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SPECIALIZATION OF LARYNGEAL MOTOR UNITS FOR THEIR FUNCTIONS

by

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DISSERTATION

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DOCTOR OF PHILOSOPHY

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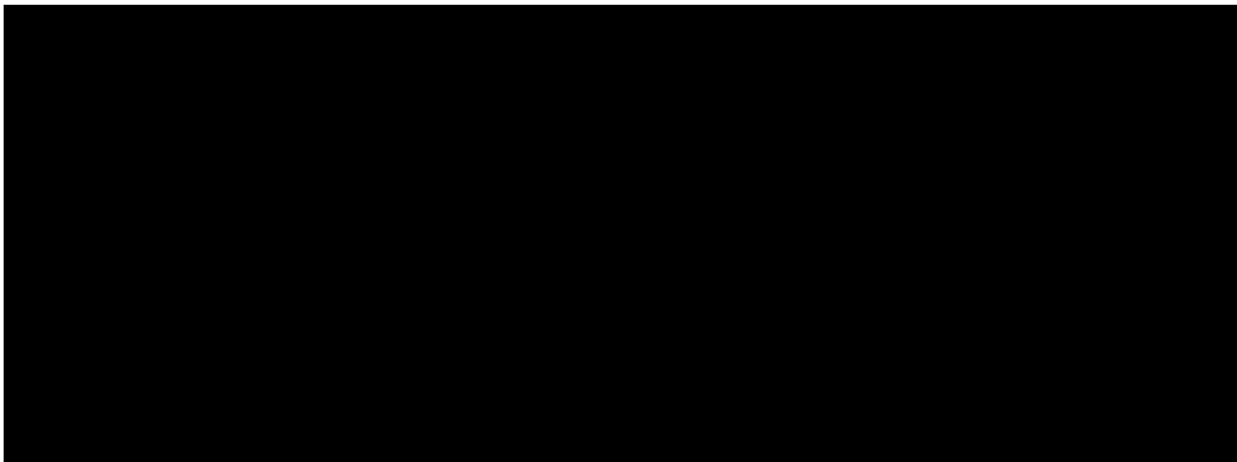

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TO MY FAMILY

ABSTRACT

Injections of HRP into laryngeal muscles have revealed that most intrinsic laryngeal muscles are innervated by motoneurons arising from two different brainstem nuclei, the nucleus ambiguous and the retrofacial nucleus. The cell bodies in these two nuclei differ considerably in their size, dendritic pattern and arrangement, so that their motoneurons may differ in their excitability and synaptic input and thus in the signals they transmit to their muscle fibers. The muscle fibers of these motoneurons (i.e. muscle units) would also be expected to differ in their contractile properties, since neuronal activity patterns largely determine these properties. The presence of different histochemical types of fibers in each laryngeal muscle has provided indirect evidence that muscle units might differ in their contractile properties.

A direct investigation of the functions and contractile properties of single motor units of one laryngeal muscle, the thyroarytenoid (TA), was undertaken using glass micropipettes. Because of the difficulty in penetrating and holding TA motoneurons in the brainstems of spontaneously breathing cats, a technique was developed for penetrating the myelin sheaths of axons in the recurrent laryngeal nerve. The technique was found to be a very stable and non-damaging approach to studying these motor units. Upon stimulation of an axon while monitoring tension development of its muscle fibers, the motor unit's contractile properties could be determined, after which recording from the axon during stimulation of mucosal afferents revealed the spiking sensitivity of the axon and thus the functional role(s) of the motor unit in TA movements.

Two distinct populations of TA motor units were observed based on differences in their tendencies to exhibit declines in tension or "sag" during unfused tetani: Fast-red and slow. Seventy-nine percent were fast-red, which was similar to the proportion of type II fibers observed histochemically in the muscle. Fast-red units were distinguished from slow units by their faster contraction times, faster conducting axons, presence of sag, and tension potentiation during the first minute of fatigue. They also differed from typical fast-red limb motor units in two respects. First, they were extremely fatigue resistant. Second, they exhibited quite pronounced sag, particularly after an initial tension peak, which resulted from synaptic depression (EMG decrease) and probably muscle fiber active state decreases as well. Slow units were also atypical in some respects. First, they exhibited a fairly large range of motor unit sizes. Second, they did not fit in the correlation between axon conduction velocity and motor unit size of fast units, but rather had slower conducting axons at any given motor unit size. Third, all the slow units observed had small or undetectable EMG responses, suggesting either that their muscle fibers didn't conduct action potentials or that their muscle units were located away from the EMG electrodes in the medial muscle division.

