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Qualitative and quantitative morphology of lateral rectus motoneurons of the principal abducens nucleus

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QUALITATIVE AND QUANTITATIVE MORPHOLOGY

OF LATERAL RECTUS MOTONEURONS

OF THE PRINCIPAL ABDUCENS NUCLEUS

by

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This thesis is dedicated to

my husband, Ken

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CURRICULUM VITAE

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ABSTRACT

Nine lateral rectus motoneurons of the principal abducens nucleus, intracellularly stained with HRP, were morphometrically analyzed by light microscopy using a new method for determining motoneuron size. Particular emphasis was placed on devising a method of estimating total dendrite size from the proximal dendritic diameter alone.

The dendrites of these cells were divided into three types. One type, the microdendrites, had a consistent diameter of 1 micrometer, variable but short lengths, and added very little to the overall cell size. The majority of the dendrites on these cells (83) were standard in appearance but they could be separated into two further types. Six dendrites differed from the other 77 in that they were tapering processes which branched minimally, had both a rostrally and a caudally directed secondary dendrite and showed a larger ratio for the sum of the secondary dendrite diameters to the proximal dendrite diameter. The remaining 77 branched extensively and traveled either rostral or caudal in the brainstem. However, the most significant difference was quantitative. The tapering dendrites were approximately 2X the size of the prevalent branching dendrites based on proximal diameter measurements. Correlation coefficients of the relation between proximal diameter and surface area or volume of the entire dendrite increased when the correlations were separated into two types. Therefore, to insure the most accurate total size

calculations, the regression lines used for estimating dendrite size were of the separate correlations. Total neuron size was calculated by adding the soma and dendrite surface areas. An intraneuronal comparison of size indicated that the size of the soma was not indicative of the size of the cell and it constituted between 2% to 7% of the total cell size. Comparison of the motoneuron size to the mechanical properties of their muscle units was inconclusive. However, a general tendency for small motoneurons to innervate muscle units of lower force output was observed. The smaller motoneurons were generally more dorsally located in the nucleus.

INTRODUCTION

It is accepted that a skeletal muscle contraction is modulated to produce the required force by the recruitment of motor units, and the rate of firing of each active unit (Burke, 1981). However, there is no clear explanation of the mechanisms which may establish a recruitment order in the intact animal. In the past twenty-five years, extensive research has been done in the spinal motor system in order to find the basis for orderly motor unit recruitment. Two main hypotheses have evolved. While neither provides a definitive explanation of recruitment order, the understanding of skeletal muscle derived during the controversy has profoundly influenced motor systems research. Spinal motor system research forms a framework other motor researchers can now employ to develop their studies as well as to test the applicability of either hypothesis.

The Spinal Motor System

The more classical concept explaining the basis for recruitment of motoneurons in a motoneuron pool is Henneman's "size principle" hypothesis. Using a variety of stimuli to the triceps surae of cat, Henneman et al. (1957, 1965a) extracellularly recorded the motoneuron responses from teased ventral root filaments. They found that these spinal motoneurons became active in what they interpreted as a size related sequence. Basing their motoneuron size determinations on the physiological parameters of extracellular spike size or conduction velocity, rather than

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morphological measurements, they claimed that the spinal motoneurons were recruited in an ascending size order, with few exceptions. Subsequent experiments (Henneman et al., 1965b, 1974; Olson et al., 1968; Somjen et al., 1965) led Henneman (1974) to state "As a consequence, the motor units of a muscle can be fired in one particular order as determined by the size of their neurons...and it is the size of the motor neuron that determines its threshold and relative excitability."

This explanation of recruitment order is acceptable but but it requires several assumptions. Underlying this explanation is the acceptance of the classical definition of the motoneuron pool as the total population of motoneurons that innervates a skeletal muscle (Creed et al., 1932). This definition suggests that the pool is a collection of cells grouped in proximity in the central nervous system. In the spinal cord, motoneuron pools are recognized as relatively defined cell columns of predictable location (Romanes, 1941). These are important concepts to Henneman's hypothesis as they support the belief that the afferent input to a motoneuron pool is uniformly distributed to all cells innervating a muscle (Henneman, 1974; Henneman and Mendell, 1981). In 1971, Mendell and Henneman recorded the excitatory post synaptic potentials (EPSPs) of motoneurons in response to stimulation of single 1A spindle fibers from the medial gastrocnemius. They found that individual EPSPs were recorded in 114 of the 122 motoneurons studied. This led

them to propose that 1A fibers are distributed to nearly all (90%) of the motoneurons in a pool. They further suggested that each motoneuron has a equal density of 1A endings (not an equal number of endings because 1A fibers differ in their number of terminals) and that 1A fibers are distributed in proportion to the surface area (i.e. size) of the motoneurons. Therefore, they postulated that if the motoneurons innervating a muscle receive the same afferents, neuronal activation is dependent on intrinsic motoneuron qualities (i.e. input resistence), which are related to motoneuron size.

In addition, if all the nerve cells to a muscle receive a homogeneously distributed input, the motoneuron pool would not be spatially divided or organized by any criterion (i.e. size) from within (Clamann and Kukulka, 1977). The existence of either of these situations would suggest selective input to specific cells, which according to Henneman's hypothesis, would not be necessary. Also, when recording from ventral root filaments, it must be assumed that the axons recorded from are representative of the response of the "classical" motoneuron pool to any stimulus (Clamann et al., 1974)

Another significant assumption of the "size principle" is that the motor units in a pool are a homogeneous population whose neural and mechanical properties vary over a continuum (Wuerker et al., 1965; McPhedran et al., 1965). The muscle unit identity is inconsequential since motoneuron

size, derived from conduction velocity, shows a relationship to muscle unit mechanical properties. Therefore, a small motoneuron innervates a small number of slow contracting fibers, a large motoneuron innervates a larger group of fast contracting fibers and there are gradations of motoneuron size to muscle unit capabilities between these extremes. In effect, the motoneuron determines the contractile properties of the muscle unit (Wuerker et al., 1965).

It should be reiterated that Henneman et al. (1965a&b, 1974; Clamann and Henneman, 1976; Bawa et al., 1984) inferred cell size from physiological parameters, rather than direct measurements. Extracellular spike size recorded from ventral root filaments was judged as a means to determine the diameter of an axon (Gasser, 1941; Clamann and Henneman, 1976). It was assumed that the diameters of axons correlated to the size of the cell bodies, based on the work of Ramon y Cajal (1909) who established this concept by measuring the cell bodies and axons of neurons in the central nervous system. In other "size principle" related studies (Wuerker et al., 1965; McPhedren et al., 1965; Bawa et al., 1984), axonal conduction velocity was used as a criterion for calculating axon diameter (Hursh, 1939) and cell size (Barrett and Crill, 1974; Clamann and Henneman, 1976; Cullheim, 1978; and Burke et al., 1982). Physiological cell size does play an important role in motoneuron recruitment. Whether it is the "factor" that determines the functional threshold of a motoneuron remains to be seen.

Whether the characteristics of a motoneuron pool necessary to support the "size principle" exist in the spinal motor system is controversial. There is disagreement as to the organization of the motoneuron pool. Evidence has been found supporting a somatotopic relation between motoneuron position within the pool and the location of its muscle unit in certain muscles (Swett et al., 1970). Burke et al (1977) showed a higher ratio of large to small alpha motoneurons distributed to the rostral third of the medial gastrocnemius nucleus than expected, based on random distribution. However, Clamann and Kukulka (1977) found that motoneurons to the plantaris and medial gastrocnemius are randomly distributed with respect to size in their motor nuclei. Sterling and Kuypers (1967,1968), based on morphological evidence, suggested that soma location may be an important determinant of connectivity. They found that motoneuron dendrites, dorsal root fibers and interneuronal fibers of the brachial spinal cord ran longitudinally in the cord. They expected that the group of motoneurons that would be activated together would be a "longitudinal array of functionally related motoneurons supplying motor units in a number of anatomically distinct muscles." Wyman et al. (1974) suggested three possible definitions of motoneuron pool in which recruitment by size could take place: all motoneurons innervating a muscle, all motoneurons in a given ventral root or all motoneurons activated by a given stimulus.

Experiments to test the hypothesis that motoneurons in one ventral root are representative of the whole pool have not been conclusive but they are convincing. Clamann et al., (1974) simultaneously recorded the monosynaptic reflexes of the triceps pool from L7 and S1 ventral roots. The integrated responses from these roots were led into an oscilloscope and displayed as an X-Y plot. They found that the two reflexes declined from their peak following posttetanic potentiation producing a nearly linear decline in the X-Y plot. The average rate of decline of the reflexes was equal in the two roots and the percent of maximal discharge in either root was a good approximation to the percent of maximal discharge in the whole triceps pool. The deviations form strict linearity were explained as being due to the unequal distribution of medial gastrocnemius, lateral gastrocnemius and soleus pools in L7 and S1. Therefore, they concluded that within limits, the triceps pool may be treated as a single pool that is sampled by the S1 ventral root. Also, a study of size and position of motoneurons in the medial gastrocnemius pool has shown that any large sample of motoneurons in an area of the nucleus will contain a sample of representative sizes (Clamann and Kukulka, 1977). However, according to Weeks and English (1982a&b), a specific subvolume or compartment of a single muscle is innervated by a specific primary nerve branch of the motor nerve which has a comparable compartment in the motor nucleus. They found (1983) that the most rostrally located

compartment nucleus consists of the largest sized motoneurons and the more caudally placed compartment nucleus contains a larger percentage of the smallest sized motoneurons. In a later study (Lucas and Binder, 1984), the medial gastrocnemius muscle was used to examine the homonymous, monosynaptic connections from lA-afferents to motoneurons innervating an intramuscular compartment. They found that homonynous lA-afferent input is topographically weighted within the medial gastrocnemius motor nucleus so that the synaptic effects in motoneurons projecting to the same compartment are greater than in other homonymous motoneurons. Also, the weighting appeared greater in experiments containing a large number of high rheobase cells (large cells). A concomitant study (Lucas, Cope and Binder, 1984) determined that this "topographical weighting" was not due to projection frequency differences between nerve branches. However, the mean EPSP amplitude was larger in the same branch group of motoneurons than in other branch groups. Again this difference was most obvious in high rheobase (large) motoneurons. They speculate that this finding reflects a qualitative difference in the organization of synaptic input to small and large motoneurons.

The possibility exists that the stimulus defines the motoneuron pool. Henneman et al. (1965b) recorded the responses of neurons in both a stretch reflex and a crossed extension reflex. They found that most motoneurons were not activated by both stimuli. Variation in the sequence of

recruitment order during voluntary contraction shows similar results. Grimby and Hennerz (1968) showed that the recruitment order of motor units on voluntary contraction of the tibialis anterior was altered by proprioceptive afferent activity and velocity of contraction. Desmedt and Godaux (1981), studying the first dorsal interosseus muscle, found a size related recruitment order during abduction which changed when the same muscle was used as a flexor.

The changes in recruitment order exhibited in the previous studies may reflect that motor unit recruitment is based on motor unit type. Liddell and Sherrington (1925) defined the motor unit as the motoneuron and the muscle cells it innervates. The importance of motor unit identity was first emphasized by Denny-Brown (1929) while working with Sherrington at Oxford. He showed that in a stretch reflex, the first motoneurons recruited (or those with the lowest thresholds) innervated muscle units that contracted slowly, were fatigue resistent and red in color. The others, which had the highest functional thresholds, were ordinarily recruited in the largest reflex responses and innervated muscle units in the fast contracting fatiguable "white" muscles.

During the decades since this pilot work, motor units have been shown to be distinct morphological, biochemical, and physiological entities (Brooke and Kaiser, 1970; Burke et al., 1967, 1971, 1973, 1974; Peter et al., 1972; Fleshman et al., 1981a; Sypert and Munson, 1981). Brooke and Kaiser

(1970) and Peter et al. (1972) developed the two major histochemical classification systems for muscle fibers. The former researchers identified three fiber types based on the alkaline versus acid stability of myofibrillar ATPase activity: Type I, high ATPase activity after acid preincubation at pH 4.3; Type IIa, high ATPase activity at pH 9.4 and low at 4.6; and Type IIb, high ATPase activity at 9.4 and at 4.6. The latter identified three types of fibers on the basis of staining intensity of specific metabolic enzymes: fast glycolytic or FG; fast oxidative glycolytic or FOG and slow oxidative or SO.

Early physiological studies demonstrated that motor unit types could be differentiated on the basis of contraction properties (Wuerker et al., 1965). It was observed that the slow contracting units developed less tension than the faster units but fatigued more slowly. It was thought that the differences found were due to the morphology of the fibers making up the units. However, subsequent work using glycogen depletion of single units, showed that all fibers making up a unit contained identical histochemical properties (Edstrom and Kugelberg, 1968). From that time, it has been assumed that a muscle unit is homogeneous in fiber type.

Burke et al. (1967, 1971, 1973, 1974) developed a tripartite motor unit classification based on the contractile properties of the motor unit. An initial study (Burke et al., 1967) defined motor units on the basis of contraction speed, fast twitch or F units and slow twitch or S units.

