracting and highly fatiguable units; fatigue index <0.25), FI (fast contracting and moderately fatiguable units; fatigue index >0.25 but < 0.75) and FR (fast contracting but resistant to fatigue units; fatigue index > 0.75).

and 43 percent to the FF type. This distribution of MU types is similar to that reported previously (Burke et al. 1973; Munson et al. 1986)

When fatigue index and tetanic force of MU types are plotted (figure 3.4), we can see that force varies from as little as 10 mN for the smallest S units to as much as 1000 mN for the largest FF units. It also shows that MU's resistant to fatigue (fatigue index > .75), including slow contracting (S) and fast contracting (FR), generally produce force of less than 300 mN, whereas easily fatiguable and fast contracting (FF and FI) units generally produce tetanic forces between 300 and 1000 mN.

3.3.2. "Sprouted" Motor Units.

a) **Whole Population.** The average tetanic force produced by MU's from partially denervated MG muscles is compared to that of normal MU's in figure 3.5. Assuming that all MU's enlarge by the same factor, these results would [illegible] increases of 1.07, 1.27 and 6.33 times for MU's from slightly, moderately and severely denervated MG muscles respectively, when compared to normal MU's. However, comparisons of arithmetic means provide only a rough estimate of the response of the MU population, because MU force is not normally but logarithmically distributed (Gordon and Stein, 1982). Forces of different MU types are therefore compared in figure 3.6, where axon potential amplitude (graphs A-D) and fatigue index (graphs E-H) are plotted as a function of log tetanic force for the 4 groups

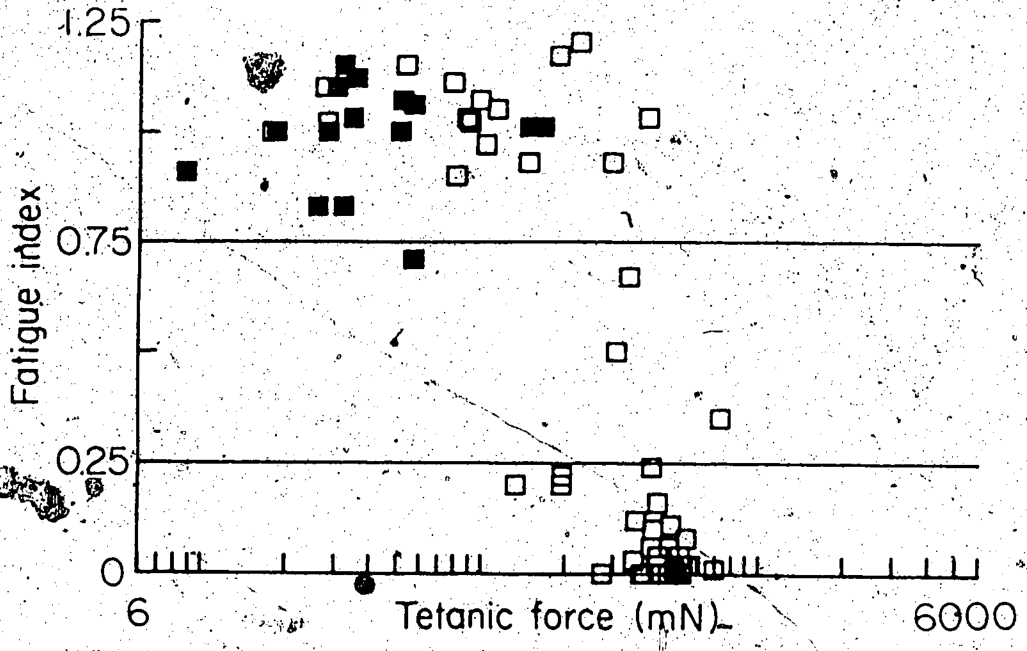


Figure 3.4. Tetanic force produced by the different MU types. Slow units are shown as solid squares and fast units are shown as open squares. The latter group is separated on the Y-axis by their fatiguability as described in figure 3.2. MU's produce force in the following order FF (mean:427.9, SD:127.7 mN) > FR (mean:112.7, SD:78.6 mN) > S (mean:54.6, SD:44.9 mN). FI units are normally very few and can produce as much force as FF.

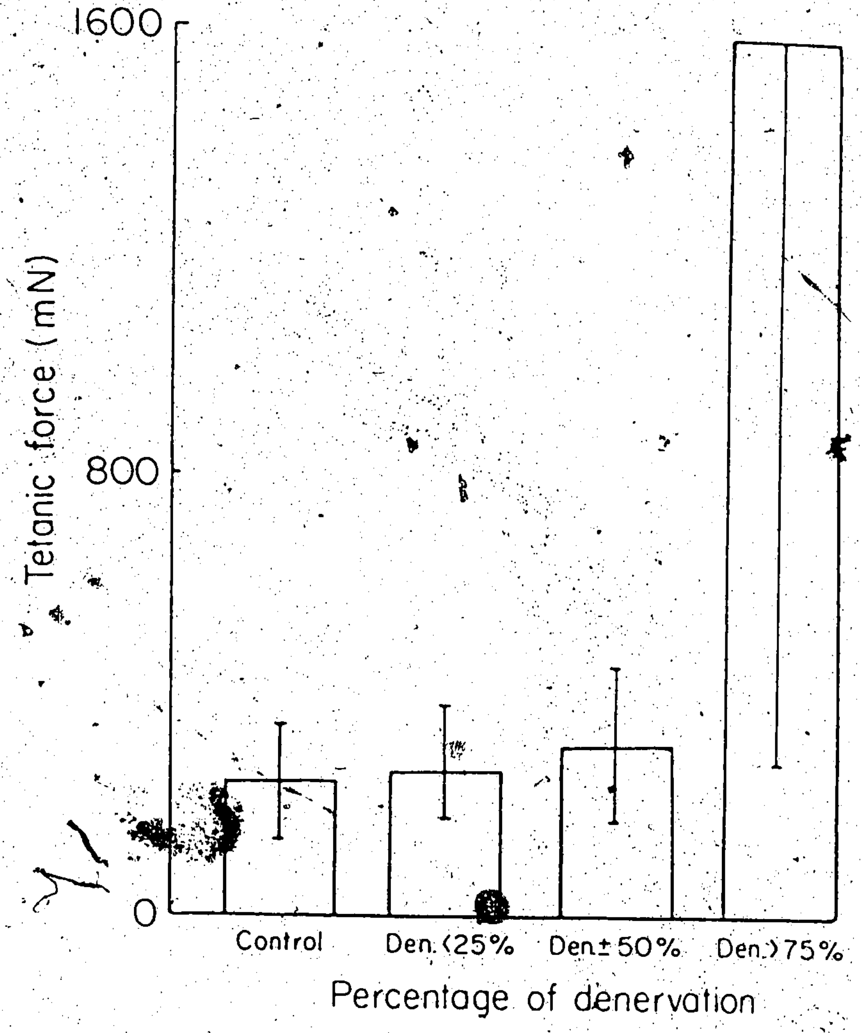


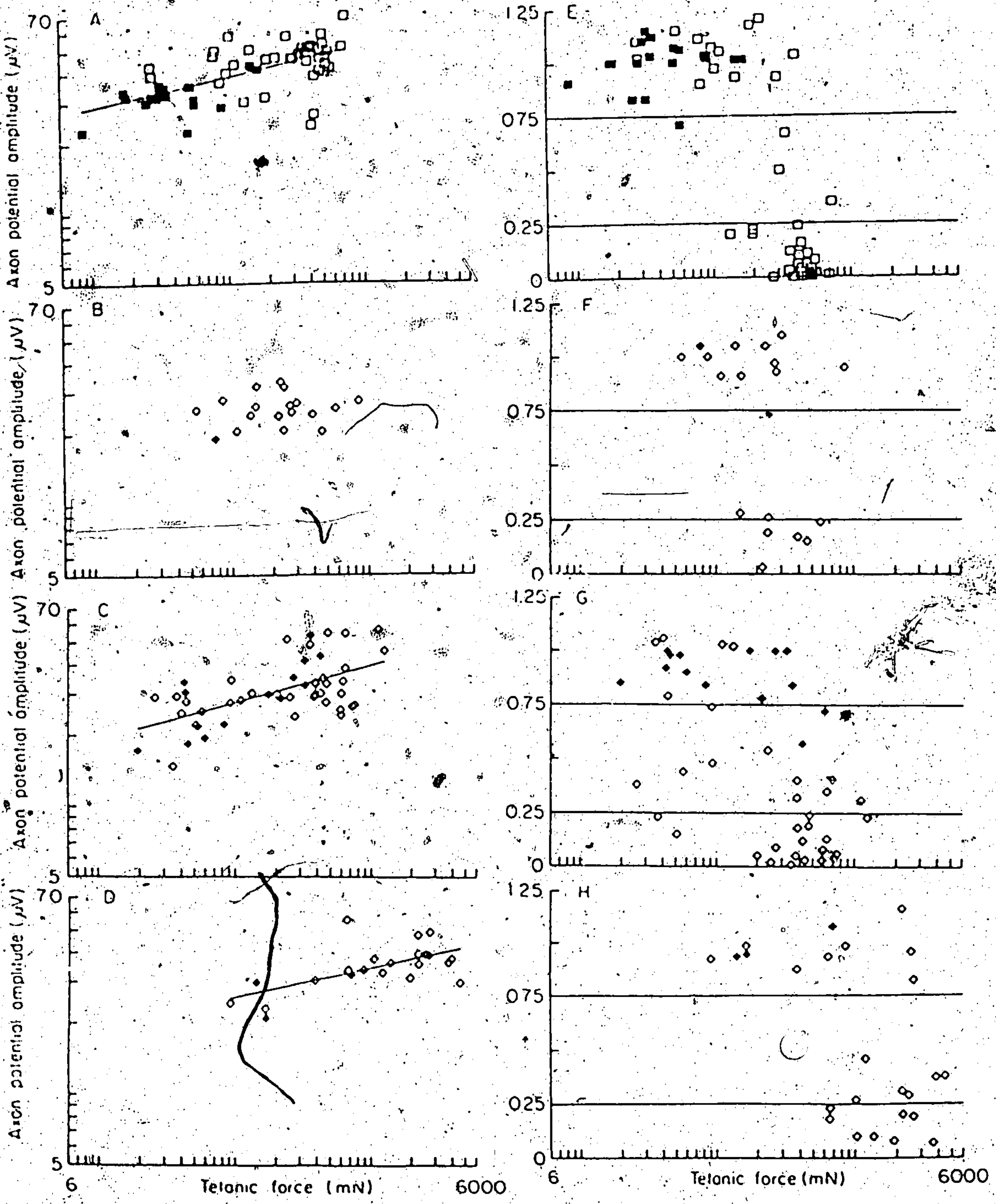
Figure 3.5. Mean (± S.E.) tetanic force produced by control and by experimental MU's from partially denervated MG muscles. MU populations from MG muscles with different extents of denervation: 0% (control, n=61), < 25%. n=25, ≈50%. n=19, and >75%. n=49.

of sprouted MU's. The whole range of MU force moves progressively to the right along the force axis as the percentage of denervation increases from less than 25 to more than 75 percent. MU force increases without alteration in axon size, since the line does not move along the y axis. The shift in the range of force is also seen when MU's fatiguability is plotted as a function of tetanic force (graphs E-H). Note that there is more overlap of MU type on the force axis.

Increases in force of MU's remaining in the partially denervated muscle is likely due to increased number of muscle fibers per unit (increased innervation ratio) and not to hypertrophy of muscle fibers, because fiber diameter of the four identified muscle fiber types, which correspond to the four MU types, were not statistically different from normal in any of the 4 groups. This is illustrated in figure 3.7 for the muscle group that includes muscles denervated by more than 75%.

3.3.2.2. Motor Unit Types. If MU response to partial denervation varies according to type, this would be most obvious in a direct comparison of the smallest S type and largest FF type. Figure 3.8 shows a proportional increase of MU size with partial denervation. Motor unit tetanic force (geometric means for all MU's in each experiment. n:61, 18, 31, 18, 16, 9) is plotted versus extent of muscle denervation. Graph A contains means for all MU types from each experiment and shows a proportional increase of MU

Figure 3.6. Enlargement of MU size with partial denervation. Graphs A-D are log plots of axon potential amplitude as a function of tetanic force for increasing percentages of partial denervation (A: 0, B:<25, C:=50, and D:>75), (r: A=.58, B=.47, C=.64, and D=60). In graphs E-H fatiguability is plotted versus tetanic force for the same groups of MU's.