With stimulation of mucosal afferents in the internal laryngeal nerve, a purely sensory nerve, reflex gag contractions of the TA muscle could be initiated. Repeated stimulation of the nerve also served as an arousal stimulus, evoking tonic expiratory activity of the muscle. Both types of units participated in the gag reflex, and were undistinguished in their responsiveness to a given sensory stimulus. Only slow units participated in tonic expiratory activity, suggesting that their moto-

neurons were more excitable. Since slow units had smaller axons and therefore smaller cell bodies, it can be concluded that the size principle holds when comparing slow and fast units. Fast units, however, were just as responsive to mucosal afferent stimulation evoking gag activity, despite their lower excitability, suggesting that they receive stronger direct synaptic input of these afferents from the periphery.

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Chapter 1: INTRODUCTION

The intrinsic laryngeal muscles have had an interesting phylogenetic history. In fishes they (or their homologues) participated in the rather simple function of swallowing. As the central nervous system evolved and the neural control of these muscles grew in sophistication, they were delegated additional functions which were more complicated than the stereotypic action of swallowing and which served other purposes besides alimentation. Thus, with budding off and formation of lungs from the alimentary canal in the first progenitors to tetrapods, they were given the roles in respiration of protecting the airway and regulating its airflow. Eventually, with the emergence of mammals, their most refined and complicated function evolved, sound production, for yet another purpose, communication.

With functions ranging in complexity and serving different goals as described, a given mammalian laryngeal muscle might be expected to comprise motor units, alpha motoneurons and their muscle fibers, which are specialized and limited in their roles in the muscle's total activities. That is, motoneurons may differ in the nature of their synaptic inputs and their excitabilities and thus in the signals they send to their muscle fibers. Their muscle fibers would also necessarily differ in certain contractile properties (e.g.s, fatigue resistance, contraction speed) in

order to meet the demands required of them in their individual roles.

In this introductory chapter, results from two whole muscle studies are presented which suggest that motor units do differ in their motoneuron synaptic input and excitabilities, on the one hand, and in their muscle fiber contractile properties, on the other. These studies are anatomical and histochemical, respectively, in nature and provide a "picture" of what laryngeal motoneurons and their muscle fibers are like and some insight as to what they do. However, direct observation of <u>individual</u> motor units in operation in a live animal was desired to gain a better appreciation of how a laryngeal muscle's actions are achieved. Following the development of a technique, described in Chapter 2, to stimulate and record from individual motoneuron axons, the contractile properties and functions of single motor units of the thyroarytenoid muscle were directly determined and are described, respectively, in Chapters 3 and 4. Since the technique has not been previously used and is, in my opinion, superior to commonly used motor unit techniques, it is described in sufficient detail for the reader to successfully duplicate. All of the studies which will be described were performed on cats. However, an attempt will be made to use the findings from these studies, as well as those of other investigators, to

describe the nature of the neural control of laryngeal muscles, in general.

Before presenting material supporting the notion that laryngeal motor units are specialized and differ somewhat in their characteristics, a description of the locations and actions of the intrinsic laryngeal muscles is provided for orientation.

I. The locations and actions of the intrinsic laryngeal muscles

In this section, the locations and actions of the human intrinsic laryngeal muscles are described and illustrated. Although the cat larynx does differ in some respects from that of the human, the following general description of the locations and actions of the laryngeal muscles also applies to the cat for the most part. However, the reader should use his own discretion in attributing specific characteristics shown to the cat larynx.