Later studies (Burke et al., 1971, 1973, and 1974) added the fatiguability of a motor unit to this classification. This resulted in three types: FF or fast twitch fatiguable, FR or fast twitch, fatigue resistant and S or slow twitch, fatigue resistant. Concommitant histochemical analysis with the physiological tests revealed (Burke et al., 1973, 1974) that the tripartite system S, FR, and FF corresponded to the muscle fiber classification system of Brooke and Kaiser (1970) of I, IIb, and IIa and Peter et al. (1972) of SO, FOG and FG, respectively. Burke (1981) and Sypert and Munson (1981) indicated that recruitment of motor units is based on motor unit type. They suggest that the intrinsic motoneuron excitability and the density of 1A synaptic current both decrease in the order of motor unit type, S>FR>FI>FF. Therefore, sequential recruitment is in order of increasing contraction strength and fatiguability in the order S, FR, FI and FF. While it appears that the thrust of the research of the adherents to the "motor unit type" hypothesis has been directed toward the characterization of the muscle unit, physiological properties of the motoneurons to these units have been studied as well. In 1981(a), Fleshman et al. measured the rheobase (an index of neuron excitability), input resistance, conduction velocity and mechanical responses of medial gastrocnemius motor units. They classified motor units on the basis of contraction time and fatigue index of the muscle units. Then, they correlated the intrinsic motoneuron characteristics of rheobase, input resistance and conduction

velocity to motor unit type, maximal tetanic tension and to each other. What they found were coefficients of determination (r x r) which showed that the variability of rheobase, input resistance and maximal tetanic tension was best accounted for by motor unit type. Conduction velocity, an accepted indicator of cell size, accounted for little of the variability (37% or less).

However, it should be mentioned that input resistance has been shown through physiological and morphological measurements to have a higher direct correlation to motoneuron surface area than that of conduction velocity to surface area (Lux et al., 1970; Barrett and Crill, 1974; Cullheim, 1978). With that in mind, review of previous correlations (Fleshman et al., 1981a) suggest that 76% of the variability in rheobase can be accounted for by motor unit type, while size (input resistance) accounts for 56%. Motor unit type accounts for 69% of the variance in size, rheobase accounts for 56%. Thus, while motoneuron size can be linked physiologically to muscle unit capabilities, other physiological characteristics have a more significant relation (and may affect the recruitment order of the motor unit).

Input resistance, as a measure of cell size, needs further discussion. Input resistance (RN), in theory, depends on the surface area of the cell, the specific membrane resistivity (RM) and the electrotonic length (LN) of the dendritic tree. Therefore, input resistance is only a reliable indicator of size if RM and LN remain constant

throughout the motoneuron pool. Kernell and Zwaagstra
(1981) found that specific membrane resistance is lower for
motoneurons with fast conducting axons than for slow
motoneurons. So, taking RM to be constant across the pool
would lead to an overestimate of size differences between
fast and slow cells based on the RN. Recently Ulfhake and
Kellerth (1984) presented the results of a study of the
passive membrane properties of triceps surae motoneurons.
Soleus motoneurons, of type S motor units and medial
gastrocnemius motoneurons of types S, FR and FF motor units
were analyzed for electrical properties and morphological
size. The results of the work indicated a difference in
specific membrane resistivity between S and F type
motoneurons. The difference was observed in neurons of
similar anatomical size. Specific membrane resistivity was
higher in S motoneurons than F motoneuron types.

The extrinsic factors of functional threshold (e.g.
organization and quality of synaptic input) are controversial
as well (Enoka and Stuart, 1984). Mendell and Henneman
(1971) found an equal density of 1A fibers on triceps surae
motoneurons. Scott and Mendell (1976) later found that there
were 1A fibers with different projection frequencies for the
homonymous muscle. One group (X) which evoked EPSPs in
almost all (80%) of homonymous motoneurons and the other (Y)
which had a lower projection frequency (65% or less) to the
homonymous muscle. This suggests that 1A fibers are not
homogeneous in their reflex activity.

Burke et al. (1970) found qualitative and quantitative differences in afferents to the triceps surae motoneuron pool. Polysynaptic cutaneous afferents in the saphenous and sural nerves predominantly caused inhibition in slow motor units and mostly excitation in fast units. Stimulation in the red nucleus caused predominantly inhibition of the slow units and excitation in the fast units. The amplitudes of the inhibitory post synaptic potentials (IPSPs) were larger in slow units.

Obviously, the intrinsic and extrinsic parameters which affect the functional threshold of a motoneuron are not completely understood. Fleshman et al. (1980, 1981b) investigated the amplitude of 1A EPSPs recorded from motoneurons to the medial gastrocnemius resulting from the stretch reflex. They measured the input resistance and conduction velocity of the motoneurons as well as the mechanical properties of the motor units. They found that mean 1A single fiber EPSP amplitude differed among motor units, rather than by motoneuron size, decreasing in the order of S>FR>FF. Fatigue resistant units (FR and S) received a larger proportion of 1A fibers than did the fatiguable units. However, Clamann et al. (1985) found something quite different. They determined the functional connectivity of 1A and Group 11 spindle afferents from the medial gastrocnemius muscle to the medial gastrocnemius motoneurons. They found that the lowest projection per-centage was obtained between the smallest afferent fibers and

smallest motoneurons and that the percentage increased as the size of the afferent fiber or the motoneurons increased.

It is apparent that some combined effect of the presynaptic and postsynaptic factors is the basis of motor unit recruitment. Whether cell size or motor unit type predominates, is not evident.

In 1981, Ulfhake and Kellerth completely reconstructed and measured ten ventrolateral dendrites of intracellularly stained triceps surae motoneurons. They found a very high correlation (r=.99) between the proximal dendritic diameter and the surface area of the entire dendrite. By using the regression line of this correlation, they estimated the surface area of the remaining dendrites by their proximal diameters alone. Finding the mean soma diameter and calculating its surface area from a geometrical formula, they could then readily determine the quantitative morphological size (receptive area) of the entire cell.

In 1982, Ulfhake and Kellerth and Burke et al., in separate studies, used this regression line (Ulfhake and Kellerth, 1981) to determine the soma-dendrite size of motoneurons to known muscle unit types of the triceps surae of cat. These studies had important results. First, the size of the soma, mean diameter or surface area, was not found to be indicative of motor unit type, or vice versa. There was overlap of soma size within the gastrocnemius motoneuron pool to the FF, FR and S motor unit types. Significant differences were found only between the triceps S

type (soleus and gastrocnemius) and the gastrocnemius FR and FF types. However, when they determined total membrane surface area, they found that the mean area varied with motor unit type in the sequence FF>FR>S. While it could be argued that the regression line used for their dendritic estimates was based on only ten dendrites and that these had the same orientation to the soma (ventrolateral), both teams seemed to agree that there is a correlation between motor unit type and motoneuron size. This is interesting considering that in a recent article, Bawa et al. (1984), determined the recruitment order of motor units in the homogeneous muscle, soleus. The type "S" motor units were recruited in order of their conduction velocity (size).

It is obvious that there is no single, simple explanation for the recruitment of motor units that is presently demonstrable in the spinal motor system. Inasmuch as the majority of researchers have used only the triceps surae of cat, it seems likely that the answer may be found in the study of other motor systems. While the techniques used to study other systems are primarily those employed in the spinal motor system, the results, particularly in the extraocular motor system, have given new insight to the recruitment order controversy.

The Extraocular Motor System

The extraocular muscles, four recti and two obliques, move the globe of the eye. In some species (e.g. cat) there is an additional muscle, the retractor bulbi, which retracts

the globe for the passive movement of the nictitating membrane (Marek et al., 1984). The regulation of muscle force output of these muscles, like those of the spinal system, is modulated by the recruitment of motor units and discharge frequency of motor units already recruited (Barmack, 1977). In contrast to spinal skeletal muscle, extraocular muscle force appears to be regulated primarily in some species (e.g. primates) by frequency modulation (Robinson, 1970). However, this issue is controversial and the existence of a size related recruitment order is being investigated.

There is minimal research of motor unit recruitment in the extraocular system. This is not surprising as the lack of agreement on motoneuron pool organization, afferent input and motor unit types is indicative of the complexity of the system. Extraocular muscle is divided into an orbital and global layer (Kato, 1938). Also, Hess (1961) and Hess and Pilar (1963) first demonstrated two basic classes of muscle cells in these muscles, singly innervated fibers and multiply innervated fibers. Singly and multiply innervated muscle fibers have been found in mammals in the extraocular muscles, the laryngeal muscles (Floyd, 1973) and the inner ear musculature (Erulker et al., 1964; Fernand and Hess 1969).

The application of either of these two major theories of recruitment to the extraocular system is premature. However, to consider the characteristics of spinal motor unit recruitment as they apply to the extraocular system, is not.

This review will emphasize the lateral rectus of cat.

The lateral rectus is said, classically, to be innervated by the principal abducens nucleus which is found beneath the floor of the IV ventricle in the brainstem (Ramon y Cajal, 1911). However, the motoneuron pool of the lateral rectus is not all contained in the principal abducens nucleus. Retrograde labeling studies, intramuscular or cut nerve, stained cells in the principal abducens nucleus (Spencer and Sterling, 1977), the accessory abducens nucleus and the oculomotor nucleus (McClung et al., 1983).

The organization of lateral rectus motoneurons within the principal abducens nucleus is controversial. Spencer and Sterling (1977) found lateral rectus motoneurons distributed throughout the rostral-caudal extent of the principal sixth nucleus with no size (soma dimension) orientation. Goldberg et al. (1981) found that these motoneurons tended to be organized according to the contraction speeds of single muscle units. Intracellular stimulation of these motoneurons produced progressively slower contraction times from dorsal to ventral in the nucleus.

As the motoneuron pool organization of the lateral rectus seems obscure, so is the definition of an extraocular motor unit. Though exceptions occur (Emonet-Denand et al., 1971), the muscle unit of a motor unit is supposed to be found in one muscle. Crandall et al. (1981) stimulating motoneurons in the principal abducens nucleus of cat, were specifically looking for retractor bulbi

motoneurons in order to study the muscle unit contractile properties. What they found was that out of forty stimulated retractor bulbi motor units, seven of the muscle units involved the contraction of retractor bulbi muscle slips as well as lateral rectus muscle fibers.

The muscle units of extraocular muscle have not been fully classified. In morphological studies, depending on species and experimental sampling procedures, subdivisions of singly and multiply innervated fibers have resulted in the description of five (Alvarado and Horn, 1974; Pachter et al., 1976: Spencer and Porter, 1981) six (Davidowitz et al., 1977; Mayr, 1971; Porter, 1980) and seven (Pachter, 1983) fiber types.

In a combined ultrastructural and histochemical analysis, Porter (1980) divided the lateral rectus into six fiber types. All of the singly innervated fibers (4) showed myofibrillar ATPase activity of type II fiber types of Brooke and Kaiser (1970). However, the oxidative enzyme histochemistry suggested further subtypes. The multiply innervated fibers (2) differed in mitochondrial content, ATPase activity and oxidative enzyme histochemistry depending on whether they were found in the orbital or global layer. The orbital layer multiply innervated fibers corresponded to types I and II of Brooke and Kaiser (1970) while the global layer multiply innervated fibers compared to type I.

Some of the contractile properties of extraocular motor units have been identified. Cooper and Eccles (1930) found

extraocular muscles to be the fastest contracting mammalian muscles. Reported contraction times for whole muscles range from 5 to 10 msec., while the fastest hindlimb muscle twitch time is approximately 25 msec. (Chiarandini and Davidowitz, 1979; Lennerstrand, 1975). However, Duke-Elder and Duke-Elder (1930) demonstrated that some of these fibers, sensitive to acetylcholine, developed a slow, long lasting contraction when exposed to cholinergic drugs. Subsequent morphological and physiological studies demonstrated that these fibers were multiply innervated and distinct from singly innervated twitch fibers (Hess, 1961: Hess and Pilar, 1963: Matyushkin, 1964). While it is accepted that the singly innervated fibers make up the twitch or phasic (fast) motor component of extraocular muscle and are capable of impulse conduction (Bach-y-Rita and Ito, 1966; Hess and Pilar, 1963; Lennerstrand, 1975), there is controversy over the characteristics of the multiply innervated fibers.

The multiply innervated (polyneuronal) muscle fibers appear to be the slow component of extraocular muscle (Chiarandini and Stefani, 1979; Chiarandini et al., 1983). In some studies they do not show tension development until stimulated tetanically (Matyushkin, 1964; Barmack et al., 1971; Nelson, 1984). In others, a slow twitch multiply innervated component was found (Bach-y-Rita and Ito, 1966; Hess and Pilar, 1963; Chiarandini et al., 1983).

Based on the mechanical properties and innervation patterns, Lennerstrand (1974) divided extraocular motor units

into three types: a fast singly innervated unit, a slow
twitch multiply innervated unit and a slow non-twitch unit.
In a subsequent study, using intracellular stimulation of
lateral rectus motor units, Goldberg et al. (1976) compared
the contraction characteristics of lateral rectus motor units
to those of the inferior oblique units in Lennerstrand's 1974
study. They found that the contraction speed and tension
properties were within the same range. However, Meredith
(1981) studying the muscles innervated by the oculomotor
nerve, found that the motor units exhibited faster
contractions and stronger tensions than the lateral rectus
units. Results of a study of the mechanical and electrical
characteristics of the motor units in the superior oblique do
not conform to the classification system of Lennerstrand
(Nelson, 1984). Nelson proposed a classification system in
which he divided units into twitch and nontwitch units.
Twitch motor units exhibited a broad range of motoneuron
muscle unit mechanical properties. However, the
interrelationship of these properties showed linearity. The
nontwitch units appeared to be a homogeneous population which
produced weak tetanic tensions, were fatigue resistant, and
were innervated by slowly conducting axons. He did not find
slow twitch multiply innervated fibers.