A
B
C
D
E
F
G
H
70
5
1.25
0.75
0.25
0
Axon potential amplitude (μV)
Tetanic force (mN)
6
6000

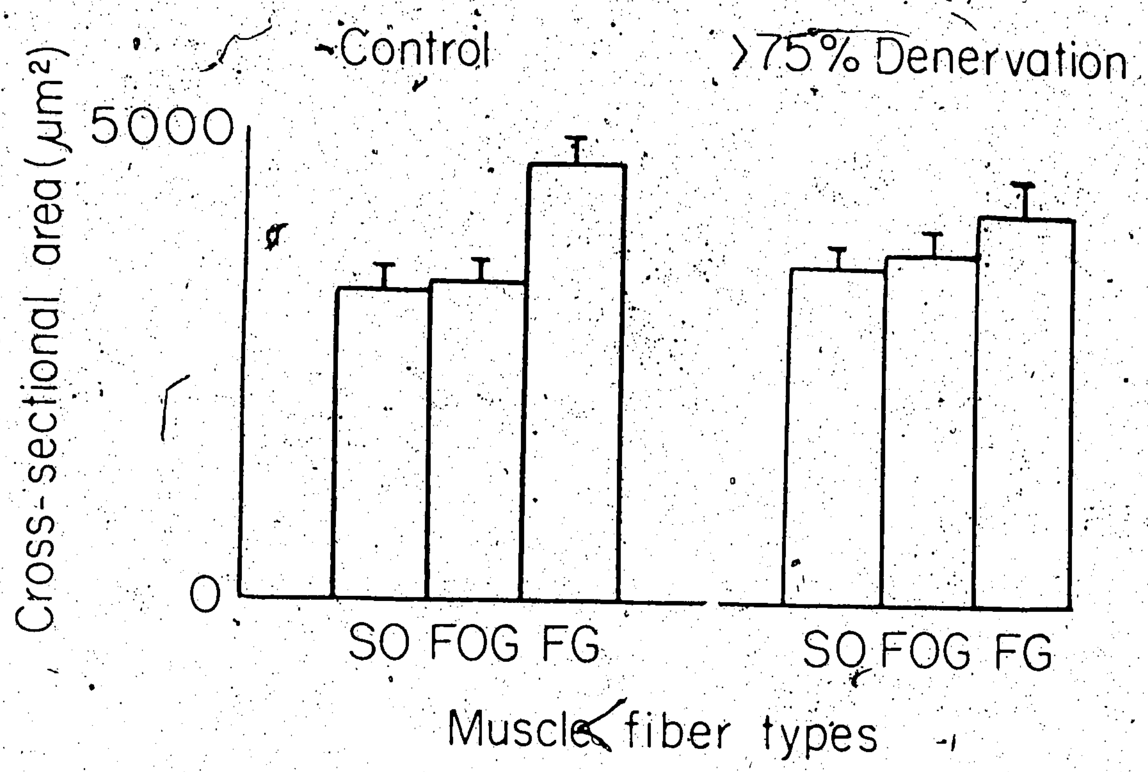


Figure 3.7. Cross-sectional area of muscle fiber types in control and partially denervated MG muscles. Means ± SE are drawn. There is no significant difference between values for control and experimental muscle fibers as determined by a t-test for differences between means ($p \leq 0.05$). The pattern of SO < FOG < FG observed in control muscles is maintained by denervated muscles. Since there is no increase in muscle fiber diameter, the increase in MU tetanic force is a good indication of muscle unit enlargement.

size with extent of denervation. However, graph B shows that this MU expansion is not equally shared by all types of MU's. The size of slow MU's increases steadily, while that of FF units appears to increase less and compensate relatively less for smaller than for larger extents of denervation. Differences in response between S and FF motor unit types are shown in figure 3.9. Graph A shows a progressive enlargement of slow MU's in response to increasing extents of muscle denervation. Graph B shows that FF enlarge predominantly in response to severe extents of denervation.

There is a decrease in the range of muscle unit speed. [illegible]ch is mainly due to an increase in slowest contracting MU's, but also to a decrease of the fastest contracting. See figure 3.10.

3.3.3. Regenerated Motor Units.

Motoneurons are capable of regenerating their axons after axotomy. After 4 months of denervation we observed axonal regeneration from the cut spinal root to the denervated muscle in 4 animals. Three out of the 4 muscles were severely denervated. In all but one (E.5) case, the muscle force elicited by stimulation of the regenerated root was only a small part of that produced by the whole muscle (<20%). In every case, recovery of force was obtained from sprouting of axons in the intact root.

Thirty-two regenerated MU's (regenerated motor axon plus rearranged muscle unit) were studied in these 4 cats

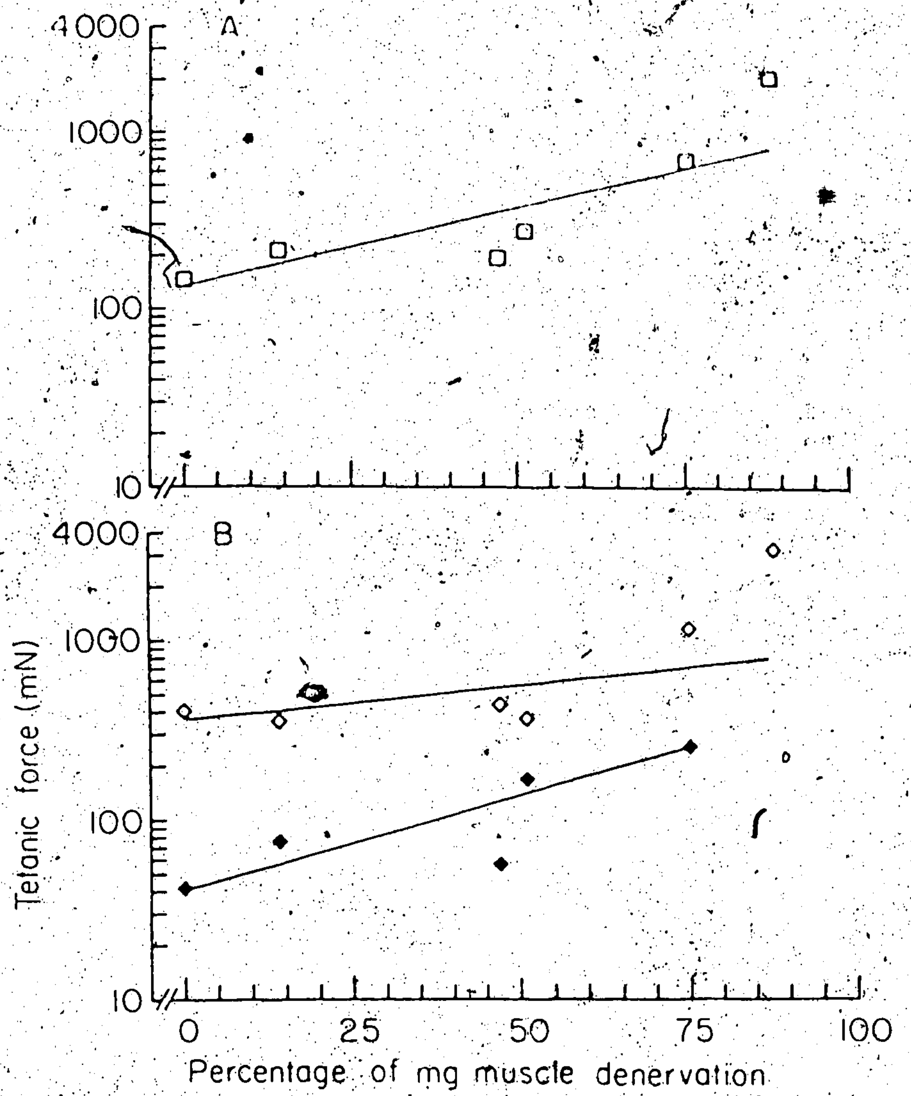


Figure 3.8. Proportional MU expansion with partial denervation. MU size, as measured by its tetanic force, increases with extent of partial denervation. Geometric means from individual populations for A) all (empty squares), and for B) FF (empty diamonds) and S (filled diamonds) units are plotted as a function of extent of denervation. Regression lines were fitted to all (n=61), all slow (n=18) and all FF (n=25) units, respectively, and not to geometric means.

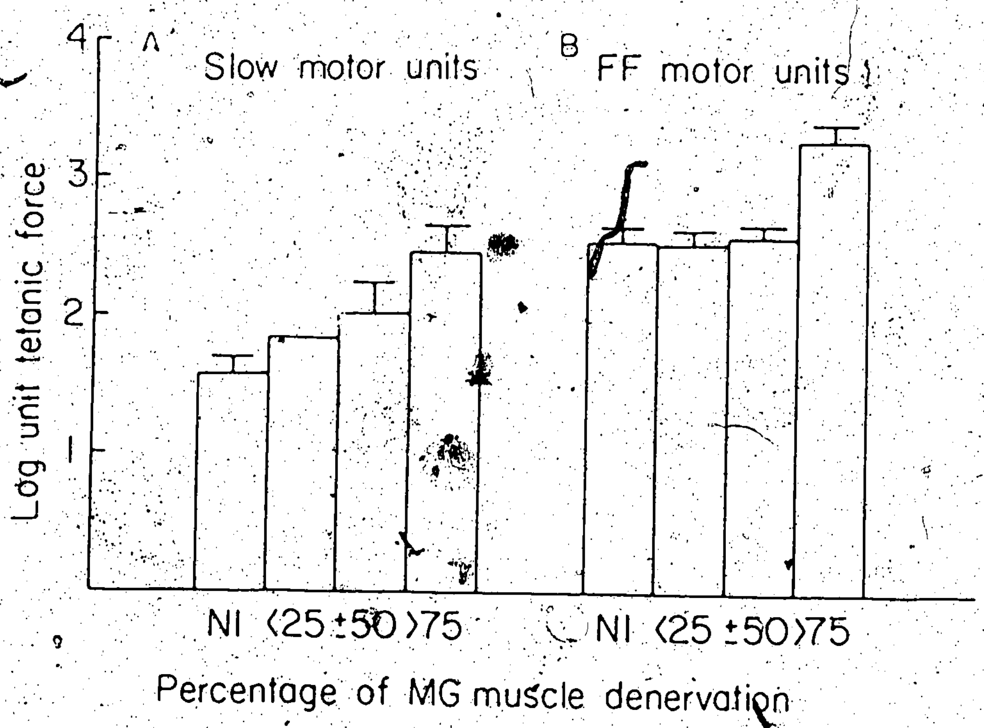


Figure 3.9. Comparison of the sprouting response of slow and FF motor unit types. Data were divided into four groups on the basis of extent of MG muscle denervation: 0 (control MU's), < 25%, approximately 50% and > 75%.

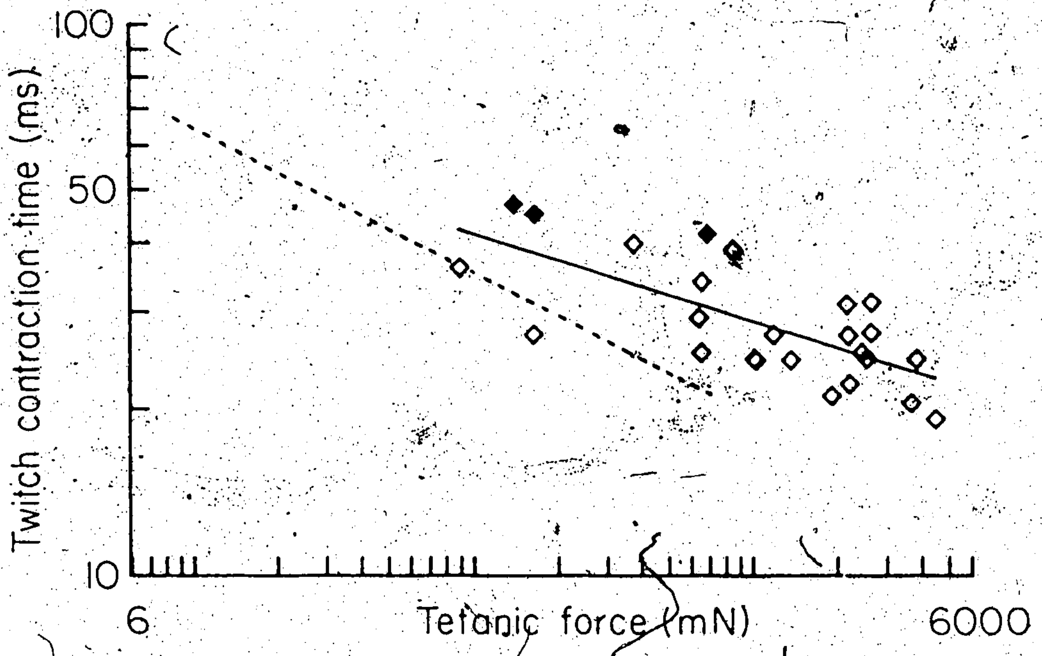


Figure 3.10. Decrease in the range of muscle unit speed. Dashed line was drawn from all control MU's. Diamonds and their regression line represent MU's from severely denervated MG muscles (>75%).

(see table 3.1) and are grouped together. Motor axon size, as measured by either axon potential amplitude or axonal conduction velocity (figure 3.11, graph A) is not recovered by most regenerated axons (graph B). However, the relationships between these 2 parameters of axon size are normal.

On the other hand, regenerated MU's were able to produce forces within the normal range (figure 3.12), even though their axons were thinner than normal (figure 3.11). Sprouted MU's (graph C), develop increased force without alteration in their axon diameter, which remains in normal limits.

Figure 3.13 shows the type distribution of regenerated MU's in relation to sag (graph A: fatiguability vs twitch contraction time). The type frequency distribution corresponds to the following percentages: S=23%, FR=23%, FI=30%, and FF=23%. These proportions are different than those found in control muscles (S:29%, FR:32%, FI:5%, and FF:43%), but more in agreement with those found in sprouted MU's (S:19%, FR:23%, FI:23% and FF:35%). The major change in both experimental groups was an increase in FI units mainly at the expense of the FF type in the regenerated group, and at the expense of all other types in the sprouted group. The same figure also shows the distribution of MU's showing sag. Sagging and non-sagging MU's can be seen in all 4 types of units, making this criterion very unreliable for classifying regenerated motor units.