The external shell of the larynx is composed of two modified tracheal rings, the cricoid and thyroid cartilages. The thyroid cartilage is an open ring, expanded in the front into a shield to protect the delicate underlying structures of the larynx (Figure 1). The cricoid cartilage, on the other hand, is a true (signet) ring, expanded into a lamina in the back upon which sit the important pair of arytenoid cartilages (Figure 1, middle drawing). Four of the five

intrinisic laryngeal muscles insert onto the body or muscular process of the arytenoid cartilage and thus effect movement of its vocal process, the origin of the vocal cord. Abduction, adduction, and stretching of the vocal cords occur only through movements of the arytenoids, since the insertion of the vocal cords is firmly attached to the ventral aspect of the thyroid cartilage, the anterior commissure.

One could envision that three kinds of movements of the arytenoids occur from the shapes of the articular facets of the cricoid and arytenoid cartilages, shown in the middle drawing of Figure 1: sliding along the long axis of the cricoid facet, rocking (obliquely) about this axis, and rotation about a vertical axis. All three of these movements (or their combinations) occur naturally and are clearly depicted, respectively, in Figure 2B, C, and D.

The intrinsic laryngeal muscles are defined as those muscles having both origin and insertion on one of the three laryngeal cartilages mentioned above. There are five pairs of intrinsics and each is named according to its origin and insertion: The posterior cricoarytenoid (PCA), the cricothyroid (CT), the thyroarytenoid (TA), the lateral cricoarytenoid (LCA), and the arytenoideus (A). The five pairs and their individual actions are shown, respectively, in Figures 3 and 4.

The posterior cricoarytenoid (PCA) is the only abductor of the vocal cord. The action is actually accomplished through more rocking than rotational motion of the arytenoid than depicted in Figure 4 (see Figures 5A and 5B and Kotby and Haugen, 1970a,b). Since the PCAs abduct the vocal cords during inspiration, opening the glottis, they have a role in respiration. They also participate in vocalization as antagonists to the adductors to provide for fine control of vocal cord position and tension.

The other four intrinsic laryngeal muscles are adductors and participate in various adductory functions of the vocal cords including airflow regulation, airway protection (egs. coughing, gagging), glottis closure for variation of intrathoracic or intrabdominal pressure (egs. defecation, valsalva maneuver), and vocalization. The cricothyroid (CT), the only muscle that does not insert upon the arytenoid, in addition to adducting the vocal cord is the primary determiner of its length and tension and thus its fundamental frequency of vibration during vocalization (see van den Berg, 1958). When contracting it tilts the thyroid towards the cricoid ventrally in a visor like action (see Figure 4A), effectively stretching the vocal cords. The medial division of the thyroarytenoid (TA), often called the vocalis muscle, composes most of the true vocal cord and also has a role in the determination of its tension. It contracts in opposition to the stretching of the CT to fine

tune vocal cord tension. Unlike the medial division, the lateral division of the TA inserts on the body and muscular process of the arytenoid cartilage, and is therefore more of an adductor than tensor of the vocal cord.

None of the intrinsic laryngeal muscles acts alone in providing for the various functions of the larynx (with the possible exception of the PCA during quite breathing). Rather, they act together synergistically, sometimes in a very precise relationship as during vocalization and at other times in a rather coarse manner as during the gag reflex.

II. Specialization of laryngeal motor units: Whole muscle studies

A. Anatomical studies suggesting that motoneurons innervating each laryngeal muscle differ in their synaptic input and excitability, and thus in the signals they transmit, as revealed by horseradish peroxidase tracing.

It is obvious that motoneurons having different synaptic input will differ in the signals they transmit. It is not so obvious that motoneurons receiving the same synaptic input can also vary in their transmitted signals, if they have different excitability.

In recording from motoneurons innervating the gastrocnemius muscle during muscle stretch, Henneman, et. al. 1965a,b and Granit, et. al. 1956a demonstrated that

the first motoneurons recruited by 1A afferent input (i.e. the most excitable) had the smallest axons and thus the smallest cell bodies (Cajal, 1909, p. 52). This inverse relationship between cell size and excitability formed the basis for the size principle. These small cells responded phasically during the onset of small prolonged stretches, but became tonically active with increased stretch, just as larger cells were being recruited to exhibit phasic activity. Within the normal physiological range of muscle spindle activity, then, small cells would tend to exhibit a tonic firing pattern while large cells would tend to exhibit a phasic firing pattern. The characteristics of these phasic and tonic motor unit types, usually referred to as "fast" and "slow" types, respectively, are shown in Figure 6. It is not certain whether they represent two distinct populations as suggested by Granit, et. al. 1956 and Eccles, et. al. 1958 (see Burke, 1967) or extremes in a continuous population as emphasized by Henneman, 1965a, based on the argument that both types can exhibit either activity pattern. In any case, the tendency is for two distinct types to exist, given a physiological range of afferent input. Looking for a moment at the level of the cell body in Figure 6, a motoneuron will exhibit either a phasic or a tonic activity pattern depending upon the nature of its afferent input (i.e. origin and pattern of input signals and types of synapses), the level of the afferent