Studies of the pathways to the extraocular muscle motor
nuclei have been used to assess afferent input similarities
or differences. Several important sources of input to the
principal abducens nucleus have been identified: the

vestibular nuclei (Highstein, 1973; Carleton and Carpenter, 1983), the contralateral oculomotor nucleus (Maciewicz et al., 1975), the pontine reticular formation (Buttner-Ennever and Henn, 1976; Highstein et al., 1976) and nucleus prepositus hypoglossi (Maciewicz et al., 1977). However, the abducens nucleus does not contain only lateral rectus motoneurons (LRMs). Besides LRMs, there are interneurons to the medial rectus subdivision of the contralateral oculomotor nucleus (Baker and Highstein, 1975) and in cat, retractor bulbi motoneurons (Crandall et al., 1981).

Spencer and Sterling (1977) labeled LRMs and interneurons in the principal nucleus. They were attempting to find cytological and synaptological differences to distinguish the two. While they did not find a structural difference between the motoneurons and interneurons or a synaptic organization which could separate the two, they did find differences within the motoneuron population. The distribution of boutons containing spheroidal vesicles (excitatory) to that containing flattened vesicles (inhibitory) is in a ratio of one to one for the motoneuron cell body and one to 2.3 for the dendrites. The boutons containing excitatory or inhibitory vesicles form homogeneous clusters. Also, the total density of boutons varied between motoneuron parts. A greater density was found on the dendrites than on the soma. Last, despite the ratio of spheroid/flat (S/F) vesicles being the same, motoneurons varied significantly in the total density of boutons.

Spencer and Sterling suggested that although there were constant features of synaptology shared by the motoneurons, other features such as total density of boutons and dendrite S/F ratio were specific for each motoneuron.

Morphological studies of LRMs in the principal VI nucleus are limited and have produced conflicting results. Somas have have been shown to be multiple in shape and range in size from 15 to 60 micrometers in mean diameter (Spencer and Sterling, 1977; Grant et al., 1979; Highstein et al., 1982). Grant et al. (1979) found a highly branched dendritic pattern which extended outside the borders of the principal abducens nucleus to distinct regions of known afferent input. Highstein et al. (1982) did a similar study using intracellular HRP staining. Like Grant et al. (1979), they found that dendrites branch close to the soma but were contained within the nucleus and tapered extensively. The relationship of soma surface area to soma-dendrite surface area was variable. Their three totally reconstructed motoneurons showed soma surface area to soma-dendrite surface area measurements of 2,400 um^2:43,600 um^2; 3,200 um^2:51,000 um^2; and 1,900 um^2:57,000 um^2. These ratios are 0.055, 0.062, and 0.033, respectively. These comparisons suggest that soma dimensions are not good indicators of the total receptive area of the cell.

Combined morphological and physiological studies of LRMs are very limited. Grantyn et al. (1977) compared the morphology of procion-dyed LRMs (soma sizes) to their conduction velocity, input resistance and rheobase. They found LRMs could be divided into two groups, fast and slow, based on conduction velocity. Within the fast conducting group, conduction velocity increased and input resistance decreased as a function of soma surface area. The regression line of their correlations could not be used for slow conducting motoneurons. If the soma size of slow LRMs was estimated from the conduction velocity using the fast conducting cells' regression line, it would result in too low a value for soma surface. They suggest that slow conducting motoneurons be considered a separate pool. In another soma size related study comparing soma sizes to the mechanical properties of LR motor units, Goldberg and McClung (1982) found a lack of correlation between the mean soma diameter and the mechanical properties of twitch units except at the extremes.

In 1978, Grantyn and Grantyn, again using intracellular procion dyes to label LRMs, compared the physiology of the motoneurons to their size. This time they determined the total surface measurement of 5 cells completely reconstructed and measured. They found a statistical relationship between the input resistance and soma surface area ($r=-0.74$) and input resistance to conduction velocity ($r=-0.78$). However, in looking at the data of their 5

reconstructed cells, there is a lack of consistency between the morphological and electrophysiological data of the fast conducting cells (25 to 84 m/sec). One cell had a very low input resistance and was second in size (surface area) but it had the lowest conduction velocity. Also, assuming that specific membrane resistivity is the same as spinal motoneurons, the input resistance recorded was two to four times less than that predicted, based on cell size. It should be mentioned that the morphological-physiological comparisons in this study are not definitive as procion dyes have been shown not to stain cells as conpletely as horseradish peroxidase (Grant et al., 1979) and lead to underestimating quantitative morphology.

In summary, Henneman's "size principle" hypothesis does not completely explain motor unit recruitment in the spinal motor system. Both presynaptic and postsynaptic factors may be involved. However, as it is related to the contractile properties of the muscle unit, motoneuron size is a significant variable in defining spinal motor units. As a recruitment mechanism cannot be proposed in the extraocular motor system because the motor units have not been delineated, the present study evolved. In order to develop qualitative and quantitative morphological knowledge of LRMs, nine cells, intracellularly stained with horseradish peroxidase were reconstructed and measured. These principal abducens motoneurons of known muscle unit capabilities were also evaluated for possible structure-function relationships.

The specific aims of this study were:

1. To determine whether there is a statistically significant relation between the size of a proximal neuronal structure (soma or proximal dendrite) and that of entire dendrite (surface area, volume or combined length).

2. To determine the total neuron size of these LR motoneurons.

3. To evaluate morphological characteristics of soma size, soma shape, dendritic tree characteristics, location in the nucleus (depth from the IV ventricle) and neuron size for a possible motoneuron type classification.

4. To compare these morphological qualities to their muscle unit's mechanical properties.

5. To compare LR motoneuron morphological data to published data on spinal cord motoneurons.

Methods and Materials

Cell Preparation

Fifty micrometer sections of nine principal abducens motoneurons to lateral rectus muscle units, prepared for light microscopy, were obtained from the collection of Dr Goldberg and Dr. McClung. The cells had been identified by antidromic stimulation of the VI nerve in the brainstem. Muscle unit confirmation was through muscle twitches elicited by intracellular motoneuron stimulation and recorded with a strain gauge. Glass electrodes filled with a 4% solution of HRP impaled each cell and the solution was iontophoresed. After a minimum of two hours, the cat was sacrificed by intracardiac perfusion and the tissue was fixed with a 1.25% glutaraldehyde/1% paraformaldehyde solution. The tissue was processed for HRP labeling according to Hanker et al. (1977). No correction was made for tissue shrinkage in the quantitative data.

Cell Reconstruction

Reconstruction of the LRMs was done using a Nikon LKE microscope fitted with a drawing tube. The magnification of the drawing set up was 15X (ocular), 100X (oil immersion objective) and 1.25X (drawing tube), resulting in 1875X total. For tracing accuracy, the scope was put into Kohler illumination after all lenses had been attached.

Cell reconstruction began with the slide section(s) containing the soma. The somas were drawn as they appeared,

two dimensionally, except in cases of the cell bodies which were split between sections. In these situations, soma shape was taken as the connection of the two segments which gave the best smooth surface.

In all cases, the proximal dendrites were drawn to the first branch point. Small caliber processes attached to the soma or proximal dendrites were drawn and noted. Since these microdendrites did not branch and were usually contained in the soma section, they were drawn to terminus.

The reconstruction of the 15 dendritic arborizations required the tracing of dendritic segments through several sections. Clues used to make proper connections between sections varied from individual cell staining intensity, anatomical landmarks to structural anomalies. Quantitative data was used also to verify sectional changes.

The terminus of a dendrite was determined as the point before the dendrite became impossible to resolve under light microscopy.

Qualitative Information Recorded

It was necessary to keep information on the source of each cell as 6 cats provided the 9 cells studied. The depth of the cell from the IV ventricle, in millimeters, was the location marker. The shape of the soma was noted in terms of a geometrical figure, e.g. triangle. The number, type and orientation to the soma was recorded for every proximal dendrite.

Along the extent of the 15 completely reconstructed

dendrites, notice was taken of spinous processes, beaded segments and any structural anomaly. The course of these structures in the brainstem; ventral, lateral, dorsal, medial, rostral, or caudal was followed to terminus. The termination of these dendrites (general area) was noted, e.g. MLF.

The mechanical properties of the attached muscle units were identified at the time of the intracellular staining. The twitch tension, maximum tetanic tension and fusion frequency of these units were the basis of another study (McClung and Goldberg, 1982), but the information was available for comparison with the morphological data.

Quantitative Information Recorded

The cells were measured on the Nikon LKE using an eyepiece micrometer, 1000X magnification. Prior to measurement, the microscope was put into Kohler illumination and the micrometer was calibrated for the 100X objective, using a stage micrometer. Before each individual measurement, the magnification adjusting ring was checked for maintained calibration.

Soma measurements:

Because the HRP staining masked the position of the nucleolus and the cell bodies were not symmetrical in shape, mean soma diameter was determined in several ways to find some level of consistency. Using a Zeiss MOP-3 digital analyzer, the 2-dimensional perimeter was found. This perimeter was equated to the circumference of a circle and

diameter was calculated from the formula, c=πd (circumference
equals diameter times pi (3.14). Second, the soma was
equated to a circle and using a set of circles of varying
diameter, one was chosen which best fit within the soma.
From this circle, a fixed point was chosen as the center
point. Again using the MOP digital analyzer, the largest
diameter and smallest diameter through the center point to
the circle outline were averaged. The third method was to
equate the soma to a rounded geometrical shape, i.e.
ellipsoid, find the center point, and draw perpendicular
lines to the outside borders. The mean of the greatest
maximal diameter and the greatest minimal diameter measured
with an eyepiece micrometer was considered the mean soma
diameter. The latter two methods consistently produced
similar results so the diameters used in statistical analyses
were those found using the eyepiece micrometer.

Proximal dendrites:

Proximal dendrites were counted and their diameters and
lengths were measured. Diameters were determined from
measuring the process after the truncated cone had tapered
to a consistent diameter before the first branching. The
length of the proximal dendrite was the distance from the
soma outline to the fork of the secondary dendrites.
Attached microdendrites, considered secondary dendrites,
were counted and their diameters and lengths measured.

Dendritic trees:

Fifteen dendritic arborizations were measured

completely. Diameters and lengths along the extent of the
process were found. At least three measurements for diameter
were made between branch points to find a consistent
diameter. In cases of tapering, specific and mean diameters
were calculated for a given length. Spinous processes
consisting of a thin pedicle topped with a bead occurred
occasionally after the proximal dendrite level. The diameter
of these structures was taken as the mean of the two parts.
At the terminal branches of many dendrites were found beaded
segments. The diameter of these structures was recorded
as the mean of the thin intersegments and the beads.

Calculations

Using the method previously described, the mean soma
diameter was calculated. The soma surface area was
quantified from the formula of Ulfhake and Kellerth (1981),

π　　　　　X Major diameter X Minor diameter.
Proximal dendritic diameters were summed for a combined
diameter, with and without adding the microdendrites. The
mean proximal diameter per cell was found (with and without
the microdendrites) as well as the mean diameter of the total
83 regular dendrites.

The combined length of a dendrite was the sum of the
dendritic segments of one proximal dendrite from the cell
outline to its terminus. The combined length of a secondary
dendrite was the same minus the length of the proximal
dendrite and was applied only to those segments related to
one secondary dendrite. The surface area and volume of a

dendrite were found by summing the individual areas and volumes calculated for segments. They were calculated from geometrical formulas for a smooth cylinder, $2\pi rh$ and $\pi r^2 h$, respectively. The total size of a motoneuron was the sum of the soma surface area and the surface areas of all of the dendrites.

Statistics

Means, ranges and standard deviations were calculated for soma and dendritic dimensions of the nine cells. Cell body sizes were compared to known LRM soma sizes of a retrograde HRP study (Spencer and Sterling, 1977) to check the reliability of the intracellular procedure. Also, they were compared to the spinal motoneuron soma dimensions found by Ulfhake and Kellerth (1981). Statistics of dendrite dimensions were calculated primarily to distinguish the two types of dendrites quantitatively. Also, they were used to compare LRM dendrites with spinal motoneuron dendrites (Ulfhake and Kellerth, 1981). Variations in means were tested for significance by calculating student t-statistics. Specific size parameters were correlated using the linear least squares method. This was done primarily to develop a linear regression line for future predictions based on one variable. In addition, these results could be directly compared to those of Ulfhake and Kellerth (1981) using spinal motoneurons. Confidence levels for correlation coefficients were found in a statistical table of Wissenschaftliche Tabellen Geigy (1980).

RESULTS

The results of this investigation will be presented in two parts. The first discusses the determination of total dendrite size from a proximal dendrite structure. This data will be presented in the form of a journal publication. The second part, in thesis format, deals with the total size of the nine lateral rectus motoneurons. Included in this section is a comparison of LRM size to the mechanical properties of its muscle unit.

The Determination of

Dendrite Morphology on Lateral Rectus

Motoneurons in Cat

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Abstract

Previously developed morphometric analysis of motoneurons (Ulfhake and Kellerth, 1981) was applied to lateral rectus motoneurons (LRMs). Total dendrite size was approximated from a single proximal dendritic measurement. Fifteen dendrites from 9 LRMs of the principal abducens nucleus, intracellularly stained with HRP were morphometrically analyzed. The diameters and lengths along the extent of the dendrite were measured to calculate the surface area, volume and combined length of the process. Linear correlation of proximal dendrite diameter to these size parameters produced r values of .80, .84 and .61, respectively.