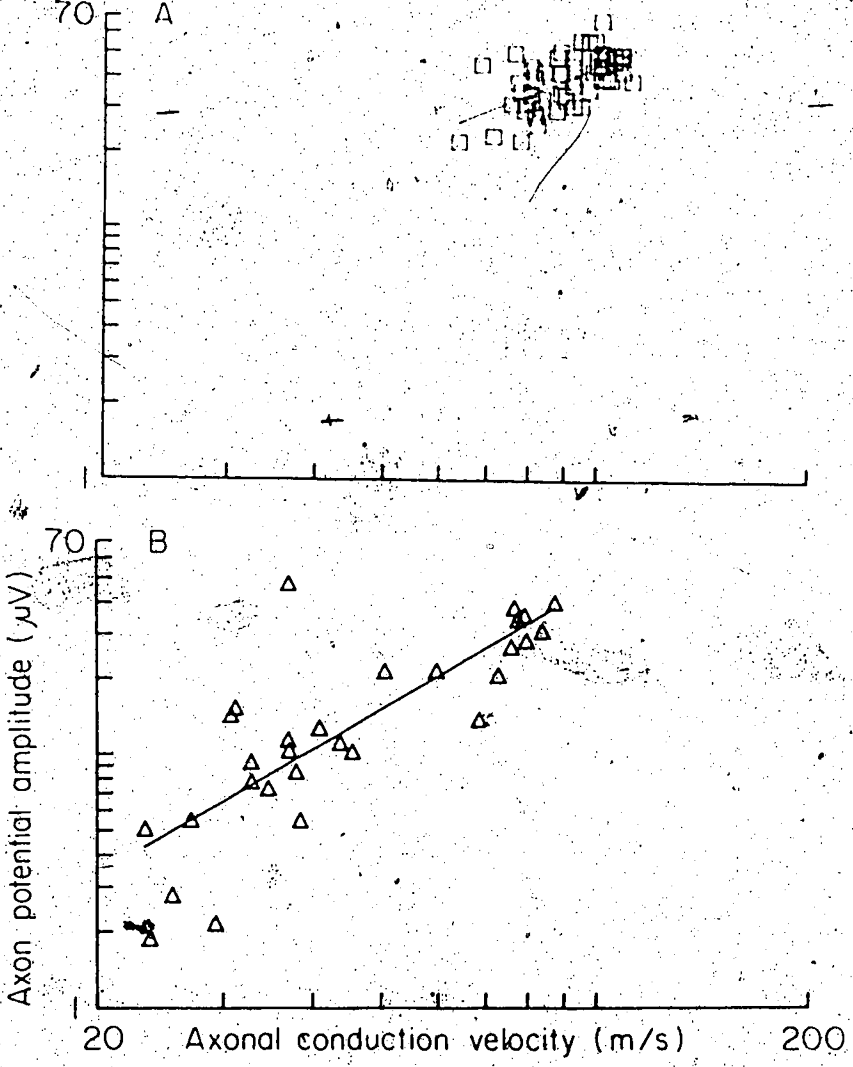


Figure 3.11. Control and regenerated axon size. Axon potential amplitude is plotted versus axonal conduction velocity for A) control (squares) and B) regenerated (triangles) motor axons.

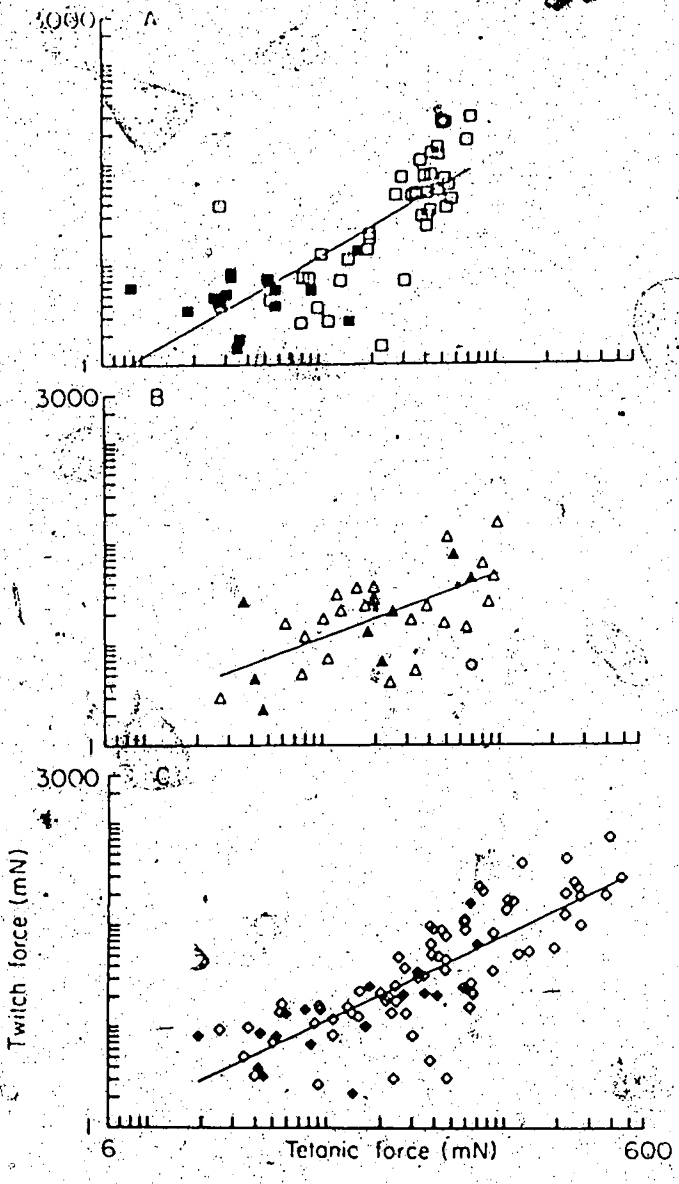


Figure 3.12. Forces of control (squares), regenerated (triangles), and sprouted (diamonds) MG motor units. Filled symbols represent slow and empty symbols represent fast units. Twitch-force is plotted versus tetanic-force for A) control, B) regenerated, and C) sprouted MG motor units. (r: A=.79, B=.64 and C=.81).

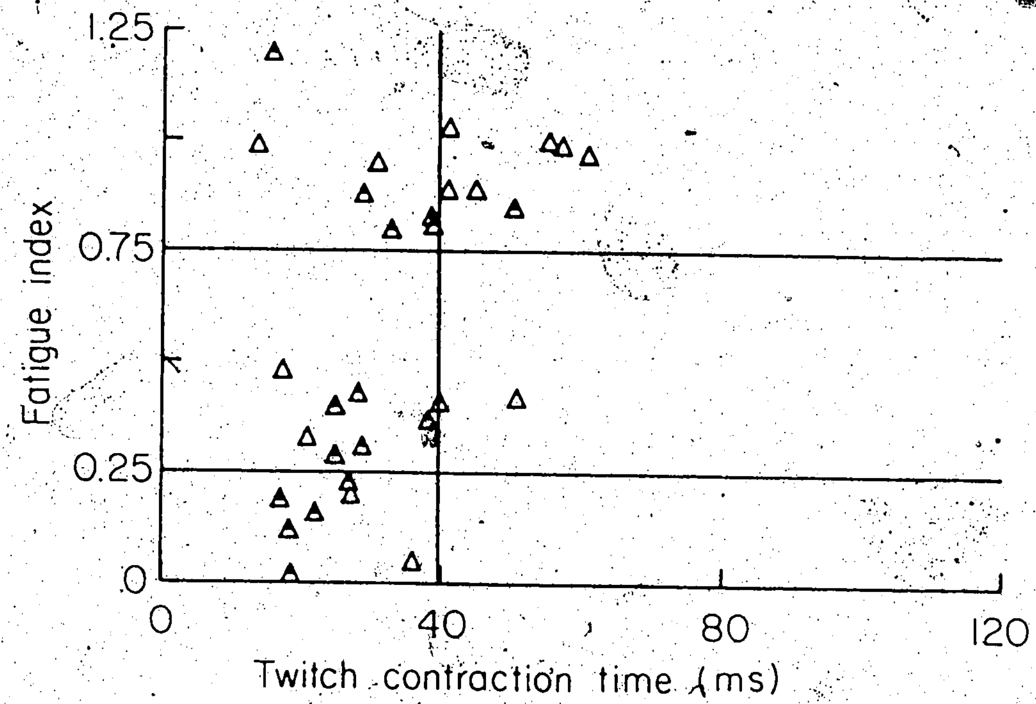


Figure 3.13. Type proportions and sag property in regenerated motor units. When MU's are divided according to twitch contraction time into slow and fast (vertical line), and the latter group is divided in types according to fatiguability (horizontal lines), the proportions of units changed. Sagging (semi-filled symbols) and non-sagging (empty symbols) are present in all types of motor units.

3.4. DISCUSSION

When part of the innervation of a muscle is permanently removed, the remaining MU's must adapt to the new situation if the muscle is to perform the same physiological role as normally and as completely as possible. One of the compensatory changes is the adoption of denervated muscle fibers by the remaining MU's (Exner, 1885; van Harreland, 1945; Edds, 1950), resulting in larger muscle units. Another possible change would be an increase in the frequency of recruitment of the remaining MU's, which in turn would modify their properties (fatigue index, speed of contraction, etc.) in order to function at its new level of activity.

3.4.1. Collateral Sprouting

The results of this study show that reinnervation of denervated muscle fibers by intact motoneurons is a surprisingly effective response to partial denervation in the cat. The efficacy of this process is evidenced in muscles which were denervated by more than 75% and their residual motor axons sprouted to reinnervate denervated muscle fibers, as evidenced by the total recovery of muscle force. MU force is the product of the number of muscle fibers (innervation ratio) and the average force generated by a muscle fiber. Since fiber force is a function of muscle fiber size and the intrinsic force capabilities of the myofilaments, any of these 3 factors could contribute to the proportional shift of MU force in response to partial denervation. Measurement of muscle fiber areas of equivalent fiber types indicated that fiber size did not increase significantly. Although changes in muscle fiber contractility can not be ruled out, most of the increase in force is due to muscle unit enlargement (reinnervation of denervated muscle fibres by intact motoneurons).

These results indicate that smaller (S and FR) MU's are the first line of defense for all degrees of partial denervation. They are able to fully compensate for small degrees of partial denervation as shown in figure 3.6, graphs B and F, where the normally smaller MU's have increased in size and the largest MU's have remained unchanged. With larger extents of denervation, small (S and FR) MU's continued to grow and showed no limitation in their capability to enlarge (see figure 3.6 and 3.9). In contrast, the larger (FF) MU's appeared to respond only after substantial muscle denervation. The extents of denervation achieved in this study did not reveal any intrinsic limitation on the capability of motoneurons to sprout. The final motor unit size (tetanic force) at any extent of denervation was still governed by motoneuron size.

When crushed nerves are subjected to electrical stimulation, a faster recovery of muscle force is observed on the experimental side (Nix and Hopf, 1983), as if motor activity enhanced [illegible] regeneration (Gutmann and Jakoubek,

1963) or the establishment of functional connections. If indeed activity (frequency of recruitment) is related to the capability of a motoneuron to regenerate and form functional connections, this would explain why slow, frequently recruited MU's are the first to enlarge.

It has long been suggested that FI units are in transition (Reinking et al., 1975). Our data are in support of this idea, but rather than FI being the only MU's in transition, all MU's under experimental conditions undergo changes and some displacement along their fatiguability range. It just happens that the fatigue index range for FI units represent half (from >.25 to <.75) of the scale and that normally very few units fall in this range, that finding a few more in that range will double or triple their number. MU transformation occurs under experimental conditions and it is probably a 2 way process in which a unit can become less or more resistant to fatigue. This MU's transformation maintains the fatigue index-tetanic force relationship, so that in any given population of MU's the largest are still the most fatiguable. A few small FI and FF units observed in graph G, figure 3.6, represent exceptions to this observation. These MU's have forces in the range of S and FR types, yet they fatigue easily; most likely they represent former S or FR units that have changed their fatiguability without increasing their size. The whole range of fatiguability decreased as seen in graphs F,G,and H of figure 3.6; the most resistant to fatigue as well as the most easily fatiguable units are in a more intermediate position.

The range of contractile speeds was decreased (figure 3.10), showing a tendency to cluster in the center of the range. Since the contractile speed of the muscle fibers is influenced by their innervating neuron (Buller, 1960), and the motor axons of these MU's have remained physically undamaged, it is likely that activity or another factor related to it is responsible for the influence of the motoneuron on the muscle unit speed.

Previous studies concerning motoneuron sprouting and MU enlargement in partially denervated muscles (Brown, 1976,1977; Thompson, 1977, 1978; Betz, 1982) were mainly carried out on rat and mouse. The difference in results presented here with regard to the limit to which a MU can enlarge, may reflect animal species and/or muscle differences. Our data tend to agree with Hatcher's (1985) results, which were also obtained from a fast muscle of young cats.

3.4.2. Axonal Regeneration

It is known that regenerating axons grow back to denervated muscles, through surviving Schwann cell sheaths (Politis et al., 1982). In partially denervated muscles, when they reach their target muscle they preferentially innervate denervated muscle fibers (Thompson, 1977; Brown, 1977), but they can also suppress newly formed synapses between collateral sprouts and muscle fibers (Brown and Ironton, 1977), especially during early stages of the sprouting process.

We first saw reinnervation of the muscle by regenerating axons 4 months after partial denervation. At this time, reinnervation of muscle by collateral sprouting is expected to be consolidated. The success of the regenerating axons to reinnervate the muscle may depend on their capability to displace collateral sprouts or upon the availability of denervated muscle fibers left by the collateral reinnervation. All cats but one (E.4) had enough time for their cut axons to regenerate and reinnervate the muscle, but only 4 showed some reinnervation by the cut axons. Three of these animals had the most denervated MG muscles. Possible explanations for the successful reinnervation by regenerating axons in some cats are: a) that all axons are capable of regenerating for long distances, but those growing back to better innervated muscles (little initial denervation) are incapable of making functional connections, and b) that successful axonal regeneration does not depend only on the intrinsic properties of the neuron and its pathway, but also on the extent of muscle denervation, so that the process of axonal regeneration is better in severely denervated muscle.

Do regenerating axons have the same capability to sprout as intact axons? Apparently not. Regenerated MU's were able to grow to a normal size range, as judged by MU force production (figure 3.12). These results are in agreement with those obtained by Gordon and Stein (1982). When muscles are partially denervated by section of part of their innervation close to the muscle (Thompson, 1978) and regenerating and sprouting terminals have similar opportunities to compete for denervated and recently reinnervated muscle fibers, regenerating axons seem to have the advantage. In our experiments, sprouted MU's had enough time to consolidate their synaptic contacts before facing competition from regenerating axons; and one could argue that their capacity to sprout was limited by the experimental conditions. However, in experiment # 7 all the innervation to the MG muscle was removed (section of L7 and S1 spinal roots), and assuming that some axons did not successfully grow back, those that made contact with the denervated muscle had the opportunity to innervate many muscle fibers. In spite of this, the size of regenerated MU's was within normal limits and the muscle was still left partially denervated. These findings suggest either that motor axons do not recover their potential for sprouting after regenerating for long distances, or that denervated muscle fibers are progressively less receptive of innervation with time.