input, and the response or excitability of the cell to the afferent input. Thus, for a given stretch or level of 1A afferent input to large and small cells without distinction in the nature of the input to the two types (see Henneman, 1965a, p. 574), small cells will tend to respond tonically because of their higher excitability and large cells phasically because of their lower excitability.

If the size principle holds for laryngeal motoneurons, large and small motoneurons could provide for both phasic and tonic activity of a laryngeal muscle, engaged in one of its functions such as vocalization, with minimal differences required in the afferent input to the two cell types. Hypothetically, slow motor units of a tensor of the vocal cord could provide for maintenance of tension during phonation at a constant fundamental frequency, while fast units could provide for modulation of tension and frequency about the maintained level.

Recent anatomical studies have demonstrated that motoneurons innervating each laryngeal muscle do differ in their cell body sizes and synaptic input. Injections of horseradish peroxidase into each intrinsic laryngeal muscle of kittens (Gacek, 1975) and cats (Zealear, unpublished observations) traced the source of their respective motoneurons to two ipsilateral nuclei, the nucleus ambiguous and the retrofacial nucleus. The retrofacial nucleus lies just rostral and ventrolateral to the nucleus ambiguous,

behind the facial nucleus, and approximately 4 mm from the obex. Nucleus ambiguous cells were large (Figure 7) while retrofacial nucleus cells were medium or small in size (Figure 8), suggesting that they may be involved in different patterns of muscular activity. The cytoarchitectonics of the two nuclei appeared quite different as well. Laryngeal motoneurons in the nucleus ambiguous were multipolar in morphology and compactly arranged, while those in the retrofacial nucleus varied in morphology and dendritic pattern and were diffusely arranged. With spatial discontinuity and different cytoarchitectonics, the nuclei undoubtedly receive different synaptic input and are probably involved in different functions or types of muscular activity.

A minority of the cells in the retrofacial nucleus are actually laryngeal motoneurons, so that many cells in this nucleus probably send and receive projections from other areas of the brain (Gacek, 1975). It might be presupposed that laryngeal motoneurons in this nucleus draw from a larger bank of information and direct more complicated types of activity of the muscles they innervate. The fact that most, if not all, motoneurons in this nucleus innervated the PCA and CT muscles (Figures 9 and 10), muscles known to be composed of a complexity of functional units responding during both phases of respiration (Suzuki and Kirchner, 1969; Suzuki, Kirchner, and Murakami, 1970), further

supports this idea. It is not even certain that the LCA and TA muscles are represented in this nucleus, since retrofacial nucleus cells containing HRP following injections of these two muscles have only been demonstrated in kittens.

In both kittens and cats, the nucleus ambiguous was observed to be further subdivided into a ventral division containing slightly smaller and more compactly arranged motoneurons innervating the PCA, the abductor muscle, and a dorsal division innervating the rest of the muscles, the adductors (Figure 11). Segregation of the abductor cells into a ventral division has both phylogenetic and clinical significance, as explained by Gacek. Before the evolution of lungs in those primitive lobe-finned fish where the larynx had only sphineteric function, nucleus ambiguous cells probably served in only an adductory capacity. With development of respiratory function, the need for inspiratory movement was fullfilled through neural reorganization of part of the brainstem reticular formation into a ventral inspiratory center and a dorsal expiratory center as described by Pitts, 1949. Parallel formation of a ventral division of the nucleus ambiguous comprised of those motoneurons which could direct opening of the newly evolved glottis during inspiration (i.e. the PCA motoneurons) allowed for convenient afferent connections to be established with the inspiratory center. Since abductor

cells are segregated, more compactly arranged and fewer in number than adductor cells by a ratio of 4 to 1, they appear to be more vulnerable to brainstem lesions. They may also be more fragile or susceptible to damage, as most systems are that appear late in phylogeny and mature late in ontogeny.