Although the regression lines could be used to estimate dendrite size from the proximal diameter, two morphologically distinct types of dendrites were measured on these cells. Six dendrites differed from the other 77. Therefore, these six and a representative sample of the more common dendrite (9) were included in the measurements. The rare dendrites consistently branched at about 40 μm from the soma into a rostrally and caudally directed secondary dendrite. The secondary dendrites branched minimally and reduced in diameter by tapering. Also, these dendrites exhibited a higher than expected total dendrite size to proximal diameter ratio compared to "regular" dendrites. Statistical correlations of proximal diameter to surface area or volume within each dendrite type showed clear increases in r values from those of all 15. Significant differences were found between the mean size parameters of the two types. These qualitative and quantitative differences must be considered in accurate motoneuron size determinations in the abducens nucleus.

Introduction

Although it is not possible at this time to explain the intrinsic
and extrinsic factors of functional threshold in terms of motoneuron
size alone (Enoka and Stuart, '84), the "size principle" of motor unit
recruitment (Henneman et al., '65) remains central to the understanding
of spinal motor control. The approaches and techniques which were
developed to test this hypothesis form the basis of research used in
other motor control systems.

The size principle hypothesis depends on the presynaptic and
postsynaptic aspects of motor unit recruitment being related to the size
of the motoneuron (Bawa et al., '84). Therefore, motoneuron size
determination is critical. Henneman et al. ('65 a,b) and colleagues
(Wuerker et al., '65; McPhedren et al., '65) used extracellular spike
height or conduction velocity to gauge the size of the motoneuron.
These physiological parameters had been shown to be indicative of axon
size (Hursh, '39; Gasser, '41) and thus assumed to indicate cell body
size (Ramon y Cajal, '09). Definitive morphological corroboration was
sought and became meaningful as intracellular staining techniques
evolved (Lux et al., '70; Barrett and Crill, '71; 74' Zwaagstra and
Kernell, '81). As total cell visualization improved by staining with
horseradish peroxidase (Cullheim and Kellerth, '76; '78; Cullheim, '78),
it became apparent that cell body or axon dimensions may not be adequate
for reliable estimation of total neuron size.

Exemplary quantitative morphology was demonstrated by Ulfhake and
Kellerth ('81). They reconstructed and measured the cell bodies and
proximal dendrites of spinal motoneurons intracellularly stained with

HRP. Also, they completely measured the diameters and lengths of ten anterolateral dendrites. Statistical correlations between size parameters indicated that a very high linear correlation existed between the proximal dendrite diameter and the surface area of the entire process (r=.99). The regression line of this correlation could be used to estimate dendritic size from the proximal diameter, making total neuron quantification more expedient. Ulfhake and Kellerth ('82) and Burke et al ('82) used this finding to show that a relationship exists between spinal motoneuron size and motor unit type.

Supported by this background the present study was done to find a comparable method of determining the size of motoneurons in the principal abducens nucleus which innervate the lateral rectus muscle. These brainstem motoneurons were qualitatively and quantitatively analyzed for soma and dendritic size. In an effort to find an acceptable way to estimate total dendrite size from a proximal measurement, particular emphasis was placed on the statistical correlations of proximal dendritic (1°) diameter to the surface area, volume or combined length of an entire process. Part of the results was presented in preliminary form (Russell et al., 1985).

Materials and Methods

    Adult cats weighing 2.5 to 3.5 kg were anesthetized with

pentobarbitone sodium (40 mg/kg IP). During the experiment additional

anesthetic was delivered through a venous cannula. The cerebellum was

aspirated to expose the floor of the IV ventricle. A pneumothorax,

tracheotomy and continued drainage of the cisterna magna were effected

to facilitate intracellar recording and stimulation. The lateral rectus

was exposed by a lateral orbital approach, isolated from the globe and

attached to a strain gauge (Pixie model 8101 Endevco). The muscle was

coated with petroleum jelly and mineral oil to conserve heat and

moisture. The rectal temperature and end tidal CO_2 levels were

monitored and maintained within normal limits.

    Intracellular recordings and HRP ionophoresis were done with

beveled glass micropipette electrodes of 0.5 to 1.5 μm tip diameter.

These were filled with 4% HRP and 0.5M KCl (electrical impedence 20–40

megohms) and inserted into the brainstem through the facial colliculus

to the abducens nucleus. The nuclear field was identified by antidromic

stimulation of the VI nerve in the brainstem with a bipolar electrode

(Horsley-Clarke coordinates: AP, −4.6; L, −1.8; H, −8.5). Cells were

impaled and single muscle units were activated using intracellular

stimulation of the motoneurons. Mechanical responses were recorded with

the strain gauge. The HRP injection parameters were 750 msec

depolarizing square pulses delivered at a rate of one per second and a

current of 15 to 20 nanoamps. The total injection time was 1 to 3

minutes. After a minimum of 2 hours, the cats were sacrificed by

transcardiac perfusion of a phosphate buffered mixture of 1%

paraformaldehyde and 1.25% glutaraldehyde. The brainstem was exposed, a
tissue block including the abducens nucleus was removed and stored
overnight at $4^{o}C$ in the fixative. Serial 50 μm coronal sections were
cut through the nucleus with a vibratome and processed for HRP according to
Hanker et al. ('77). Sections were mounted on glass slides and
coverslipped. No correction for tissue shrinkage was made in the
quantitative data because of the tight correlation between the
stereotaxically and microscopically measured depth of the cells below
the IV ventricle.

The cells used for reconstruction and quantification lacked light
microscopic signs of damage. Sections of individual cells presented
consistent staining intensity. The cell bodies and proximal dendrites
(to first branching) of all cells were reconstructed and analysed. In
addition, 15 dendrites of random orientation to the soma were completely
drawn and measured along the extent of the dendritic arborization.

The reconstructions of the cell bodies and dendrites were made at
1875X magnification in a Nikon LKE fitted with a drawing tube. The
quantitative measurements of the dendrites were made at 1000X
magnification with an eyepiece micrometer. Because HRP staining masked
the position of the nucleolus and the cell bodies were not symmetrical,
several methods to determine soma diameter were attempted. The most
consistent results were obtained by determining the mean of the greatest
major and minor diameters through the center point of a geometrical
shape fitted to the cell body. Due to the subjectivity involved in
determining the cut off point between the cell body and an extending
process, proximal dendrite diameters were determined from measurements

taken after the truncated cone extended to a consistent diameter prior to the first branching.

The 15 completely reconstructed dendrites were measured for diameter and length between branch points. Each segment of dendrite was measured in at least three places for a consistent diameter and in cases of tapering, diameters were determined for the appropriate lengths. Three of the cells exhibited tiny proximal dendrites (microdendrites) usually contained within the plane of the soma. These were measured for diameter consistency. Along the extent of many of the standard dendrites, small spinous projections were observed. The diameter of these structures was determined by calculating the mean diameter along their length. The terminal branches of most of the dendrites showed a section of "beaded appearance." The diameter of these segments was obtained from the mean of the swellings and thin intersegments. Length corrections for the oblique passage of dendrites through the tissue were calculated using the Pythagorean theorem. The terminus of a dendrite was determined as the point before the dendrite became impossible to resolve under light microscopy.

The combined length of a dendrite was determined by adding the lengths of all dendritic segments of one proximal dendrite from the cell outline to the terminus. Dendritic surface area and volume were calculated from geometrical formulas for a smooth cylinder, $2\pi rh$ and $\pi r^2 h$, respectively.

Statistical relations between various size parameters were investigated using linear functions. Levels of significance were determined from tables or student t-statistics.

Results

    All of the cell bodies were located in the abducens nucleus. They were found at depths between 0.876 to 2.08 millimeters from the floor of IV ventricle. The geometrical shape of the cell bodies varied from triangular, spheroid, ovoid to quadrilateral. The mean soma diameter of the nine cells was 40.69 µm from a range of 27.5 to 50.9 µm.

    The dendrites of these cells were not homogeneous. Three of the cells contained small caliber dendrites which had a consistent diameter of approximately one micrometer. These microdendrites did not branch, were usually contained in the plane of the soma and contributed little to the overall surface area of the cell compared to the standard-looking dendrites (Fig. 1). The regular dendrites ranged in number from 7 to 11 per cell, with a mean number of 9.2. The mean proximal diameter of the total population (83) of the nine cells was 3.85.

    Fifteen of the regular dendrites were completely reconstructed and measured. During this evaluation, this group was subtyped. Nine of the 15 were comparable to the majority of the population (77). Six of the 15 were, morphologically, distinct from the other 77. The most apparent difference was that the majority of dendrites decreased in diameter by branching whereas the six unique dendrites branched minimally and reduced in diameter by tapering.

Branching Dendrites (Fig. 2)

    The proximal dendrites of branching dendrites were variable in length, 8.8 µm to 265.8 µm. The mean proximal diameter of these processes was 3.28 µm, in a range of 1.7 to 5.75 µm. The ratio of the

sum of daughter dendrite diameters ($\Sigma d_1 + d_2$) to the proximal dendrite diameter (D_1) was usually approximately equal, $.8 \leq \dfrac{\Sigma d_1 + d_2}{D_1} \leq 1.3$.

Branching dendrites had no specific orientation to the soma and were directed either rostral or caudal. Spines, consisting of a thin pedicle topped with a bead, were occasionally found along the extent of these processes. These dendrites often terminated with a beaded appearance.

The average surface area of these nine dendrites was 9265.8 μm^2 (3660.4 S.D.) with a range of 3,516.76 to 14,435.99 μm^2. The mean volume was 4,066.66 μm^3 (2144.99 S.D.) and a range of 1,254.83 to 7,853 μm^3. The combined length of these dendrites averaged 2,255.49 μm (902.98 S.D.) in a range of 747.6 to 3,488.78 μm.

Tapering Dendrites (Fig. 3)

These rare dendrites were found on four cells. Two cells each contained two such dendrites and two cells contained one each. The proximal dendrites of these processes consistently branched at approximately 40 μm. The mean proximal diameter of the tapering type was 4.38 μm, range 2.69 to 6.9 μm. The ratio of the sum of the secondary dendrite diameters to the proximal diameter was greater than one and significantly different from that of the branching dendrites

$$\frac{\Sigma d_1 + d_2}{D_1} \geq 1.5, \ p < .05$$

These dendrites had a medial or lateral orientation to the soma. The proximal dendrite divided into secondary dendrites, one of which

traveled caudal, the other rostral. These dendrites also occasionally

had attached spines as well as beaded terminals.

The mean measurements of these unique dendrites were: surface area

22,251 μm^2 range, 12,987.75-36,815.1 μm^2 (9140.3 S.D.), volume

11,061.32 μm^3, range, 5342.83-20,990.32 μm^3 (6680.31 S.D.) and combined

length, 4610.36 μm, range, 2,184.5-6624.7 μm (1829.84 S.D.) As is

indicated by the figures above, the most important quantitative effect

of these dendrites on total cell size was that the overall size of the

dendrite was approximately 2X that of the branching dendrites. For

example, a branching dendrite had a proximal diameter of 2.6 μm and a

total surface area of 10,078 μm^2. A tapering dendrite with a similar

diameter, 2.69 μm, had a total surface area of 21,440 μm^2. The linear

correlation of proximal diameter to total surface area of the two types

produced equations for the regression lines that are very different:

$y = 2,537.45x+940.15$, for branching and $y=4,911.64x+730.59$, for tapering

dendrites.

Statistics

Because of the difference in size between the two dendritic types

linear correlations were constructed in three ways: 1) the proximal⁻

dendritic diameter to total size parameters (surface area, volume, or

combined length) of all measured dendrites (n=15); 2) proximal dendritic

diameter to total size parameters of branching dendrites, n=9 and 3)

proximal dendrite diameter to total size parameters of the tapering,

n=6. The statistical results of these correlations are shown in Table 1.

An additional set of correlations (not shown) was done to see if the

proximal tapering dendrites should be considered as extended soma. The

results of these correlations were very similar to those of all 15,

Table 1A. Therefore, regardless of where one measures proximal diameter, there is a distinction in the size correlations based on type.

A student's t-statistic was calculated from the mean surface area, volume or combined length for the two types. The probability of finding a larger t was .01 < p < .001 (surface area) and .02 < p < .01 (volume and combined length).

Discussion

The intracellular injection of HRP into motoneurons allows the most complete visualization of the dendritic tree and best light microscopic resolution of any staining method currently available (Ulfhake and Kellerth, '81, '82, '83, '84; Cullheim and Kellerth, '76; 78; Ulfhake and Cullheim, '81; Cullheim and Ulfhake, '79). It has been suggested that the intracellular staining method is biased toward large cells (Ulfhake and Kellerth, '83). However, judging from the range of soma diameters found among these nine lateral rectus motoneurons (27.5 μm to 50.9 μm) and the range of LRM soma sizes known to exist in the abducens nucleus of 15μm to 60 μm (Spencer and Sterling, '77), it is evident that a representative sample of cells was impaled. Also, intracellular impaling did not cause significant structural modification of the cell bodies such that their mean diameters were unusual.