Regenerated MU's regained normal force production without simultaneously regaining axon size. This difficulty in recovering axon size has not been found when axons have regenerated for shorter distances (Gordon and Stein, 1982; Foehring et al, 1986). Such a difference could mean that the capacity to recover axon size is similar in both cases but the long-distance regenerating axons are thinner than short-distance regenerating axons before making contact with the muscle, and if enough time was allowed axon size would be recorded in both cases; or that in fact the cell bodies of long-distance regenerating axons have made a maximum effort in regenerating the axons to the target that are not capable of recovering axonal size.

The altered distribution of MU type shown in figure 3.13 is probably the result of MU interconversion, since it is also present in sprouted units, rather than the result of an advantage of axons of a certain MU type.

The plasticity exhibited by the cat MG motor units under partial denervation of the muscle is surprising. First, residual motor axons innervate denervated muscle fibers, enlarging the size of the muscle unit by several times. This process by itself compensates for most extents of denervation. Second, regenerating axons can reinnervate the muscle as a complementary process -contribution of this process to the total recovery of the muscle depends on the distance between denervated muscle and site of axon section, and probably on the extent of muscle denervation as well. In summary, the MG muscle of the cat is adequately reinnervated after partial denervation.

CHAPTER FOUR

MOTOR UNITS OF PARTIALLY DENERVATED LATERAL GASTROCNEMIUS AND SOLEUS MUSCLES OF THE CAT

4.1. INTRODUCTION

The lateral gastrocnemius (LG) and the soleus (Sol.) muscles are synergists which extend the ankle, are in proximity to each other, receive innervation from the same nerve and share the same tendon of insertion. Yet, their MU composition is quite different. These two muscles offer an attractive combination to study the response of a fast-twitch (LG) and a slow-twitch (Sol.) muscle to partial denervation. Traditionally, fast flexor muscles of the ankle have been compared with the slow extensor soleus muscle. Results from synergists muscles, therefore, would rule out muscle function as a determining factor in any difference that might exist between a fast and a slow muscle.

Partial denervation introduces a change in the amount of physical activity that the remaining MU's perform. The altered recruitment, as discussed in chapter 3, may influence the properties of MU's after partial denervation. We studied the changes (enlargement and interconversion) of these MU's.

Hatcher el al. (1985) have shown that residual motor axons of the partially denervated flexor digitorum longus muscle in the adult cat do not increase in size despite the great increase in muscle unit size. These results have been confirmed in this study in the MG muscle of the adult cat (chapter 3). Other studies on regenerated MU's have shown that axons recover their original size (Gordon and Stein, 1982; Foehring et al., 1986) after making functional connections with the muscle. Are these findings indicating that the maximum axon size is entirely governed by the motoneuron and that nerve-muscle interaction can only help in the expression of that size? We re-addressed the question of whether the muscle unit enlargement can influence motor axon size (see chapter 3) when partial denervation is performed before polyneuronal innervation is completely eliminated.

Sprouting after partial denervation has often been viewed as a dedifferentiation to the neonatal status (Brown et al., 1976), where muscle fibers are polyneuronally innervated and motor axons innervate about 5 times as many muscle fibers as in mature muscles. During the first 6 weeks of postnatal life, this polyneuronal innervation is eliminated in the kitten (Bagust et al., 1973). If partial denervation is performed during this period, will the remaining MU's enlarge less than in adult cats, as they do in immature rats (Thompson and Jansen, 1977).

4.2. MATERIALS AND METHODS

Experiments were conducted on 11 cats of both sexes. At the time of partial denervation the cats were between 1 and 12 months of age. Final acute experiments were carried out between 4 and 18 months after partial denervation. Weight of cats at final operation was between 2.9 and 5.1 Kg.

4.2.1. Chronic Partial Denervation was performed under general anesthesia and aseptic conditions. The experimental animals underwent a laminectomy from S1 to L7. On the right side, either S1 or L7 spinal root was cut extradurally. No attempt was made to prevent regeneration. Animals were maintained freely moving in large cages.

4.2.2. Extent of Muscle Denervation was estimated as previously described for MG muscle, taking into account electrical charge and muscle force. In most experiments LG and Sol. muscles were simultaneously studied, since the LGS nerve is common to LG and Sol. muscles and both share the same tendon of insertion, the individual contributions L7 and S1 spinal roots to the innervation was assumed to be similar for both muscles.

4.2.3. Acute Experiments were carried out as described in general methods (see chapter 2). In the experiments in which both muscles were studied, the LGS nerve was dissected free for a length of about 20-25 mm proximal to the LG muscle. An electromyogram probe was sutured unto the surface of each muscle. The muscles were dissected free except for their proximal attachments and both were tied to a force transducer through the same piece of calcaneous bone. After identifying a single axon, its corresponding muscle unit was located on LG or Sol. muscle by EMG and visual inspection of the muscle contraction. In those experiments in which only Sol. muscle was studied (see table 4.3), this was dissected free from the LG muscle and the Sol. component of the LGS nerve was also dissected free from that of the LG.

4.3. RESULTS

4.3.1. Lateral Gastrocnemius Muscle.

The motor axons innervating the LG muscle left the spinal cord through S1 and L7 ventral roots. Nine of the 11 cats had a predominance of motor axons exiting through L7 ventral root as judged by the contribution of that root to LG innervation on the control side.

A total of 135 MU's was studied. Twenty-eight control MU's in 2 cats. Forty-six "sprouted" and 61 regenerated MU's in 5 cats (see table 4.1).

4.3.1.1. Control Lateral Gastrocnemius Motor Units. When MU's of the LG muscle are classified on the basis of twitch contraction time and fatigue index (figure 4.1, graph A), the range of the constellation of MU's is similar to that of MG muscles (figure 4.1, graph B). However, the LG motor units of the FR type are faster than those of the same type in the MG muscle, and are as fast as the rest of fast LG units. There is also a smaller number of slow and FF, and an increase in FR MU's in the LG muscle. Our control data are similar to those of McPhedran et al., 1965 and Thomas et al., 1986.

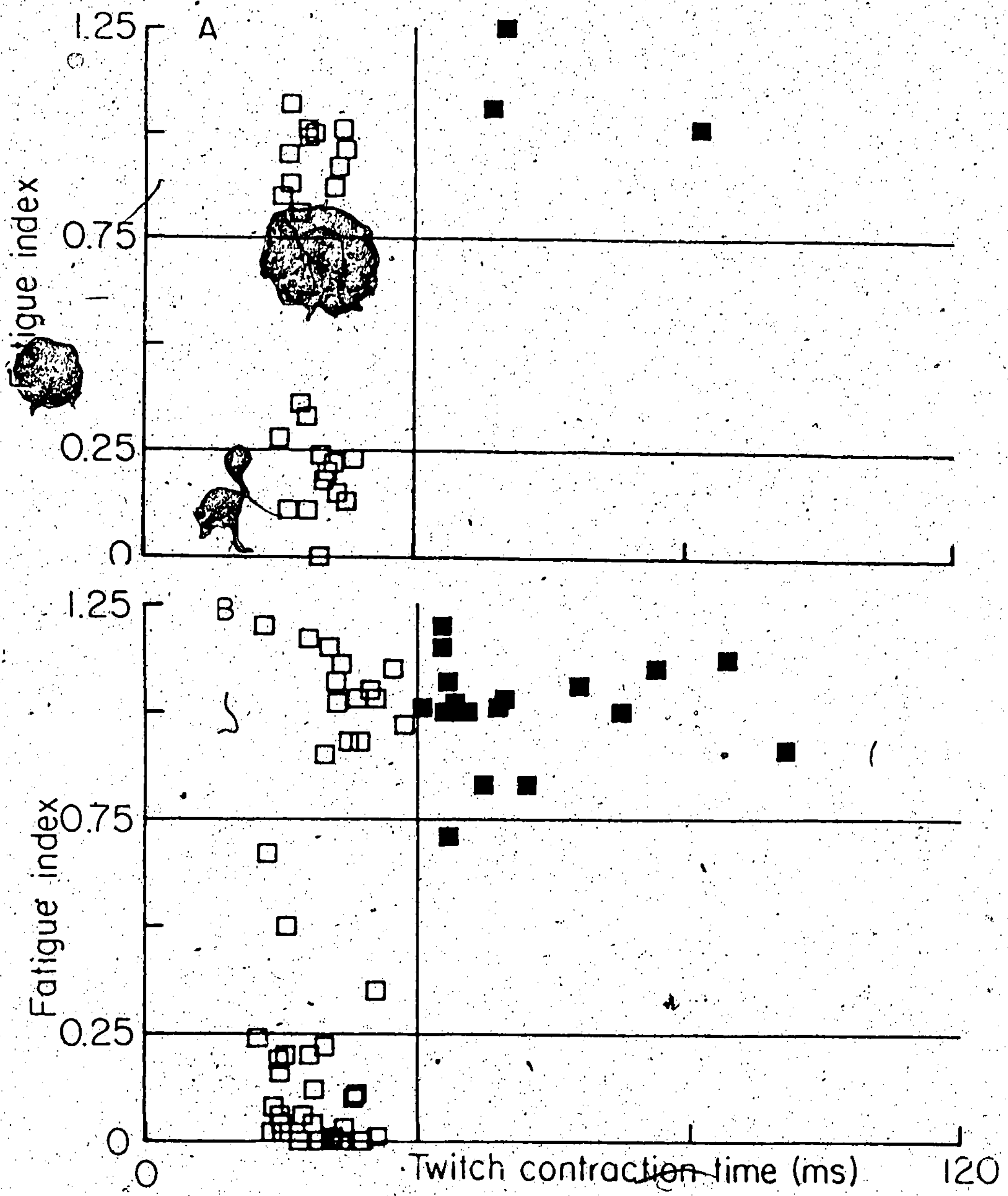


Figure 4.1. Comparison between LG and MG motor unit types distribution. Fatigue index is plotted as a function of twitch contraction time for control LG (graph A) and control MG (graph B) motor units. Filled symbols: slow units, empty symbols: fast units.

TABLE 4.1. SUMMARY OF LG DATA

	Age at:		Time after den.	Weight at exp.	Spinal root cut	% of den.	Number of motor units studied	
	den.	exp.						
Cat	(---months		---)	(kg)				Group
C.1	-	-	8	3.7	-	0	24	I
C.2	-	-	9	3.2	-	0	4	I
E.1	8	12	4	4.0	S1	7	31	II
E.2	1	19	18	4.0	L7	69	1	III
E.3	1	11.5	10.5	2.9	S1	88	8	III
E.4	12	19	6.2	4.2	L7	93	5	IV
E.5	1	8	7	3.3	L7	94	1	IV

Table 4.1 Summary table of LG sprouted motor units. Two control (C.) and 5 experimental (E.) cats were studied. Enough survival time after initial operation was allowed for regeneration of the cut root to occur. No regeneration occurred in the cat with little denervation (E.1) and substantial regeneration occurred in the rest of animals (see also table 4.4). Data were grouped as control MU's (group I), MU's from muscle with slight (group II), moderate (group III) and severe (group IV) denervation.

When fatigue index is plotted as a function of log tetanic force, the range of tetanic force of LG motor units is similar to that of MG motor units (figure 4.2). However, there is less overlap between forces of slow and FR units due to a bigger size of FR units of the LG muscle. FF motor units of LG muscles showed a wider range of force. The average tetanic force of the fast group of LG motor units was bigger than that of the same group of units in the MG muscle (see also table 4.2).

4.3.1.1.1. Sag Phenomenon. The general correspondence between the sag and the twitch contraction time criteria for grouping normal MG motor units into fast and slow is not as prominent in normal LG muscles. Sagging and non sagging MU's are displayed on a fatigue index-twitch contraction time graph (figure 4.3). MU's of all types sagged, while some fast MU's did not sag.

4.3.1.2. Sprouted Lateral Gastrocnemius Motor Units.

4.3.1.2.1. Motor Unit Enlargement. When MU tetanic force is displayed on a log scale (figure 4.4) as a function of the of fatigue index (A-D) and twitch contraction time (E-H) for different extents of denervation. The entire range of MU shifted to the right along the force axis proportionally to the extent of denervation. There was [illegible] limitation to the capability of MU's to sprout (graphs A-D) as shown for the MG muscle in chapter 3. These results differ from those of previous experiments (Brown and Ironton, 19[illegible] Thompson, 1977) performed on different muscles and animal species.

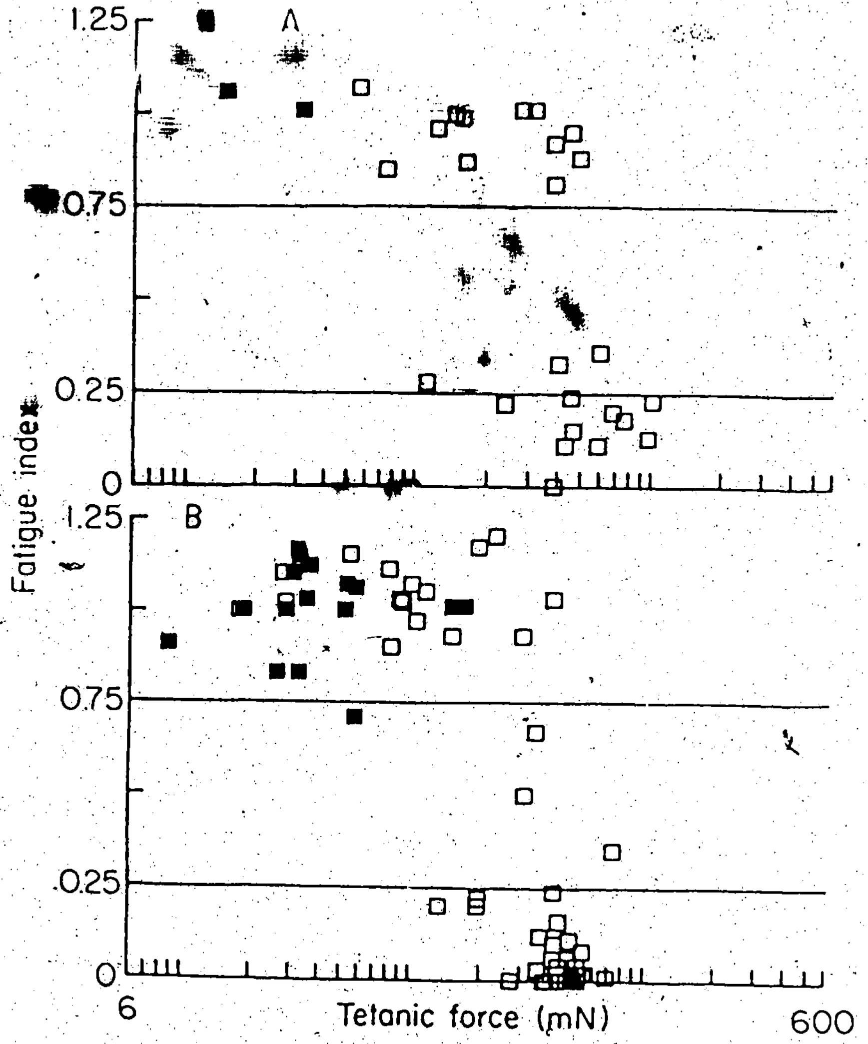


Figure 4.2. Fatiguability and force production of LG and MG motor units. Fatigue index is plotted as a function of tetanic force for control LG (graph A) and control MG (graph B) motor units. Filled symbols: slow units, empty symbols: fast units.