Several aspects of the observed motoneuron morphology are not unique to LRMs. While beaded dendrites and spinous processes were reported in a similar LRM study (Grant et al., '79), they were also found in spinal motoneurons (Ulfhake and Kellerth, '81; Burke et al., '79; Ramon y Cajal, '09). Microdendrites have been reported in retractor bulbi muscle motoneurons of the accessory abducens nucleus (Spencer et al., '80). While the above characteristics may prove significant in defining LRM types, they do not contribute appreciably to the cell size and will not be emphasized here.

Lateral rectus motoneurons have been shown to be smaller, on average, than spinal motoneurons (Grantyn et al, '77; Grantyn and Grantyn, '78). In the principal abducens nucleus, the LRM mean soma

diameters ranged (as previously stated) from 15μm to 60μm (Spencer and Sterling, '77); triceps surae motoneuron soma diameters ranged from 47.5 to 75.5 μm (Ulfhake and Kellerth, '81). The mean combined length of spinal motoneuron dendrites was 4,653 μm (Ulfhake and Kellerth, '81). In these LRMs, the mean combined length was 2255 μm, for branching dendrites and 4610 μm, for tapering dendrites. The mean surface area and volume estimates for spinal motoneuron dendrites were $33,040 \mu m^2$ and $27,220 \ \mu m^3$, respectively. The ranges for these values were; surface area, $8,598-76,097 \ \mu m^2$ and $3,762-62,266 \mu m^3$, for volume. When comparing the same size parameters of LRM dendrites to spinal motoneuron dendrites, the motoneuron types differed in dendritic size. The differentiation depended on the type of LRM dendrite. The branching dendrites had an average surface area of $9265 \ \mu m^2$ and a mean volume of $4066.66 \ \mu m^3$, while the tapering dendrites had an average surface area of $22,251 \ \mu m^2$ and $11,060 \ \mu m^3$ in mean volume. The ranges of surface area and volume found in branching dendrites were less than that reported for spinal motoneuron dendrites. But the tapering dendrites ranged from $12,987 \ \mu m^2$ to $36,815 \ \mu m^2$ (surface area) and $5342 \ \mu m^3$ to $20,990 \ \mu m^3$ (volume), both of which were within the spinal dendrite's size ranges. While it may be speculated that tapering dendrites may be comparable to spinal motoneuron dendrites, this doesn't appear to be the case. Recently, Ulfhake and Kellerth ('84) presented the formula they used to calculate unknown surface areas from proximal dendritic diameters. Using this formula and these LRM proximal diameters, predictions of surface area were compared to actual values. In all 15 comparisons, the values differed. In the case of the tapering dendrites, the predicted

values were always less than the actual, while the predicted branching
dendrite values were usually greater.

The main thrust of this work was to find a convenient method to
determine lateral rectus motoneuron size. It is obvious that the LRMs
and spinal motoneurons differ in quantitative morphology, and the
established linear correlation between proximal diameter to surface area
(or volume) for spinal motoneurons is not applicable. It is evident
also that LRM dendrites are not a homogenous population. The branching
and tapering dendrites differ morphologically and these differences
significantly affect their size. As was indicated in Table 1A, the
correlation between proximal dendrite diameter to any of the three size
parameters produces positive r values such that the regression lines
could be used for estimates. The correlation coefficients would
probably improve with additional branching dendrite data as there was
not a representative sample used in those comparisons, i.e., 9/77 versus
6/6. However, it is obvious in Table 1B + C that the surface area and
volume correlations increase in r values when the dendrites are
subtyped. In fact, they approach the values Ulfhake and Kellerth ('81)
found for spinal motoneuron dendrites. As soma-dendrite measurements
are done in order to determine the total receptive area of a neuron, it
would seem logical to find the best estimate for surface area.
Therefore, in determining LRM size, the differentiation between dendrite
subtypes should produce the most accurate size determination.

This differentiation should not be restricted to intracellularly
stained LRMs alone. Retrograde HRP filling, for determining size ranges
in the nucleus, should be applicable with minor modifications.
Reconstruction of the entire dendrite tree is not necessary to discern a

difference in type. Specifically, to be considered tapering, a proximal dendrite should branch at about 40 μm, be in a medial or lateral orientation to the soma and the $\dfrac{\Sigma d_1 + d_2}{D_1} \geq 1.5$.

The possible significance of the dendritic subtypes is unknown. The morphology of either type is characteristic of the isodendritic cell (Ramon-Moliner and Nauta, '66). The branching patterns of these two are not unlike motoneurons found in the cervical (Rose, '82) and lumbosacral spinal cord (Egger and Egger, '82). Ulfhake and Kellerth ('81) might have found the same differentiation in spinal dendrite quantitation if their dendrites had not been restricted to a ventrolateral position.

Rose ('82) said that stem dendrites which branch to regions of different afferent input integrate that input on the dendrite rather than the soma. Therefore, he suggested this afferent organization facilitated the optimal interactions between certain sets of inputs. LRM tapering proximal dendrites branch into a rostrally and a caudally directed secondary dendrite. Also, they either course ventral or dorsal to sites which may be of different afferent input. Considering their scarcity in the dendrite population (6 out of 83), it is intriguing to think that their occurrence may indicate that cells with this dendritic type have a functional role dependent on specific afferent inputs.

Very recently, Cameron et al. (1985) quantitatively analyzed the dendrites of 4 cat phrenic motoneurons, intracellularly stained with HRP. They found that a high positive correlation existed between the proximal dendrite diameter and the surface area of the entire dendrite (r=.88). However, when they used the power equation of the correlation to predict known values, their predictions differed from the measured

values. They found two reasons for this discrepancy. First, the exponents of the power equations for surface area of dendrites differed with each motoneuron. Therefore, the motoneurons were not a homogenous population. Also, and more relevant to the present study, the dendrites were divided into 5 groups depending on their general terminal field. An analysis of variance between size parameters of each dendrite type often resulted in a significant F-ratio, (P <.05). Therefore, it could also be said that phrenic motoneuron dendrites are not a homogenous population.

An important consideration of this dendrite heterogeneity, particularly in a motoneuron, is the relationship of motoneuron structure to motoneuron electrophysiological properties. Also, the comparison of motoneuron morphology to muscle unit mechanical properties may show correlations. It may be possible that with this information, a structure (in addition to size) function correlation could be found in the motor unit.

Acknowledgements

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Table 1. Statistical comparison of branching and tapering dendrites

	Size Parameters: 1° dendrite diameter/ total dendrite	Correlation coefficient	Coefficient of determination	Standard error of estimate	Level of significance
A Mixed dendrite types n=15	1° dia./surface area 1° dia./volume 1° dia./length	r=.80 r=.84 r=.61	r^2=.64 r^2=.72 r^2=.36	5,566.44μm^2 3,060.22μm^3 1,449.59μm	p<.001 p<.001 .01<p<.05
B Branching dendrites n=9	1° dia./surface area 1° dia./volume 1° dia./length	r=.93 r=.86 r=.81	r^2=.86 r^2=.74 r^2=.66	1,474.37μm^2 1,172.59μm^3 565.15μm	p<.001 .001<p<.01 .001<p<.01
C Tapering dendrites n=6	1° dia./surface area 1° dia./volume 1° dia./length	r=.87 r=.97 r=.36	r^2=.76 r^2=.94 r^2=.13	5,017.06μm^2 1,809.09μm^3 1,908.99μm	.01<p<.05 p<.001 NS

Figure 1.   This is a photomicrograph of one part of a split
            cell body, (complete soma is illustrated in Figure
            2).   The arrow points to a microdendrite.
            Adjacent is another microdendrite attached to a
            proximal dendrite.   Microdendrites were
            approximately one micrometer in diameter and
            varied in length from 6.8 to 38.7 micrometers,
            with a mean length of 19.5 micrometers.
            (----------, bar = 25 um)
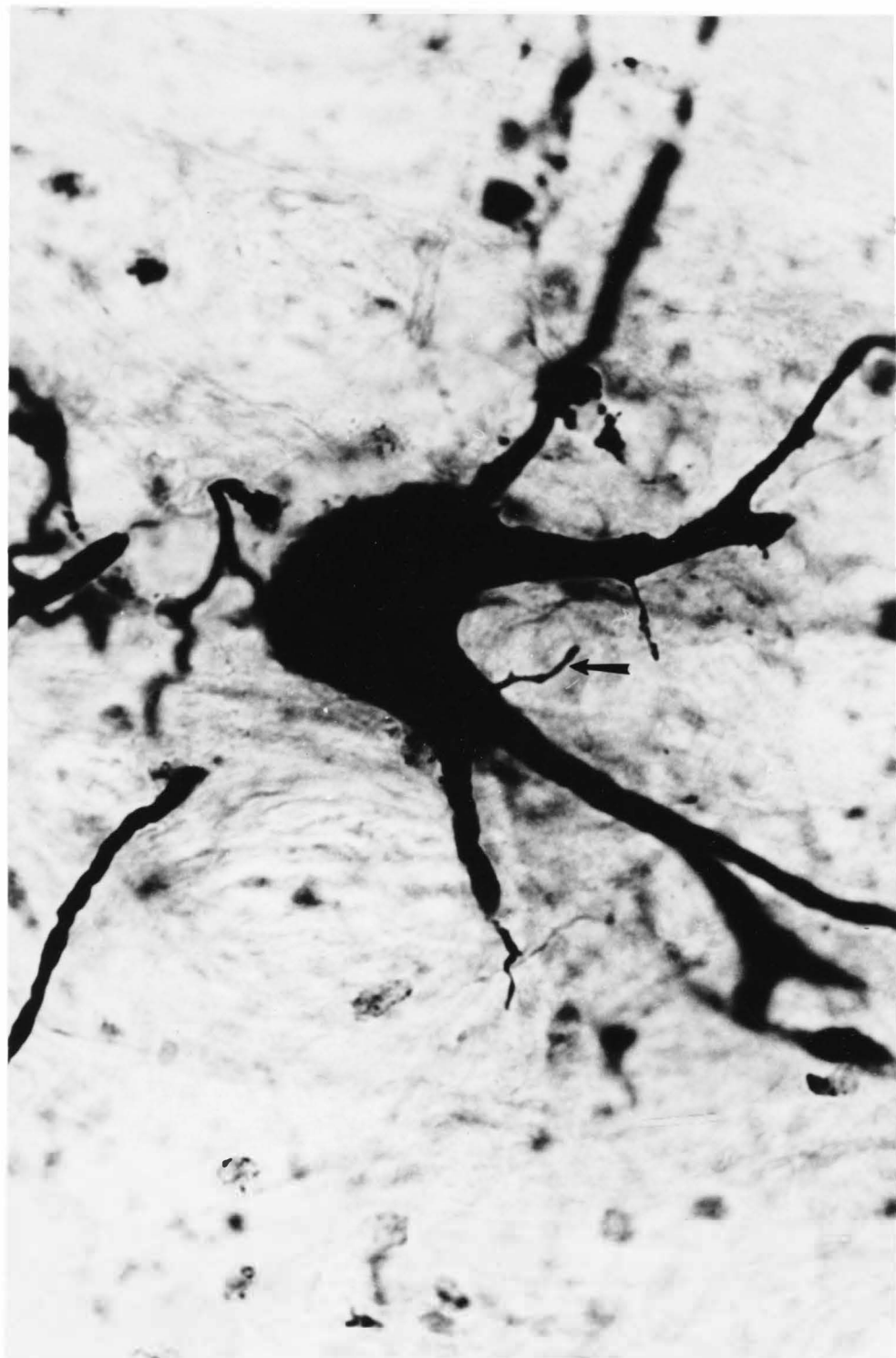
Figure 2.   This ink drawing is a reconstruction of a
            branching dendrite, illustrating the general
            morphology of LRM dendrites. A long proximal
            dendrite, several third and fourth degree
            dendritic branches and reduction in diameter
            primarily by branching, are visible characteristic
            differences from tapering dendrites.

            (--------, bar = 100 um)

Figure 3.   This ink drawing is a reconstruction of a
            tapering dendrite, illustrating their unique
            morphology. Visibly apparent of this type is
            a proximal dendrite which branches at
            approximately 40 micrometers, minimal dendritic
            branching and reduction in diameter by acute
            tapering. (--------, bar = 100 um)
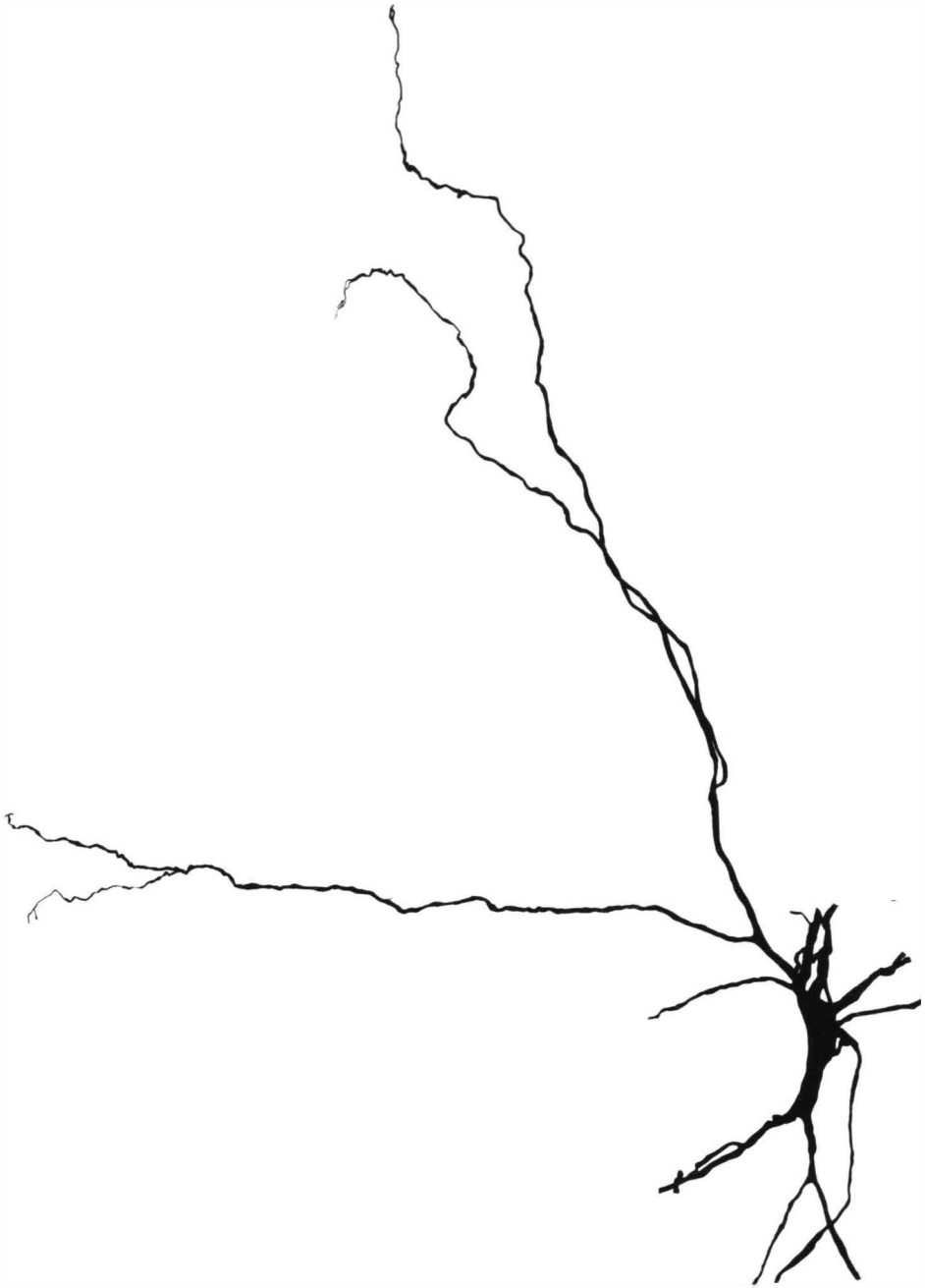
Lateral_Rectus_Motoneuron Morphology

Soma and proximal dendrites

The cell bodies of all nine LRMs were found in the principal
abducens nucleus at depths of 0.88 to 2.08 millimeters from
the IV ventricle. They varied in shape from spheroid, ovoid,
triangular to quadrilateral. The range of mean soma
diameters was 27.5 to 50.9 um, x=40.6. Soma surface area was
calculated from the formula, π X Major diameter X Minor
diameter. The surface areas ranged from 2,357,73 um^2 to
7,188.12 um^2, (See Table 1).

The proximal dendrites were divided into three types.
One type, the microdendrite, was usually contained in the
plane of the soma and consistently measured about 1 um in
diameter. The microdendrite did not branch and added little
to the overall size of the neuron receptive area compared to
standard dendrites. However, calculations and statistical
analyses of the data were done with and without including
these structures. The other two types were standard-looking
dendrites and referred to as branching and tapering.

The mean number of standard dendrites per cell was 9.2,
range 7 to 11. Three of the cells contained microdendrites
in addition to a normal number of standard dendrites. The
ratios of microdendrites to standard dendrites of the three
cells were 6/9, 6/9 and 8/11, respectively. The mean
diameter of the standard proximal dendrite was 3.85 um, range
1.4 to 8.2 um. The combined diameter of the standard
dendrites ranged from 20.5 to 44.8 um for the nine cells.

Statistical correlations of the soma diameter to the number
of proximal dendrites (without and with microdendrites) are
shown in Figures 1 and 2. The correlation coefficients were
r=.33 (without microdendrites) and r=.11 (with micro-
dendrites). The linear correlation of soma diameter to mean
proximal diameter was moderately positive when microdendrites
were excluded (r=.50) and low when they were included
(r=.27) (See figures 3 and 4). The relationship of soma
diameter to combined dendrite diameter per cell produced r
values of .53, without microdendrites counted, and r=.52,
with microdendrites counted (See figures 5 and 6).

General information on Dendritic trees

Fifteen standard dendrites, randomly oriented to the
soma, were completely reconstructed and measured. The
proximal dendrites always branched dichotomously, whereas
trichotomous branching could be seen after the primary
dendrite level. However, in such cases, one branch was
usually a short terminal one. This type of branching pattern
is not to be confused with microdendrites which very seldom
were found attached to proximal dendrites. They were
considered secondary dendrites, even though the diameter
of the main trunk showed minimal change in diameter after
microdendrite attachment and subsequently divided into two
standard-looking secondary dendrites. Spinous processes,
consisting of a thin pedicle (of various lengths) topped with
a bead were found occasionally after the primary dendrite
level. The terminal branches of many of the dendrites

Figure 1. Linear correlation of the mean soma diameter to
the number of proximal dendrites, not including
the microdendrites. The equation of the best
fitted line is y=6.89 + .057x.

Coefficient of correlation (r) =   .33

Coefficient of determination   =   .11

Standard error of estimate   = 1.31

Significance of r value   = N.S.

Figure 2. Linear correlation of the mean soma diameter to
the number of proximal dendrites, including the
microdendrites. The equation of the best fitted
line is y= 9.029 + .059x.

Coefficient of correlation (r) =   .11

Coefficient of determination   =   .01

Standard error of estimate   = 4.25

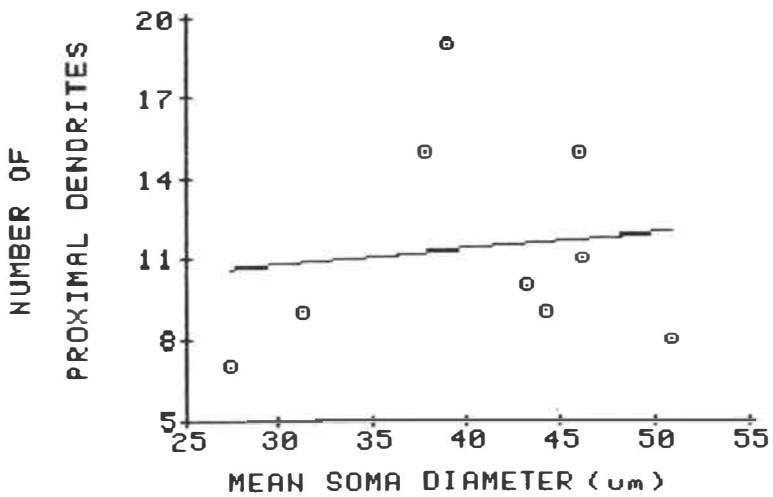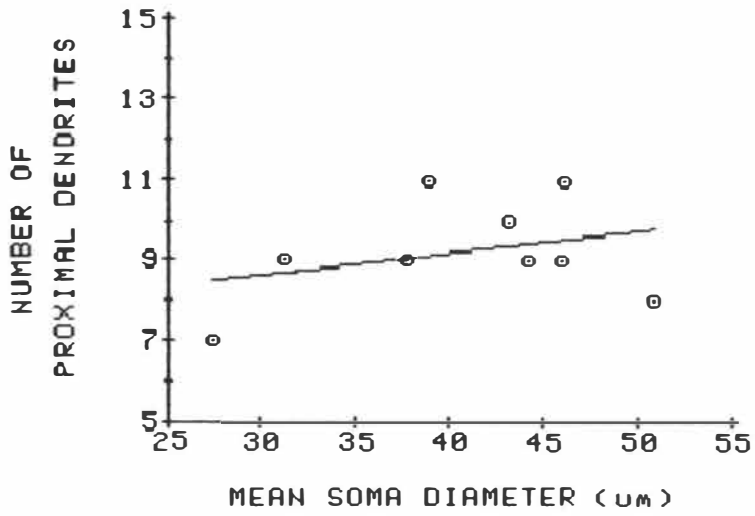Significance of r value   = N.S.

Figure 3.   Linear correlation of the mean soma diameter to
the mean proximal dendrite diameter, not including
the microdendrites. The equation of the best
fitted line is y=2.48 + .033x.

Coefficient of correlation (r)   =   .50

Coefficient of determination   =   .25

Standard error of estimate   =   .47

Significance of r value   =   N.S.

Figure 4.   Linear correlation of the mean soma diameter to
the mean proximal dendrite diameter, including the
microdendrites. The equation of the best fitted
line is y=2.18 + .013x.

Coefficient of correlation (r)   =   .27

Coefficient of determination   =   .074

Standard error of estimate   =   .90
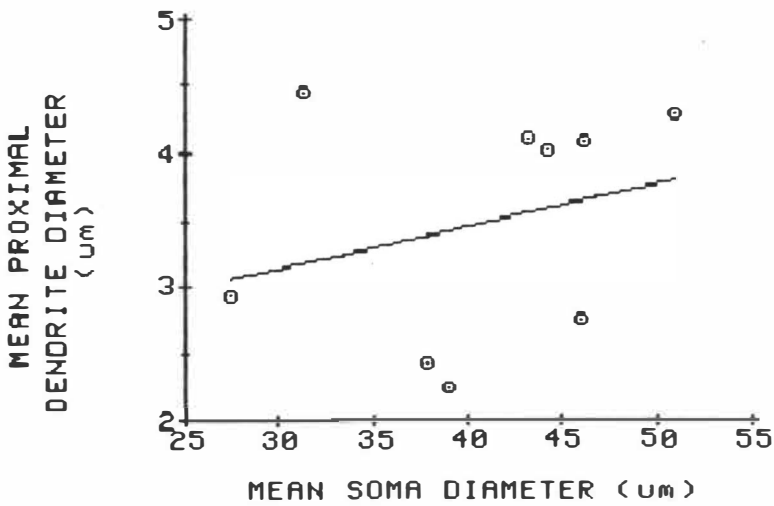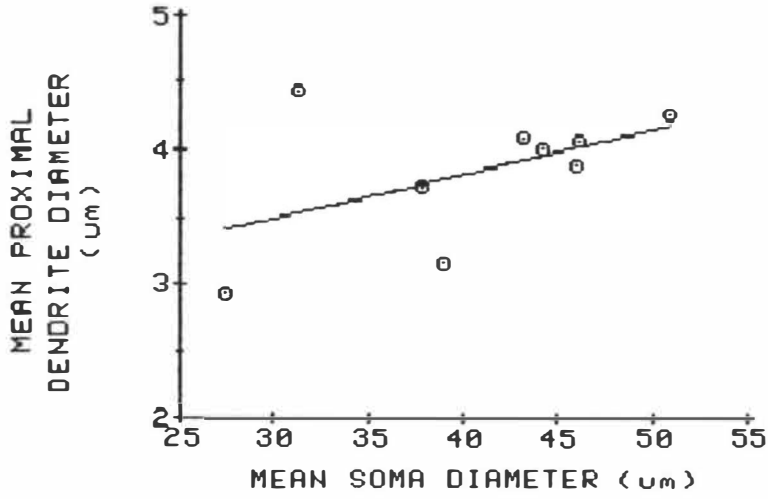
Significance of r value   =   N.S.

Figure 5. Linear correlation of the mean soma diameter to
the combined proximal dendrite diameters, not
including the microdendrites. The equation of the
best fitted line is y= 15.23 + .49x.

Coefficient of correlation (r)   =   .53

Coefficient of determination   =   .28

Standard error of estimate   = 6.36
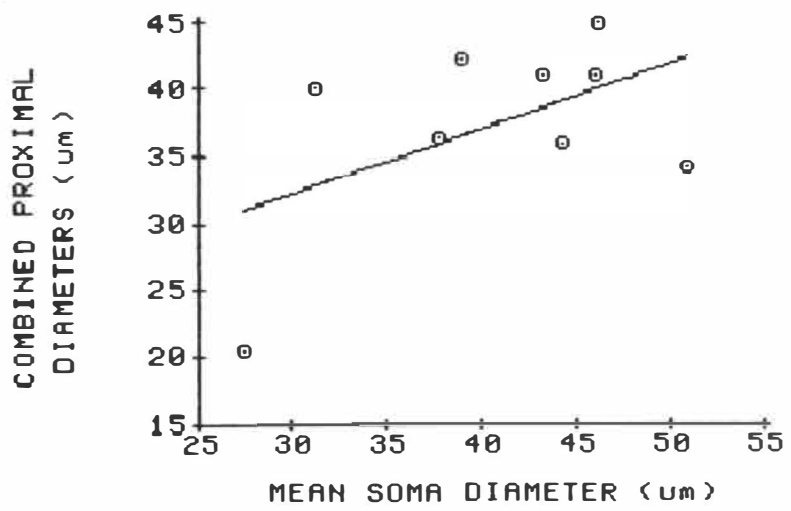
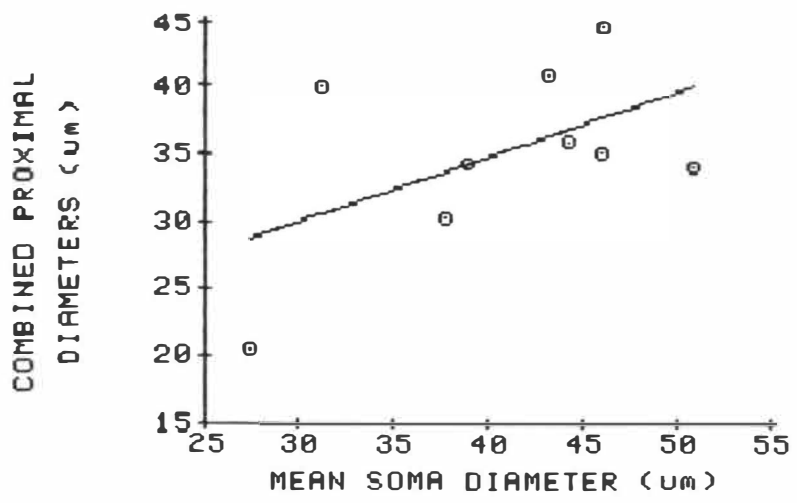Significance of r value   = N.S.