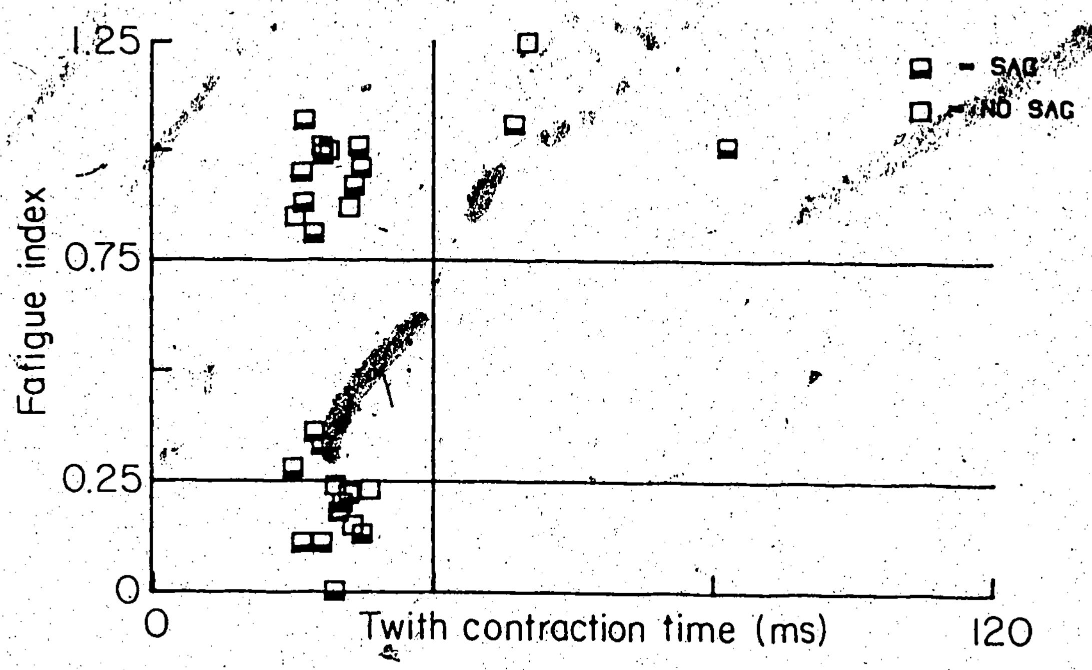


Figure 4.3. Non specificity of the "Sag Phenomenon". Control, sagging (semi-filled symbols) and non-sagging (empty symbols), motor units are displayed on a fatigue index-twitch contraction graph. Not all fast MU's sagged and, on the other hand, some slow contracting MU's did sag.

TABLE 4.2. COMPARISON OF LG AND MG MOTOR UNITS

	LG muscle					MG muscle				
MU type	Slow	FR	FI	FF	All	Slow	FR	FI	FF	All
Percentage of MU type (%)	11	43	11	35	100	29	23	5	43	100
Tetanic force (mN)										
Mean	19	249	371	600	363	54	113	459	428	253
S.E.	11	149	246	255	274	45	79	236	128	205
Twitch contraction time (ms)										
Mean	71	25	22	27	29	56	30	24	21	36
S.E.	19	3	2	3	13	16	5	8	3	17

Table 4.2. Comparison of LG and MG motor units. Mean values for MU tetanic force and twitch contraction time are given, standard deviations of the means are given within parentheses.

Graph D shows data from the most denervated LG muscles (93%) in which the remaining MU's increased their original size by about 9 times. For the loss of small extents of innervation (graph B), the muscle response is an in increase in size of slow MU's. Graph C (78% denervation) shows some enlargement of fast MU's while slow MU's have enlarged several times their original size. It is not until denervation is severe (graph D) that fast MU's show a substantial size enlargement.

4.3.1.1.1. Motor Unit Transformation. The normal distribution of LG motor unit types was shown in figure 4.1. This distribution corresponds to the following proportions: slow= 20.7%, FR= 42.9%, FI= 10.7%, and FF= 35.7%. If muscles are subjected to partial denervation, MU's move not only along the force axis, but also along the fatiguability axis (figure 4.5), consequently a greater proportion (20.5%) of MU's is detected in the FI range ((>0.25 but <.75) in the sprouted group (graph B) Graph 4.5. also shows the rare increase in fatiguability of 2 slowly contracting MU's.

4.3.1.4. Axon Size in Kittens. The axon size of sprouted MU's in LG muscles of adult cats did not change. But when axon potential amplitude is plotted as a function of tetanic force for kittens (figure 4.6), we can see a proportional increase of axon size with muscle unit size.

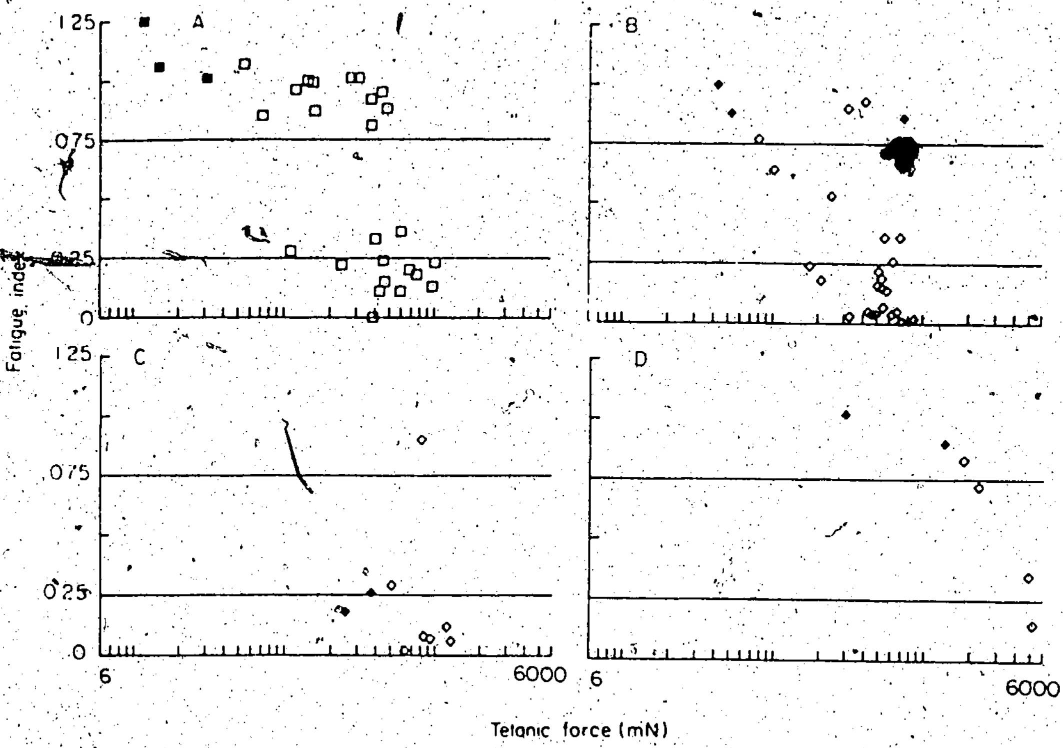


Figure 4.4. Proportional motor unit enlargement with extent of muscle denervation. Fatigue index (graphs A-D) is plotted as a function of tetanic force for different extents of LG denervation: A=0 (control), B=7%, C=78%, and D=93% of denervation. (Slow: filled symbols, Fast: empty symbols).

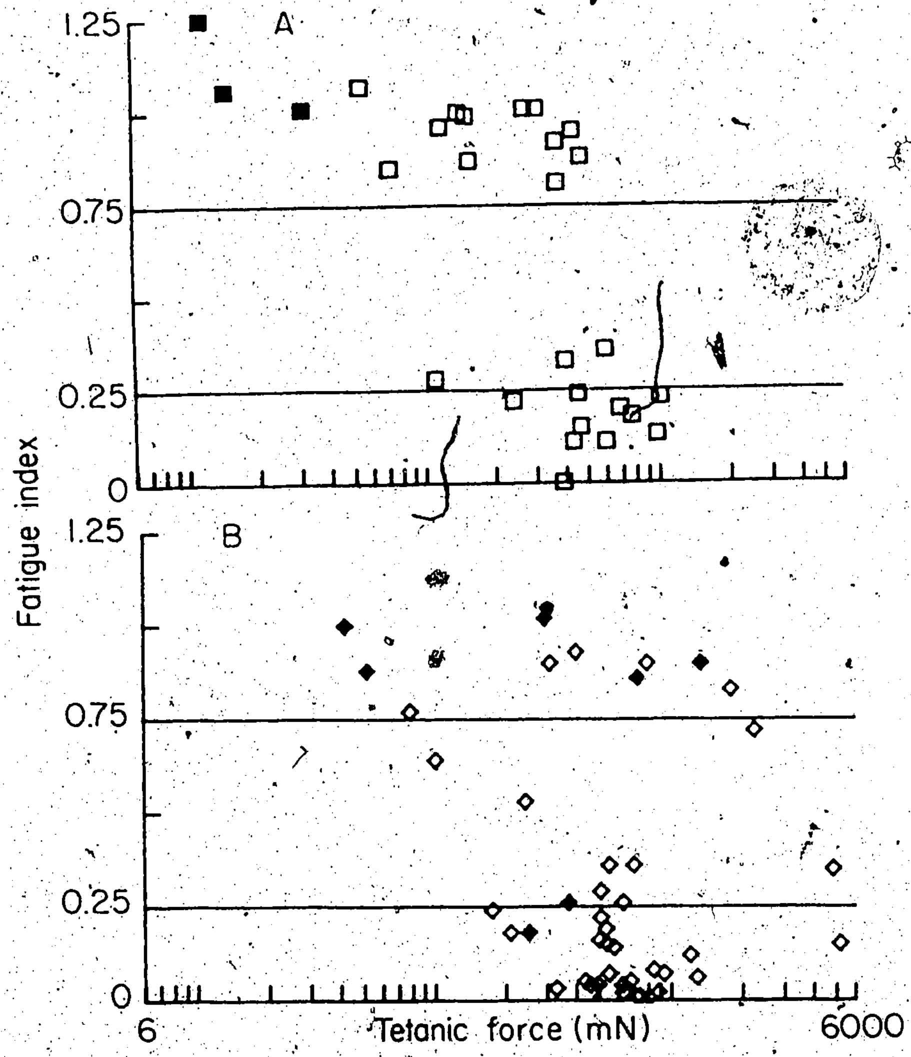


Figure 4.5. Changes in motor unit proportions during denervation. Fatigue index is shown as a function of tetanic force for control (graph A) and experimental (graph B) motor units. Slow: filled symbols, fast: empty symbols.

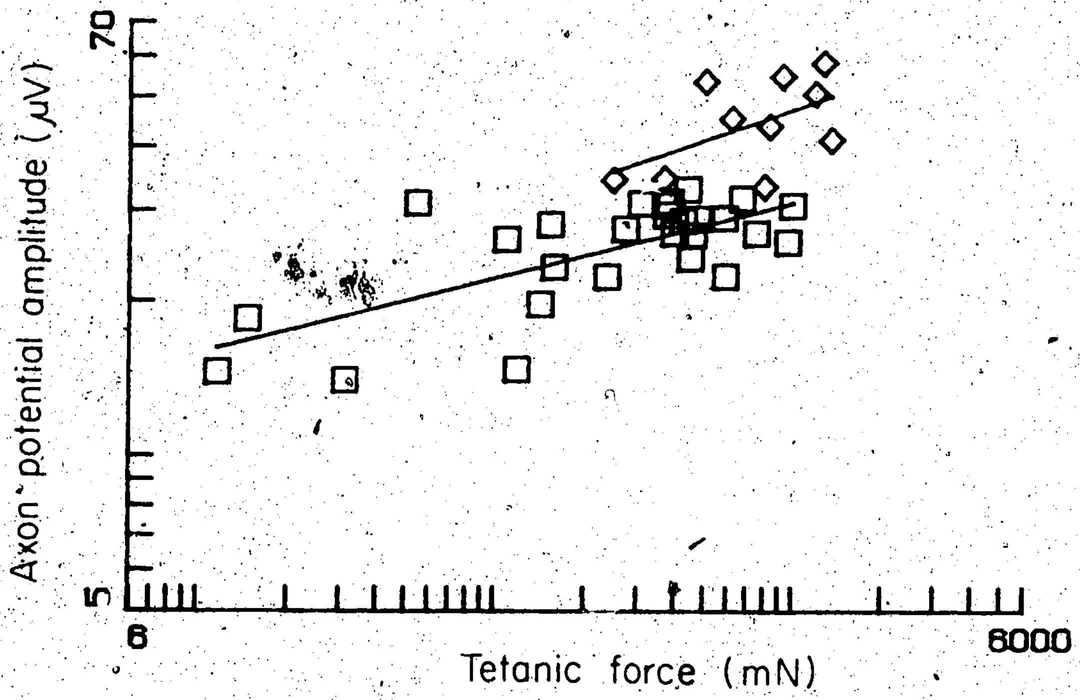


Figure 4.6. - Axon size increases in sprouted motor units of the kitten. Axon potential, as a measure of axon diamenter, is displayed versus tetanic force of sprouted MU's of the kitten (diamonds), and control MU's are also drawn for comparison. All LG muscles were highly denervated and studied after long periods of time.

4.3.2. SOLEUS MUSCLE

Soleus muscles were extensively denervated (>68%) except for 1 (E.6), in which only 12% denervation was achieved.

Contrary to MG and LG muscles, the partially denervated soleus muscle frequently failed to recover its original force (table 4.3, E.6-8).

Axonal regeneration to soleus muscle was minimum. Most of the regenerated axons made contact with the LG muscle and only very few reinnervated the soleus muscle. In 3 experiments the soleus muscle was studied independently to determine the proportion of regenerated axons reinnervating this muscle. Regenerated MU's were present in 1 of the 3 experiments and elicited less than 1 % of the total muscle force.

One hundred and twenty-five MU's were studied. Control:38, "sprouted":83, and regenerated:4. All units were slow and resistent to fatigue.

4.3.2.1. Control Soleus Motor Units. The soleus muscle contains a more homogeneous population of MU's. The properties of these MU's varied over narrow ranges as shown in figure 4.7, where all MU's exhibited twitch contraction times greater than 40 ms and fatigue indexes of around 1(graph A), tetanic force varied between 10 and 200 mN.(graph B), and most axonal conduction velocities were between 60 and 90 m/s (graph C), potential amplitudes were between 7 and 25 uV .

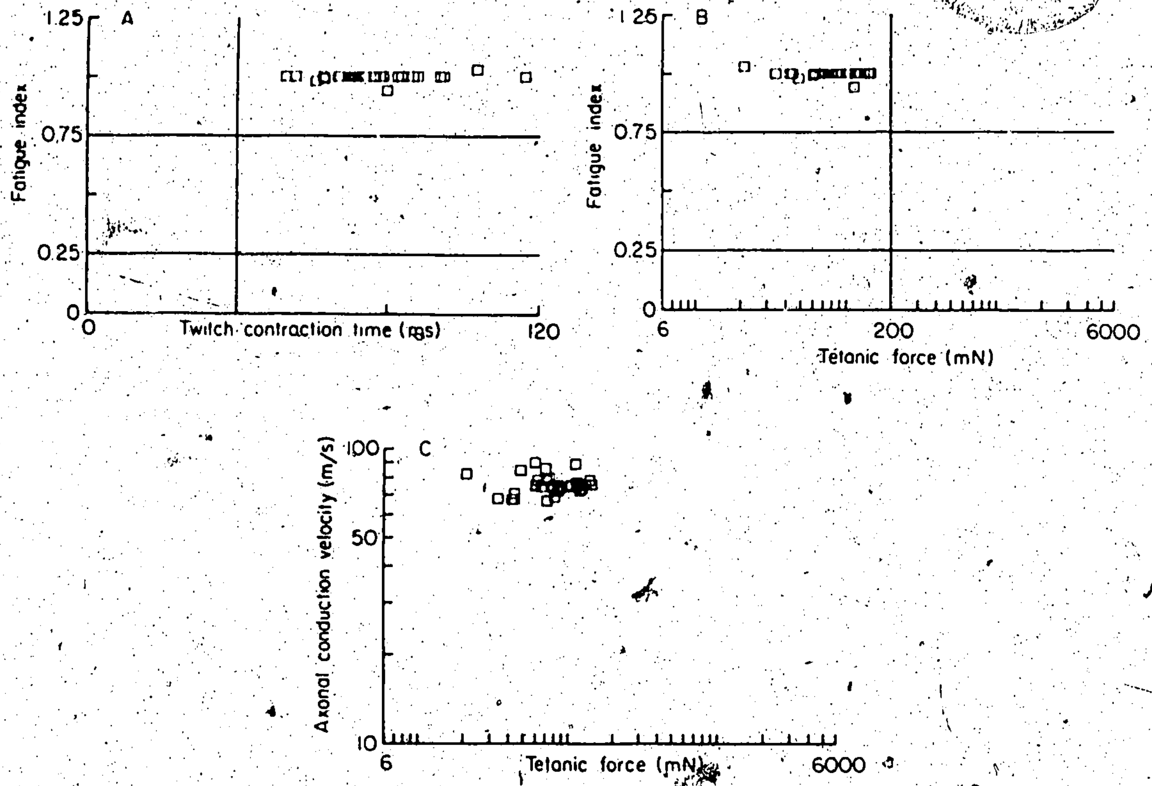


Figure 4.7. Control soleus motor units. Fatigue index is plotted vs twitch contraction time (graph A), fatigue index of 1 was given to all MU's in one experiment. Graph B shows fatiguability vs tetanic force, all MU's produced forces between 10 and 200 mN. Graph C shows axonal conduction velocity vs force.

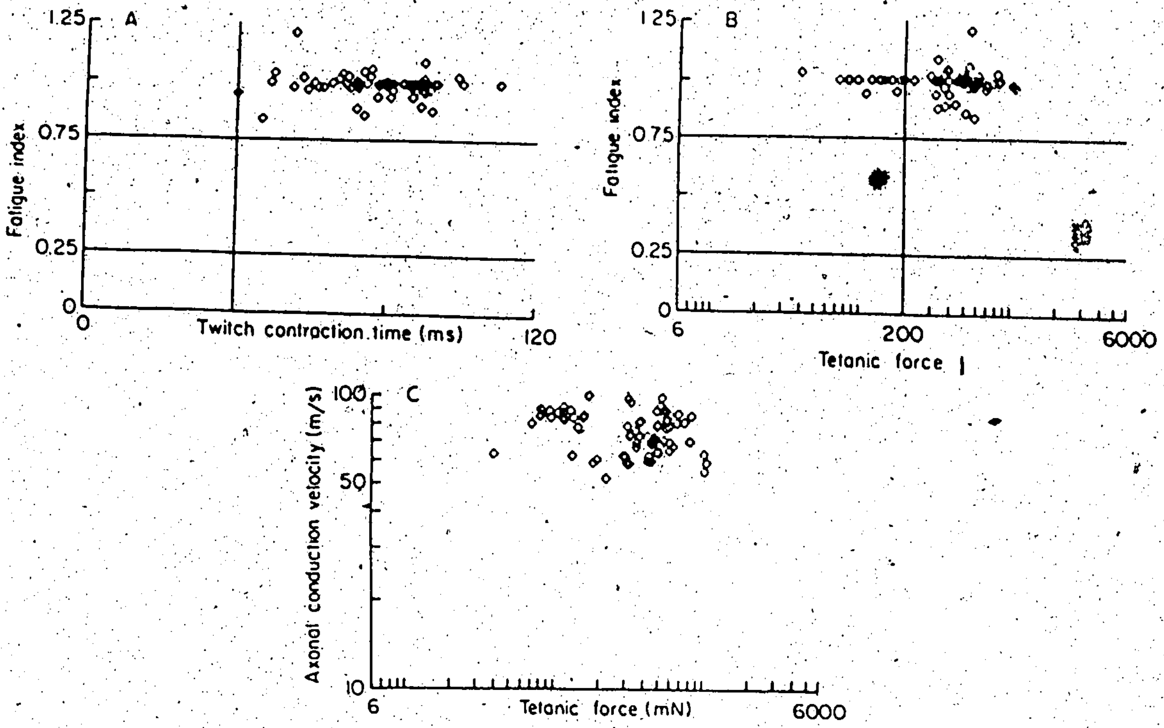


Figure 4.8. Sprouted soleus motor units. Properties of sprouted soleus motor units are displayed in this figure. Graph A: fatigue index vs twitch contraction time; fatigue index of 1 was given to all MU's of one experiment. Graph B: fatigue index vs tetanic force. Graph C: axonal conduction velocity, as a measure of axon diameter, vs tetanic force. MU's properties did not change with muscle unit enlargement.

TABLE 4.3. SUMMARY OF SOLEUS DATA

Cat	Age at dener. (---months	Time after dener. ---)	Weight at final exp.(Kg)	Spinal root cut	% of dener.	Number of Motor Units studied.	% of control force	Axon Regen.
C#2	--	--	3.2	--	0	4	---	---
C#3	--	--	4.4	--	0	34	---	----
E#2	1	18	4.0	L7	69	16	---	yes
E#4	12	6.2	4.2	L7	93	4	---	yes
E#5	1	7	3.3	L7	94	9	---	yes
E#6	9	7.2	3.1	S1	12	18	92	
E#7	5	5.8	5.1	L7	76	18	83	no
E#8	11	10	4.8	S1	90	17	58	yes

TABLE 4.3. SUMMARY OF SOLEUS DATA. This table summarizes sprouted MU's of the soleus muscle. Experimental MU's were studied in 6 cats (E#) ranging in weight from 3.1 to 5.1 Kg. Control MU's were studied in 2 cats (C#) of comparable weight. Partial denervation was performed by section of either L7 or S1 spinal root in 2 kittens and 4 young adult cats. The interoperative interval was long enough (>5 months) for axonal regeneration to occur. The percentage of denervation was high except in 1 animal (E#6). In 3 experiments (E#2,4 and 5) LG and Sol. muscles were studied together and neither the recovery of soleus muscle force, nor, the contribution of axonal regeneration to the reinnervation of that muscle could be properly evaluated. In the last 3 animals, soleus muscle was studied independently, all muscles failed to recover their original force. Only E#8 presented regenerated MU's (2 MU's=< 1% of the total force).

4.3.2.2. Sprouted Soleus Motor Units. Sprouted soleus MU's did not exhibit drastic changes in their properties, except for the increase in force. Figure 4.8 displays graphs similar to those in figure 4.7. Twitch contraction time and fatigue index remained within normal limits (graph A), MU tetanic force enlarged by up to 6 times with no changes in fatiguability (graph B), and axon diameter, as measured by axonal conduction velocity, did not increase with muscle unit enlargement (graph C).

Enlargement of intact soleus units was not as great as in the previous 2 muscles, and muscles frequently failed to recover their original forces. Figure 4.9 shows that most MU's (diamonds) from extensively denervated muscles (from 69 to 94 % denervation) tended to cluster between 300 and 700 mN. Control MUs (squares) are also drawn for comparison. Only one animal (exp. # 7) exhibited an increase in mean tetanic force greater than 6 fold (7.8 fold increase). This animal was the largest studied (5.1 kg of body weight).

4.3.3. Regenerated Lateral Gastrocnemius and Soleus Motor Units.

Regenerated MU's of these 2 muscles are analyzed together in this section.

As previously mentioned, axonal regeneration to soleus muscle was limited and only 4 MU's were studied.

Fatigue index vs twitch contraction time of control LGS units are plotted in figure 4.10, graph A. Contraction

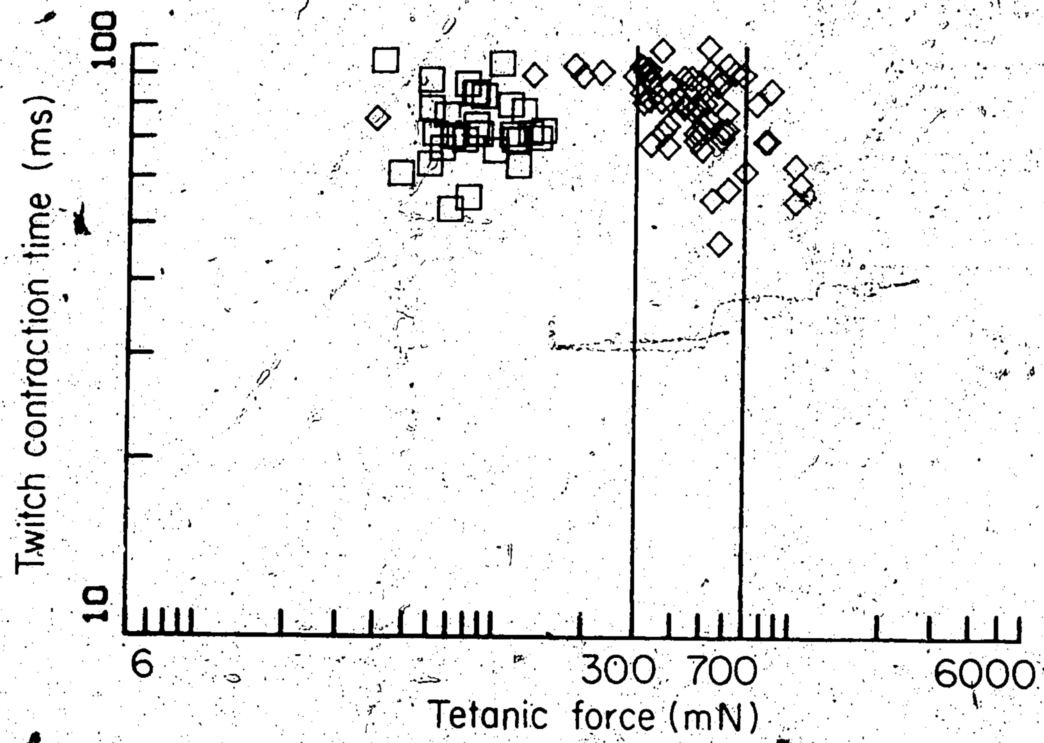


Figure 4.9. Limitation to enlargement of soleus motor units. Twitch contraction time is plotted vs tetanic force of MU's from control muscles (squares) and from 5 well denervated (69-94% of denervation) muscles (diamonds). Most MU's enlarged to a size between 300 and 700 mN.

times for fast MU's are faster than 35 ms, and there are only 3 units with fatigue index between 0.25 and 0.75. When regenerated (graph B), and sprouted (graph C) MU's are plotted in the same type of graph (graph B), it is evident that MU's from the fast group are more slow than normal and that their fatiguability is more scattered, mainly due to a decrease in the fatigue index of the FR group. Consequently, FI units increase in number.