Figure 6. Linear correlation of the mean soma diameter to
the combined proximal dendrite diameters,
including the microdendrites. The equation of the
best fitted line is y= 17.33 + .49x.

Coefficient of correlation (r)   .52

Coefficient of determination   =   .27

Standard error of estimate   6.57

Significance of r value   N.S.

contained sections of beaded appearance. The beads and/or

intersegments were not always the same size in a chain.

A terminal branch did not always end in a beaded appearance

and a beaded segment did not always mean the end of a

dendrite but it did mean the terminus was near.

Branching dendrites

Seventy-seven of the 83 standard dendrites were

branching dendrites. The proximal dendrites showed all

possible orientations to the soma, branched close or far from

the soma and the ratio of the sum of daughter dendrite

diameters to the proximal diameter was, on average,

approximately one, $.8<[(d1 + d2)/D1]<1.3$. These dendrites

reduced in diameter by branching.

The mean proximal diameter of all the branching

dendrites was 3.28, range 1.4 to 5.75 um. The mean combined

length of these dendrites was 2255.49 um, range 747.6 to

3,488.78 (902.98 SD). Figure 7 is the correlation of

proximal dendritic diameter to the combined length of the

dendrite. The surface area averaged 9265.8 um^2 and

ranged from 3516.76 to 14,435.99 um^2 for these dendrites,

(3660.4 um^2 SD). The mean volume for these dendrites was

4,066.66 um^3 (2133.99 SD), range 1,254.83 to 7,853

um^3. The linear correlations for surface area and volume

are shown in figures, 8 and 9.

Tapering dendrites

Proximal dendrites of this type of standard dendrite

were oriented medial or lateral to the soma, branched at

Figure 7. Linear correlation of the proximal dendrite diameter to the combined length of branching dendrites, n = 9. The equation of the best fitted line is y= 457.94 + 547.84x.

Coefficient of correlation (r)   =   .81

Coefficient of determination   =   .66

Standard error of estimate   =   565.15

Significance of r value   = .001<p<.01

Figure 10. Linear correlation of the proximal dendrite diameter to the combined length of tapering dendrites, n = 6. The equation of the best fitted line is y= 2832.1 + 405.84x.

Coefficient of correlation (r)   =   .36

Coefficient of determination   =   .13

Standard error of estimate   =   1908.99

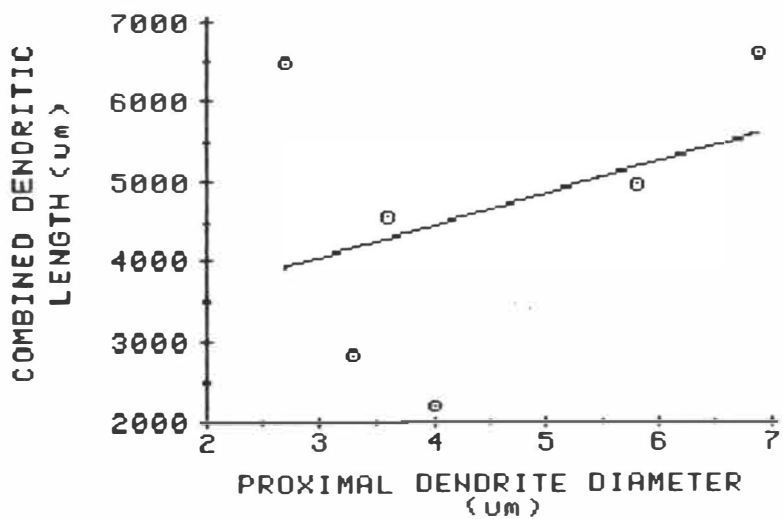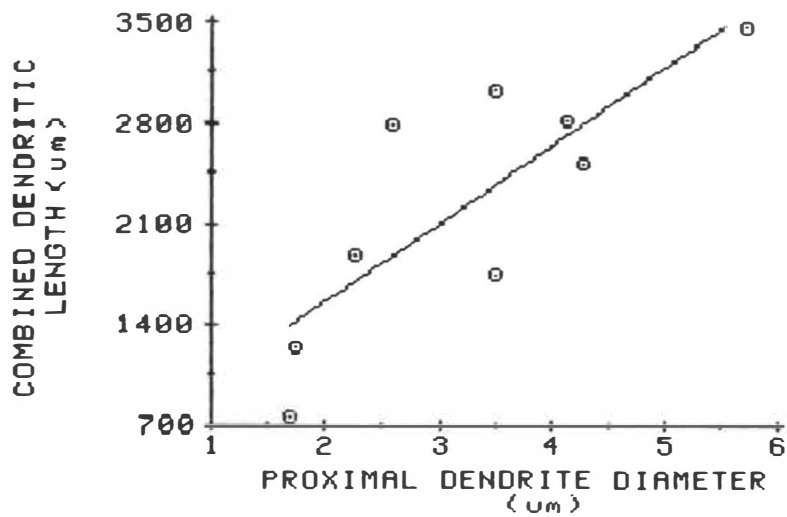Significance of r value   =   N.S.

Figure 8. Linear correlation of the proximal dendrite
diameter to the total dendrite surface area of
branching dendrites, n=9. The equation of the
best fitted line is y=940.15 + 2537.45x.

Coefficient of correlation (r)   =   .93

Coefficient of determination   =   .86

Standard error of estimate   =   1,474.37

Significance of r value   =   p<.001

Figure 12. Linear correlation of the proximal dendrite
diameter to the total dendrite surface area of
tapering dendrites, n=6. The equation of the
best fitted line is y=730.52 + 4911.65x.

Coefficient of correlation (r)   =   .87

Coefficient of determination   =   .76

Standard error of estimate   =   5,017.09

Significance of r value   = .01<p<.05
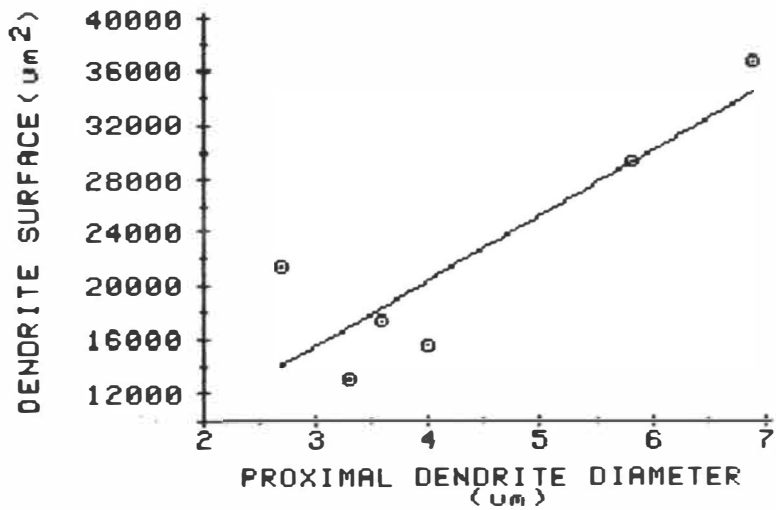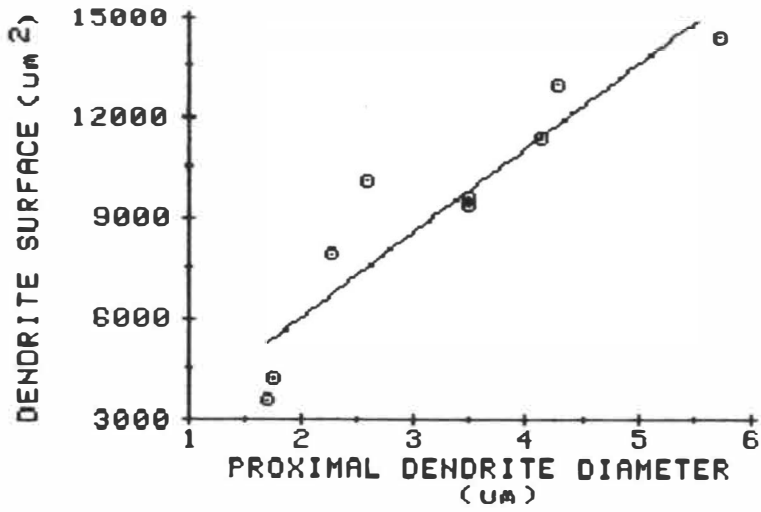
Figure 9. Linear correlation between the proximal dendrite
            diameter and the total volume of branching
            dendrites, n = 9. The equation of the best
            fitted line is y= 459.61 + 1379.49x.

            Coefficient of correlation (r)   =   .86

            Coefficient of determination   =   .74

            Standard error of estimate   =   1,172.59

            Significance of r value   = .001<p<.01

Figure 11. Linear correlation between the proximal dendrite
            diameter and the total volume of tapering
            dendrites, n = 6. The equation of the best
            fitted line is y= -6455.67 + 3997.79x

            Coefficient of correlation (r)   =   .97

            Coefficient of determination   =   .94

            Standard error of estimate   =   1809.09
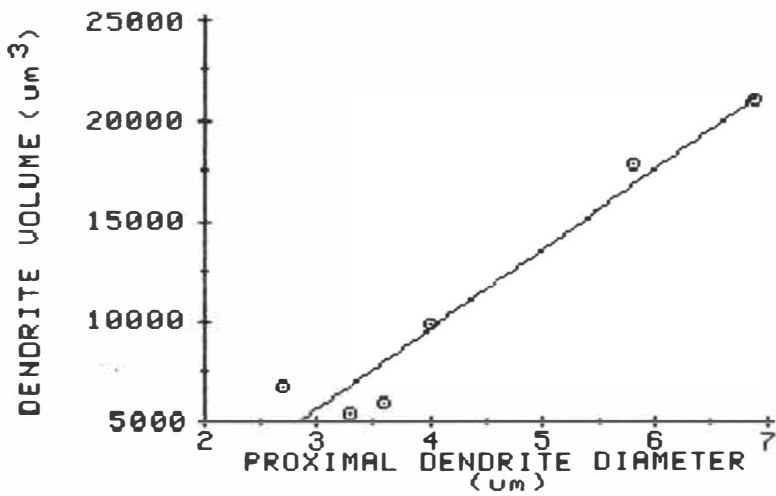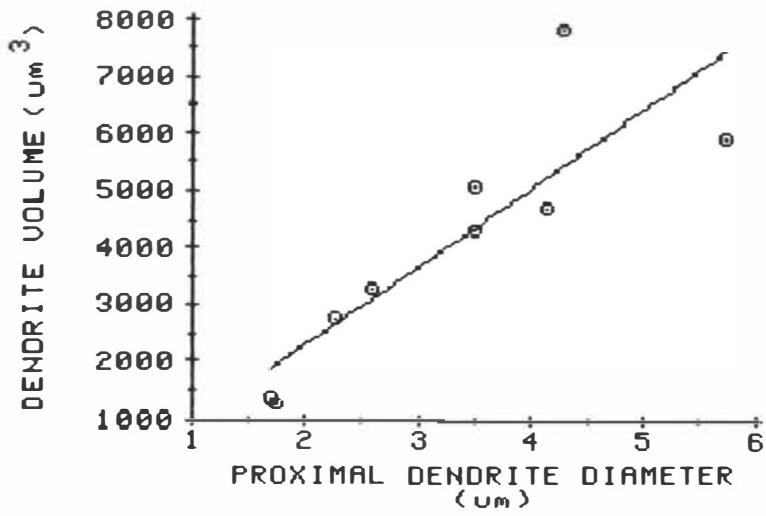
            Significance of r value   =   p<.001

approximately 40 um and (d1 + d2)/D1 > 1.5. Six of the 83 standard dendrites branched seldom and reduced in diameter by tapering, therefore they were designated as "tapering" dendrites. The size of the total dendrite was 2X that expected from the proximal diameter compared to branching dendrites. The mean combined length was 4,610.4 um (1829.8 SD), range 2,184.5 to 6,624.7 um. The volume averaged 11,061.3 um^3 (6680.3 SD), range of 5,342.8 to 20,990.3 um^3. The mean surface area was 22,251 um^2, (9140.3 SD) with a range of 12,987.8 to 36,815.1 um^2. Figures 10, 11, and 12 show the linear correlations of proximal diameter to the total dendrite size parameters of these tapering processes.