The relationship shown in figure 4.11, graph A, between tetanic force and twitch contraction time for control MU's depends on the existence of a slow group of units. After axonal regeneration (graph B), slow MU's enlarged their sizes beyond the normal range, and the relationship is lost.

The relationship between axon potential and tetanic force (figure 4.12, graph A) is also lost for regenerated MU's (figure 4.12, graph B). In contrast to regenerated MG units, in which all axons were smaller than normal, some axons have reached or surpassed the normal axon size range. While others, like in the MG muscle, are generating forces in the normal or slightly above the normal range with very small axons. These 2 groups of units have different origins, the first comes from cats denervated at 1 month of age, and the second comes from cats denervated during adulthood. In figure 4.13, motor units from the kittens are grouped together. Normal relationships between tetanic force-axon potential (graph A) and tetanic force-

Figure 4.10. Control, regenerated, and sprouted LGS motor units. Fatigue index is plotted vs contraction time for A: control (squares), for B: regenerated (triangles), and C: sprouted (diamonds) LGS units. Graph A contains MU's from 2 experiments in which both muscles (LG and Soleus) were studied. There was a prolongation of twitch contraction time for fast MU's, and an increase in the number of FI units for regenerated and sprouted MU's (graph B).

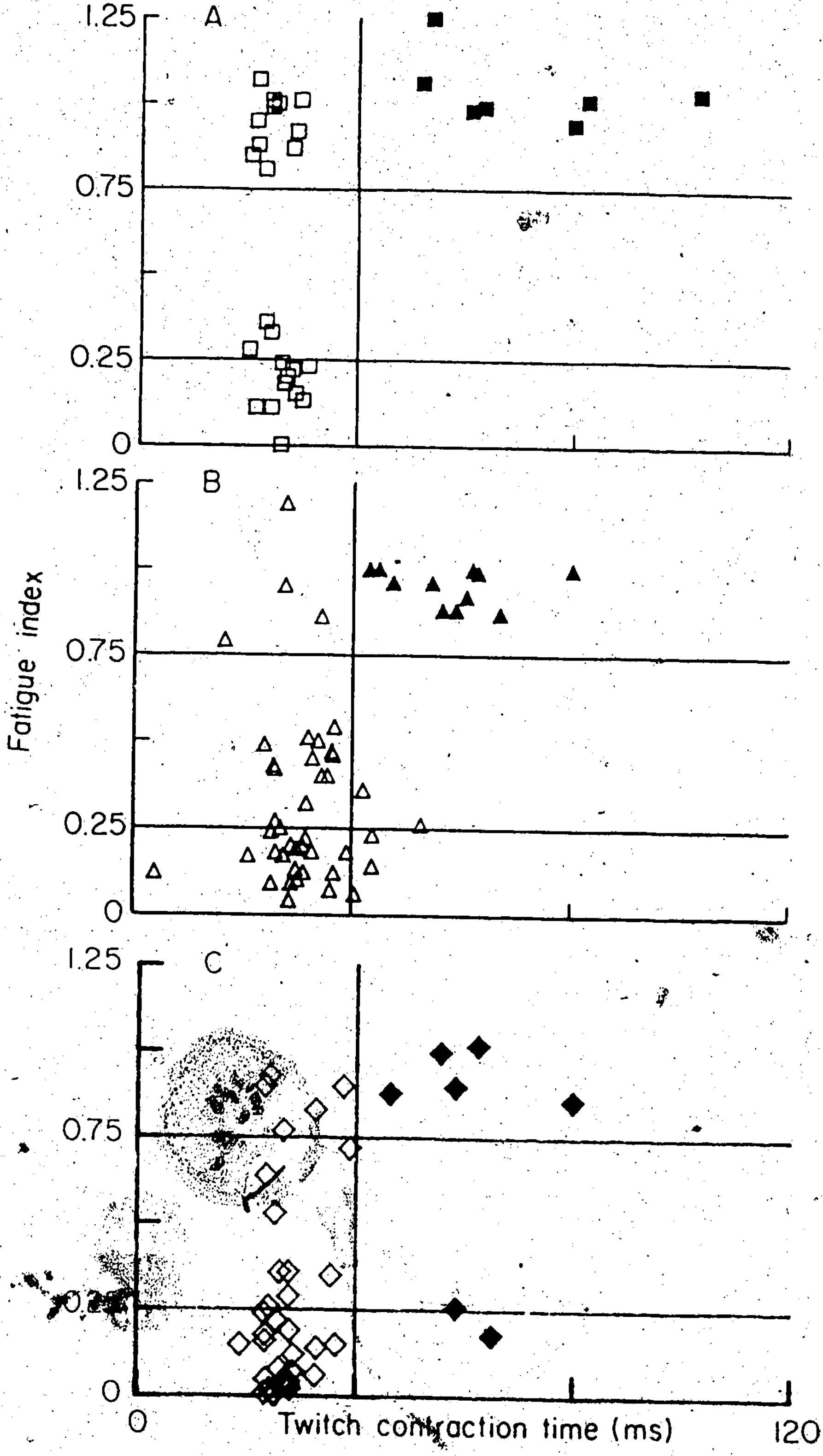
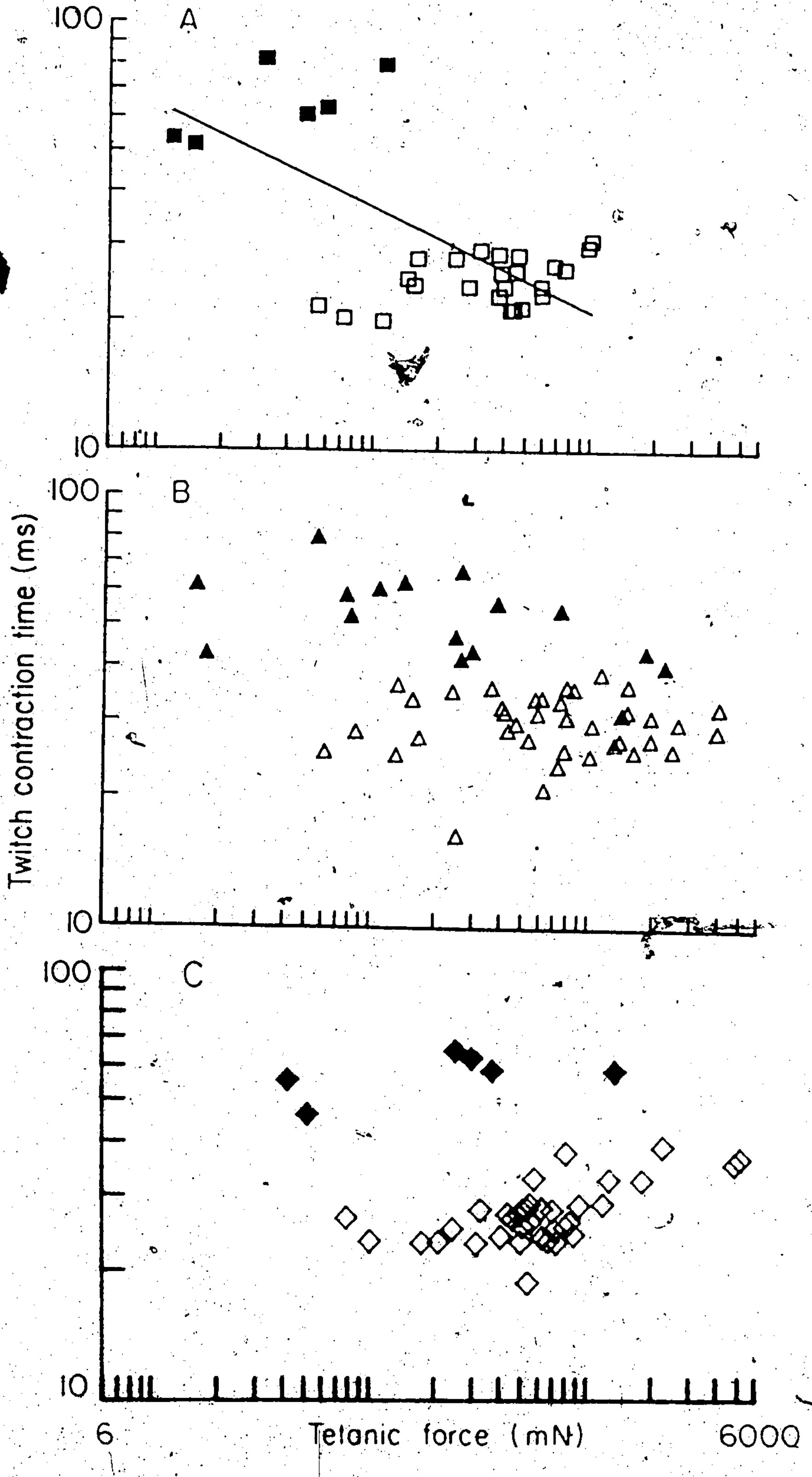

A
B
C
1.25
0.75
0.25
0
Fatigue index
0
120
Twitch contraction time (ms)

Figure 4.11. Size of control, regenerated, and sprouted LGS motor units. Twitch contraction time is plotted as a function of tetanic force for A: control (squares), B: regenerated (triangles), and C: sprouted (diamonds) LGS motor units. Regenerated MU's enlarged beyond the normal range, but not as much as sprouted MU's. Slow (filled symbols) MU's seemed to have enlarged relatively more.


100
A
10
100
B
10
100
C
10
Twitch contraction time (ms)
6
Tetanic force (mN)
6000

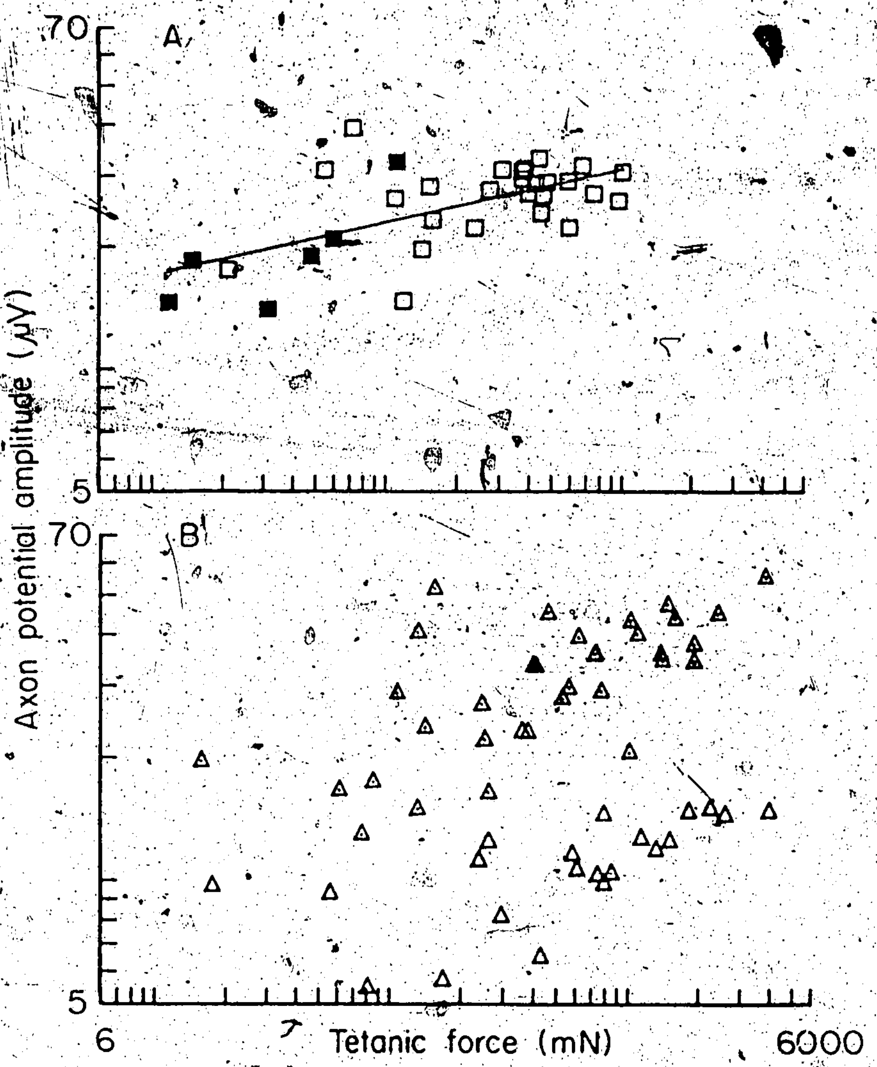
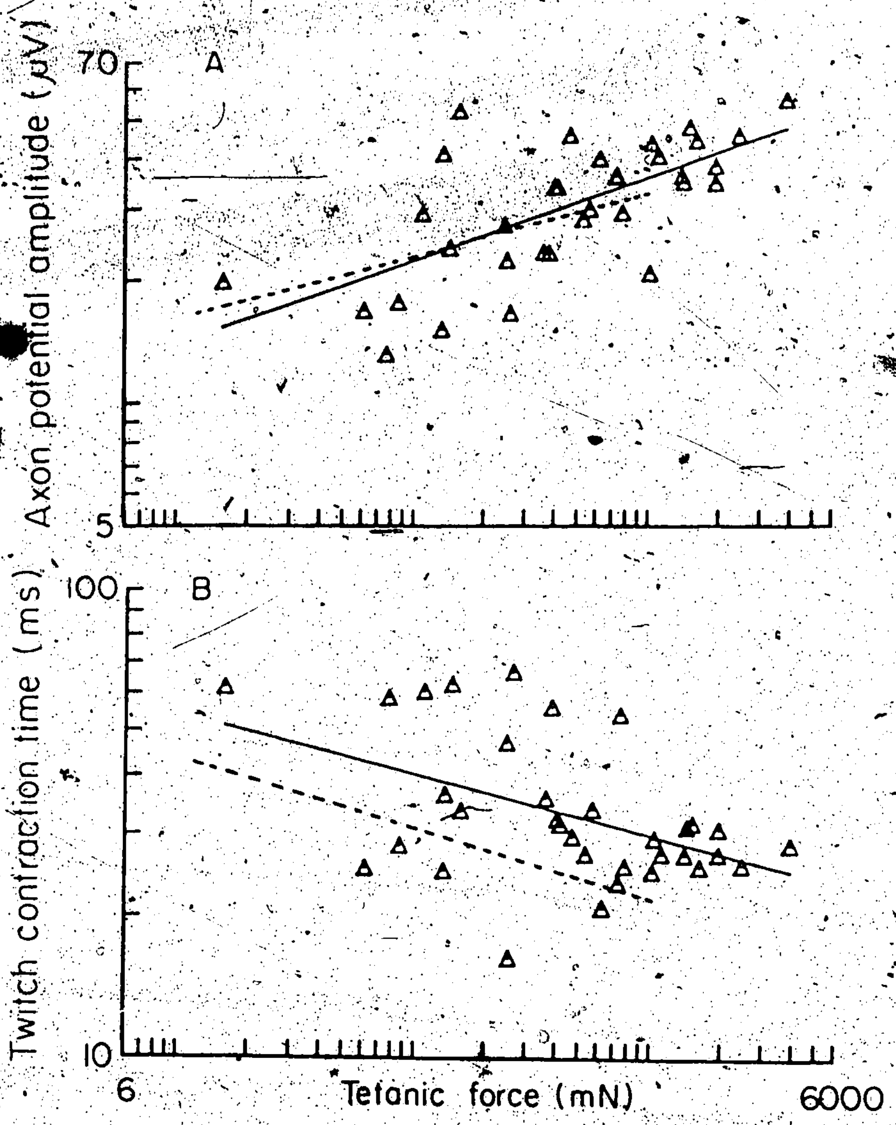


Figure 4.12. Axon and muscle unit size of control and regenerated LGS units. Axon potential amplitude is plotted as a function of tetanic force for control (graph A), and regenerated LGS units (graph B). The normal relationship of these 2 properties seems to be lost in regenerated MU's (graph B); however this graph includes MU's from cats of different ages, (empty triangles) adult cats and (dotted triangles) kittens.



Figures 4.13. Recovery of properties by regenerated motor LGS units in the kitten. Axon potential amplitude (graph A) and twitch contraction time (graph B) are plotted as a function of tetanic force for regenerated MU's. Interrupted lines represent the correlations for control data. Both properties have been re-established.

contraction time (graph B) are re-established. Also notice that these regenerated axons are of normal size and are innervating muscle units bigger than normal.

4.4. DISCUSSION

4.4.1. Reinnervation of Partially Denervated Lateral gastrocnemius muscle. The process of reinnervation of partially denervated LG muscle was similar to that of partially denervated MG muscle.

4.4.1.1. Collateral Sprouting. All LG muscles recovered their original forces, and residual MU's enlarged proportionally to the extent of muscle denervation. Slow, frequently recruited MU's were the first to increase their force and compensate for small degrees of denervation; while FF units required severed extents of denervation to increase their force. No limitation on the capability of motoneurons to sprout was observed for the extents of denervation achieved in these experiments.

Reinnervation of denervated muscle fibers by residual motor axons in the partially denervated LG muscle of the cat is an effective compensatory response. The capability of these remaining MU's to enlarge was much greater than previously found in other muscles and animal species (Brown and Ironton, 1978; Thompson and Jansen, 1977), and more in agreement with that found in fast muscles of the cat (Westerman et al., 1979; Hatcher et al., 1986). These results suggest that the number of denervated muscle fibers an axon can innervate during adulthood is not limited by the size of the MU during the postnatal period, as originally thought. In fact, the size to which MU's enlarged was such that the idea of an intrinsic limitation of the capability of the motor axon to sprout becomes questionable. If a maximum size for sprouting motor axons is found, the causes of this limitation must be sought outside the neuron: obstacles, such as connective tissue and distance between sprouting axon and denervated muscle fiber, amount of "stimulus" for sprouting received by the sprouting axon, and/or "innervability" of the denervated muscle fibers.

The earlier participation of slow MU's in compensating for partial denervation found in this muscle was also found on the MG muscle. A possible explanation for this difference in response between slow and fast contracting MU's could be the amount of activity (frequency of recruitment) that they perform. So that during small extents of denervation, slow, frequently recruited units enlarge first, as denervation increases the remaining MU's are recruited more often and FF motor units enlarged as well.

Innervation of denervated muscle fibers by residual axons is a long lasting process, since animals were studied after long periods of denervation and the size of MU's was as expected for the extent of denervation, in other words, the size originally achieved by the remaining MU's did not decrease with time.

Redistribution of MU types has been previously observed (Reinking et al., 1975; Westerman et al., 1979; Thomas et al., 1986). An increase in the relative number of MU's of intermediate fatiguability was observed, this change is not an interconversion of MU types, but an adaptive response to best perform the function of the muscle. This redistribution is permanent, rather than transitional, since it remained for long periods of time.

In agreement with Hatcher et al. (1986), we found on LG as well as on MG muscle in the adult cat that motor axons do not increase in diameter despite the muscle unit enlargement. However, when muscles were denervated at 1 month of age, the size of the axon increased in diameter, as measure by axon potential amplitude. Most studies on reinervation after nerve section (Kuno et al., 1974; Gordon et al., 1982; Foehring et al., 1986) have enphasized the recovery of axon measures and properties after muscle reinnervation, but less attention has been given to the possible influence of muscle on axons. Our results in the kitten suggest that the final size of axons is not all genetically predetermined, but it can be influenced by the muscle.

Our data on kittens also showed that remaining MU's enlarged as much as remaining MU's in the adult cats. These findings contradict those of Westerman et al. (1979), who found that soleus MU's of kittens denervated at 2 weeks of age did not enlarge as much as those in soleus muscles denervated when cats were adults. Their results were in support of the idea that the process of polyneuronal elimination continues after partial denervation in development animals, and that the size of residual MU's in these animals is due to an incomplete polyneuronal elimination and not to a sprouting process. A possible explanation for the difference in results is that we denervated the muscle at a later animal age, perhaps when most of the polyneuronal elimination had taken place and axons have probably achieved adult capacity to sprout.

4.4.1.2. Axonal Regeneration. The contribution of axonal regeneration to the reinnervation of LG was larger than for MG muscle. The inclusion of kittens on the LGS experiments could account for this difference, since stimulation of the regenerated ventral root in the 3 kittens produced forces (20, 40% of the total muscle force) larger than those produced by regenerated roots in adult cats (usually < 20% of the total muscle force). The greater capacity of kittens to successfully regenerate severed motor axons could be either the result of an age related advantage, or the result of smaller distances between site of axon section and denervated muscle. Only the muscle with the lowest denervation was not reinnervated by regenerating axons; this phenomenon was also observed in the MG muscle. We have postulated (chapter 3) that axons regenerating for long distance do not have the same capability to sprout, either because of limited supply of materials by the cell body, or because the "stimulus" for doing so has decreased by the time axons reach the muscle. This "stimulus" would be expected to decrease in the more "effectively" reinnervated muscles and also explain why axonal regeneration plays a greater role in the most denervated muscles.

4.4.2. Reinnervation of Partially Denervated Soleus Muscle.

Contrary to what happened in the fast-twitch MG and LG muscles, the soleus muscle frequently failed to recover its original force.

4.4.2.1. Collateral Sprouting, Residual MU's of the partially denervated soleus muscle enlarged by up to 6 times their original size, but were unable to adequately enlarge beyond a 4-6 fold increase (300-700mN). These results compare well with those of Thompson and Jansen (1977) and Brown and Ironton (1978), who have emphasized similar upper limits as the maximum capability of MU's to sprout. From the results on the previous 2 muscles, MG and LG, we know that intrinsic limitation is not present in the axons, and that slow MU's (similar to those constituting soleus muscle) are capable of enlarging their size many times. This difference suggests that denervated muscles may play a role in determining the extent to which residual axons can sprout. Karpati and Engel (1968) observed that muscle fibers reinnervated by regenerating axons were grouped together and that intramuscular connective tissue was determining, to a certain extent, the boundaries of the muscle unit. Later Tomanek (1974) observed that connective tissue was normally more abundant than in fast muscles, and that it increases significantly more in soleus than other muscles after denervation. These 2 separate observation are in favour of the idea of connective tissue acting as intramuscular barriers for axonal sprouting. These "barriers" could mechanically interfer with the passage of sprouts through it, or decreasing the diffusion of a soluble sprouting "stimulus".

4.4.2.2. **Axonal Regeneration.** Reinnervation of soleus muscle by regenerating axons was very limited. After long survival times (>5 months), only 1 of the 3 soleus muscles studied independently was reinnervated by regenerating axons, the stimulation of which produced <1% of the total muscle force. If extent of denervation offers an enhancing stimulus to the inherent capacity of cut axons to regenerate, soleus must offer less of such stimulus than LG and MG muscles. The anatomical relationships of these 2 muscles could have also contributed to the poor axonal regeneration to soleus muscle. The few axons regenerating through LGS nerve first have the opportunity to make contact with denervated LG muscle, as they run across it before reaching the soleus muscle.

Our results suggest that reinnervation of LG muscle of the cat by collateral sprouting and axonal regeneration is an effective process, that residual MU's enlarge proportionally to the extent of muscle denervation, and that axonal regeneration plays a complementary role in this process, depending on animal age, distance between axon section and denervated muscle, and extent of muscle denervation. Reinnervation of soleus was not as complete as in LG muscle. Neither collateral sprouting nor axonal regeneration was as efficient as in LG. More emphasis should be given to the muscle when studying limitations of MU's to enlarge.

CHAPTER FIVE: GENERAL DISCUSSION AND CONCLUSIONS.

In 1885 Exner observed that partially denervated muscles recovered their original size, he proposed that residual motor axons were responsible for reinnervating denervated muscle fibers. His observation was later confirmed by van Harreveld (1945) and Weiss and Edds (1946). Cut axons, on the other hand, regenerate (Ramon y Cajal, 1928) and may reinnervate muscle fibers constituting a complementary response to partial denervation. Thus, when part of the innervation to a muscle is removed, denervated muscle fibers can be reinnervated by sprouting of residual motor axons (collateral sprouting) and by regenerating axons (axonal regeneration).

Residual MU's were initially found to enlarge by up to 3-7 times their original size (Kugelberg et al., 1970; Thompson and Jansen, 1977; Brown and Ironton, 1978), and a 5-fold increase has been accepted as the upper limit to which MU's can enlarge. More recently (Hatcher, et al., 1986), however, MU's have been found to enlarge beyond this limit. Our results on LG and MG muscles showed that residual MU's have no intrinsic limitation on their capability to enlarge and that they do so proportionally to the extent of muscle denervation. Slow MU's were the first to enlarge after partial denervation and were able to compensate for small extents of denervation; whereas fast MU's enlarged only [illegible] severe extents of denervation. The readiness of slow MU's to enlarge and the increase in the capability to enlarge of fast MU's with increasing extents of denervation suggest that MU activity may play a role in determining its capability to enlarge. Initially only slow, frequently recruited units enlarged, as extents of denervation increased and fast MU's were recruited more often, they also enlarged.

Residual MU's of the soleus muscle were not very effective at increasing their size and often failed to adequately compensate for denervation. The reason for this difference in behavior between slow MU's of the LG and MG muscles and the slow MU's of the soleus muscle may reside in the muscle itself. Either an increase in intramuscular "barriers" (Kugelberg et al., 1970) or less sprouting stimulus in the soleus muscle would result on less collateral sprouting.

The actual stimulus for collateral sprouting is not known, but the existing evidence suggest that denervated muscle fibers may play a role in initiating and directing nerve sprouts (van Harreveld, 1947; Lomo et al., 1976; Brown and Ironton, 1981; Pestronk and Drackman, 1982). Denervated fibers are supposed to either start releasing a sprout-eliciting factor or stop releasing a sprout-inhibitory factor. Our results show that collateral sprouting is more efficient in MG and LG muscles, as if these fast muscles containing bigger and normally less active muscle fibers (type II) were more successful at attracting axonal sprouts. If denervated muscle fibers of bigger diameter were more "innervable" than those of smaller diameter, such a difference would also offer an alternative explanation as to why slow sprouted and regenerated MU's enlarge relatively more. Collateral sprouting would preferentially innervate bigger muscle fibers and result in relatively greater increases in force for the small MU's.

Regeneration of the axon is an inherent capacity of the motoneuron. After a few days the proximal segment of the axons crosses the site of section an enters a cylinder formed by Schwann cells and surrounding connective tissue, this cylinder is crucial to the success of the regenerative growth. When axons are cut close to the muscle (Thompson and Jansen, 1977; Brown and Ironton, 1978) they not only reinnervate denervated muscle fibers but also can displace collateral sprouts from newly formed synapses. In our experiments axons had to regenerate for long distances and their advantage when competing for reinnervated fibers was not observed. Reinnervation by regenerating axons took place only in severely denervated muscles and was not able to effectively displace collateral sprouts. Presumably time elapsed before axonal arrival allowed collateral sprouts to consolidate their synapses. Denervated muscle probably offers an extra stimulus to the intrinsic capability of motoneurons to regenerate their axons.

The changes observed in MU's properties, speed of contraction and fatiguability, are adaptive responses to the demands imposed upon the residual and regenerated MU's.

Reinnervation of partially denervated muscles of the cat by collateral sprouting and axonal regeneration is an effective process. Collateral sprouting is a rapid response and can successfully compensate for most extents of denervation. The participation of axonal regeneration in muscle reinnervation is less predictable because it depends on several factors. Limitations on motor axons to sprout or to regenerate are most likely extrinsic to the motoneuron.

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