Total cell size determination

Total cell size was determined as the sum of the soma and dendritic surface areas. Unknown dendrite surface was estimated from the regression line constructed for the correlation of branched dendrite proximal diameter to total dendrite surface area (Fig. 8). Total cell surface area was calculated with and without including the microdendrites. However, the addition of the microdendrite surface area did not change the total cell size sufficiently to alter the cells position among the others. Table 1 is an intraneuronal size comparison, listed in ascending cell size. Table 2 is a comparison of motoneuron size parameters to the muscle unit mechanical properties and depth location.

Table 1. Neuronal cell size comparisons

Cell #	Soma diam. (um)	Soma surface (um^2)	Dendrite surface (um^2)*	Total cell size *, **
1	27.5	2,357.73	79,935.37	82,293.1
2	37.85	4,441.27	90,028.01	94,469.28
3	50.9	7,188.12	93,808.94	100,997.06
4	44.3	4,970.75	98,791.29	103,762.04
5	38.9	4,762.65	100,390.89	105,153.54
6	43.25	4,457.9	112,758.72	117,216.64
7	46.1	5,852.02	119,657.52	125,991.78
8	31.25	2,684.37	124,912.23	127,596.6
9	46.2	6,416.01	124,004.49	130,420.5

* Denotes the addition of microdendrites.

** Denotes soma-dendrite size in um^2.

Table 2. Comparison of motoneuron morphology to
the mechanical properties of the muscle unit.

Cell #	Total cell size (um^2)	Depth from IV vent. (mm) *	Twitch tension (mg) *	Max tetanic tension (mg) *	Fusion freqency (pps) *
1.	82,293.1	1.004	5.98	59.82	100
2.	94,469.28	0.922	4.99	No data	No data
3.	100,997.06	0.876	27.42	119.65	125-150
4.	103,762.04	Mean **	Mean	Mean	Mean
5.	105,153.54	1.26	Nontwitch	49.0	<50
6.	117,216.64	2.08	123.2	642.17	125-150
7.	125,991.78	1.5	78.96	413.62	100-125
8.	127,596.6	1.95	10.04	159.81	150-175
9.	130,420.5	1.44	5.64	319.62	175-200

*   Denotes data borrowed from the files of Drs. S.J.
    Goldberg and J. Ross McClung.

** Mean denotes those values that were approximations within
    the average range of LRM muscle units

DISCUSSION

As stated in the journal article in the first part of the results, intracellular injection of HRP allows the most complete visualization of the dendritic tree and the best light microscopic resolution of any staining method currently available (Ulfhake and Kellerth, 1981, 1982, 1983, 1984; Cullheim and Kellerth, 1976, 1978; Ulfhake and Cullheim, 1981; Cullheim and Ulfhake, 1979.

Individual cells stained with the same intensity but differed from each other. This assisted in following specific dendrites through different sections of tissue to their terminus. This was important because it meant that proper HRP processing had been done to achieve consistent staining. It also meant that the cell had been filled from an electrode stuck in the soma and not in an axon. Backfilled cells from an axon do not show a fully defined dendritic tree.

The object resolution of the Nikon LKE at a 1000X is 0.22-0.44 um. The terminus of a dendrite was determined as the last visible point before the process could not be resolved. Terminal diameters were generally between 0.3 to 0.5 um. Therefore, the terminus of a dendrite was the closest possible to the real end visible on light microscopy and any additional dendrite would add minimally to its size compared to that already resolved.

While it was suggested that intracellular staining is bias toward large cells, the soma sizes found in this study

(27.5 to 50.9 um) cover the medium to large range of LRM soma

sizes found in a retrograde HRP study (15 to 60 um; Spencer

and Sterling, 1977) The total soma-dendrite size of these 9

cells agrees with that found by Highstein et al. (see

Introduction, page 22). While the 3 soma-dendrite

measurements of their cells are smaller than any of this

study, the ratio of soma to soma-dendrite area (3 - 6%) is

within the range found in this study (2 - 7 %)

Neuronal Size Comparison

As is illustrated in Table 1, soma diameter is not

indicative of the total receptive area of a motoneuron. From

Figures 1 through 6, it is clear that soma diameter does not

give any absolute information on the size of the dendritic

tree. From Table 1, it can be seen that soma surface area,

with exceptions, generally does follow a size relationship to

the total cell size. In only one case was the total dendrite

surface area not directly proportional to total cell size

(Table 1, item 9). However, considering the percentage of

total neuron surface area attributed to soma, (2 to 7%),

total dendrite surface is the more important measure.

It should be stated that soma size may not have been a

good indicator of cell size because of the methodology used

to determine it. The manner of calculating the mean soma

diameter had an inherent error, since it was only a two

dimensional estimate. Also, two of the cells (#5 and #9) had

somas which were split between two sections. Although every

effort was made to construct the cell bodies properly, these

soma measurements could be incorrect. The soma surface area calculation was a crude estimation and as Ulfhake and Kellerth (1981) suggest, not meant to be used in statistical analysis. Although any or all of these situations could have distorted the soma dimensions, it is also just as likely that cell body size is not a reliable indicator of total neuron size. Two LRM studies, Grantyn and Grantyn (1978) and Highstein et al. (1982) present conflicting results on this issue. Whereas the former gave evidence supporting a direct linear relation of soma surface to total neuron surface, the latter presented data which did not. Highstein et al. (1982) measured several reconstructed lateral rectus motoneurons, and presented data on 3 cells (see Introduction, page 22). One of the cells had the smallest soma surface area but the largest soma-dendrite surface area. However, the percentage of total cell surface area attributed to the LRM somas in their study was 3 to 6%, which agrees with the 2 to 7% found in the present study. Therefore, it can be assumed that the soma is a small part of the total neuron surface but the soma size cannot be used to make absolute statements about the size of the cell.

In a recent publication, Ulfhake (1984) reevaluated the approaches used to determine soma size in cat spinal motoneurons. He compared direct measurements of soma surface area to indirect calculations from formulas using diametric measurements. He found that the soma diameter which best correlated to soma surface area or volume was the three

dimensional mean. This was determined by the mean of the sum of the two greatest perpendicular (horizontal plane) diameters through the nucleus and the rostral-caudal diameter of the soma, followed through thin sections of known thickness. He also found that the indirect method of determining soma surface area which produced results comparable to the direct measurement was that using the formula for an oblate spheroid. Therefore, LRM soma dimensions may prove to be indicators of cell size if the three dimensional mean diameter could be determined and surface area was calculated from the formula,

$$2\pi a^2 + \pi b^2 \ \ln \frac{1+E}{E} \ \frac{}{1-E}, \quad \text{where } E=\sqrt{a^2 - b^2}.$$

(E is the eccentricity, a is the major axis and b is the minor axis). This formula could be used after measuring the tranverse axes only but the diametric calculation would require thin sections, which were not available in this study.

The importance that total dendrite surface area played in the total neuron surface was significant. In these nine cells, the dendritic surface area alone was between 93 to 98% of the cell size. This agrees well with the 97% that Ulfhake and Kellerth (1981) found for spinal motoneuron dendrites. Unique dendritic structures, such as microdendrites, did not influence overall size significantly. However, cells containing tapering dendrites tended to be the larger cells (#1, #6, #7, #8).

Table 1 also shows evidence suggesting a relation between total cell size and cell depth from the floor of the IV ventricle. Although, there is not a tight correlation of size to depth, in general, it can be seen that the smaller cells are dorsally located and the larger cells are more ventrally located. This evidence is totally speculative as nine cells are not representative of the LRM pool in the principal VI nucleus. However, it is intriguing to think a size oriented organization of the pool exists.

Table 2 compares the size of these motoneurons to the mechanical properties of their LR muscle units. As is immediately obvious, there is no apparent relationship between motoneuron size and twitch tension. This is not surprising as there is not an obvious relation between twitch tension, maximum tetanic tension or fusion frequency of these units. Obviously, some unknown factors not within the scope of the present work are involved. However, if cell #5 is separated from the other eight because it is of a nontwitch unit, the cells fall into two groups based on motoneuron size, depth from the IV ventricle and muscle unit force characteristics. In general, the relationship of total cell size to maximum tetanic tension suggests that LR motor units with small motoneurons innervate muscle units with lower total force output than larger cells. Also, the data suggests that the motoneurons located more ventrally, innervate muscle units with larger force. Again, it must be said that although this data shows tendencies, it is

inconclusive due to the limited sample. However, if this
data is taken at face value, it is important to remember that
it does not show a definitive statistical relationship
between motoneuron size and muscle unit capabilities. There
must be other factors besides motoneuron size which explain
the physiological variations.

Extraocular Motor Unit Recruitment

This study provides further evidence that spinal and
extraocular motor units are not completely comparable. These
differences are related to the recruitment order controversy.
First, as was presented in the publication accompanying the
results, LRM dendrites are not a homogeneous population.
There is a suggestion that they differ in size depending on
their terminal field. Ulfhake and Kellerth (1983) found no
major differences between spinal motoneuron dendrites
extending into different terminal fields. Second, there is a
lack of firm relation between motoneuron size and muscle unit
mechanical properties. In essence, this suggests that there
is not the relationship of motoneuron size to motor unit type
as was found in the spinal system (Ulfhake and Kellerth,
1982; Burke et al., 1982). Last, there is a possible
motoneuron pool organization; the smaller cells being found
more dorsal, the larger cells, more ventral. All of these
findings are inconsistent to a greater or lesser degree
with tenets of Henneman's "size principle" of motoneuron
recruitment in the extraocular system. However, the general
tendency for smaller motoneurons to innervate muscle units

with lower force output and vice-versa, is in good agreement
with predictions of the size principle.

Motoneuron size does play a part in LRM characterization
but there are other morphological considerations which may be
equally significant. The microdendrites, which did not add
appreciably to the total surface area of the cell, were not
common to all cells. However, they are not artifact, they
have been found before (Spencer et al., 1980) and they may
have a physiological explanation (Baker et al., 1980). It is
interesting that they were found by Spencer et al. (1980) on
retractor bulbi motoneurons (RBMs) in the accessory abducens
nucleus. As it has been shown that RBMs of the principal VI
nucleus innervate muscle units which can be split between
retractor bulbi and lateral rectus muscles (Crandall et al.,
1981), it seems logical to think that LRMs may do the same
and that these microdendrites are a morphological indicator.
However, as the retractor bulbi muscle slips were attached to
the strain gauge and did not twitch when these motoneurons
were stimulated (personal communication from Dr. Goldberg),
that possibility does not seem probable. However, the
microdendrite could be significant to the motoneuron's
physiology and could impart a special function to the motor
unit.

The tapering dendrites are found on four cells, one of
which also has microdendrites, so the two structures are not
mutually exclusive. While the above notion for
LRM-RB-microdendrite involvement remains to be shown, their

morphological significance, like the standard dendrite differentiation, is not evident at this time. Because the tapering and branching dendrite patterns are similar to those seen in the spinal cord (Rose, 1982; Egger and Egger, 1982) and there is no apparent relation between cells having tapering dendrites and motoneuron size, depth from the IV ventricle or muscle unit mechanical properties, it is probable that these are not unique to a motor unit type, but may be characteristic of a motoneuron type.

The thrust of this work was to find a convenient method of determining LRM size. This primary goal was accomplished. Then the size of the motoneuron was compared to the mechanical properties of the muscle unit. If the motoneuron's size was related to its muscle unit's mechanical properties, then motor unit recruitment as explained by the "size principle" could have a place in the extraocular system. Much more research is required before the latter considerations can be decided. Table 2 indicates that a general motoneuron size/mechanical properties relationship exists, but this is complicated by the inconsistencies within the mechanical properties themselves. Therefore, although motoneuron size appears to be related to muscle unit physiology, at least in these nine cells, it is not the total explanation for the variability in muscle unit properties. These brainstem motoneurons cannot be morphologically described in terms of size alone. Microdendrites, which add little to the total size of a cell have morphological-

physiological implications just by their existence. The structural variation in dendrites (i.e. tapering) which can be related to its possible afferents, support the hypothesis that total motoneuron size is insufficient in defining motoneuron type.

In a recent article, Cameron et al. (1985) presented similar results from a study of four totally reconstructed and measured phrenic motoneurons. They found a high positive correlation coefficient, r=.88, between proximal diameter and total dendrite surface area but when they used the same regression line to predict a previously measured value, the estimates were not within 10% of the measured value. They suggested two reasons for this disparity. First, as the power equation for the proximal dendrite diameter/total dendrite surface area relation derived from a single motoneuron's dendrites resulted in an exponent unique to that cell (differing among the four cells), they suggested a heterogeneity in the motoneuron population. Second, they knew that phrenic motoneuron dendrites project to five different terminal fields. From a quantitative comparison of the five directional dendritic types, analysis of variance showed a significant F-ratio (p<.05), indicating that the phrenic motoneuronal dendrites are not a homogeneous population (based on size). They suggested these variations may be due to their location in the nucleus and/or their functions. Considering the evidence of the Cameron et al. (1985) study and the present extraocular motoneuron study,

there appears to be a need to evaluate motoneuron types on a qualitative and quantitative basis, with particular emphasis on the dendritic pattern.

At present, in the extraocular system, a scheme for motor unit recruitment based on the "size principle" alone, appears unlikely. Motor unit typing, based on the mechanical properties of the muscle units, total motoneuron size and cell location in the nucleus, is not definitive. This complex motor system requires further studies which examine the morphology and physiology of both elements of the motor unit. A basis for orderly recruitment may then be readily evident.

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