In two out of a total of six injections into the CT muscle in cats, HRP containing cells were observed in a third nucleus, the ipsilateral lateral division of the nucleus solitarius (Figure 12). In these two experiments, more sensitive indicators of HRP were used, dianisidine and Hanker-Yates reagent. The cells were localized lateral to the spinal canal just caudal to the obex and were either small and spindle shaped or large and multipolar. Use of these more sensitive substrates should help establish whether motoneurons of other laryngeal muscles are also located in this nucleus or in other nuclei of the brainstem.

B. Histochemical studies suggesting that the muscle fibers of motor units comprising each laryngeal muscle differ in their contractile properties.

Considerable time has elapsed since Ranvier (1874) and Kronecker and Stirling (1878) first discovered that red striated muscle has a slower contraction than white striated muscle. Observing the relative movements of these muscles, it was generally believed that slow-red muscles were

specially concerned with tonic postural movements of the limbs while fast-white with quick phasic movements. After Granit's (1956a) description of two motoneuron types, tonic and phasic, Eccles et. al. (1958) provided firm support for the relationship between the activity pattern of a muscle and its contraction time with his demonstration that tonically firing motoneurons innervated slow-red muscles and phasically firing motoneurons innervated fast-white muscles.

Eccles did not actually determine the activity pattern (usually evoked through muscle stretch) of the motoneurons he studied. He made this inference having demonstrated that small, slowly conducting axons innervated slow-red muscles and knowing, from Granit's studies, that small axons had tonic firing patterns. That phasic motoneurons innervated fast-white muscles was, likewise, inferred on the basis of their large axon size. Since Eccles was recording intracellularly inside motoneurons, he also noted that small axons stemmed from cell bodies exhibiting a long duration afterhyperpolarization following invasion by an antidromic spike, while large axons a short afterhyperpolarization. Furthermore, he attributed the lower maximum discharge rate of tonic motoneurons (10-20/second compared to 30-60/second for phasic motoneurons) to their longer afterhyperpolarization, suggesting that the difference in the activity patterns of tonic and phasic motoneurons is at least in part a result of their differences in the duration of the afterhyperpolarization.

It is a fortunate circumstance in nature that tonic motoneurons, with their lower discharge rate came to be matched with slowly contracting muscle fibers, and that phasic motoneurons, with their higher discharge rate, came to be matched with faster contracting muscle fibers. Any mismatching would result in efficiency. For example, activation of a slow muscle at higher frequencies

characteristic of phasic motoneurons would be a waste of energy and only serve to fatigue muscle fibers at no return in higher tension. Likewise, activation of a fast muscle at lower frequencies characteristic of tonic motoneurons would be inefficient in fusing the individual twitch responses to give an effective tetanic contraction. How is it that appropriate matching occurs? Do muscle fibers exert an influence upon their motoneurons to control their spiking frequency, or is the reverse true, that motoneurons exert an influence upon their muscle fibers to control their contraction speeds?

In a pair of classic studies by Buller, Eccles, and Eccles (1960a,b), it was shown that the latter is true, that the contraction speeds of muscles are determined by the types of nerves that innervate them. They observed that in the newborn animal all muscles were equally slow, but over the course of several weeks muscles gradually differentiated to obtain their adult contraction speeds, considerably greater changes being required in the differentiation of fast muscles. The differentiation into fast and slow muscles was apparently controlled by their nerves, for if they were denervated and cross reinnervated with each others nerves, they switched and developed contractions speeds in accord with their new nerve types. Thus, muscles normally predestined to become slow ended up fast, and those predestined to become fast ended up slow. Adult muscles, as

well, exhibited plasticity with respect to their contraction speeds in face of new innervation. As with developing muscles, when adult denervated fast and slow muscles were reinnervated by each others nerves, a nearly complete switch occurred in their contraction speeds. Since muscles were observed to be so adaptable to their new innervation, it was not surprising to find that the characteristics of motoneurons were unaffected by the reinnervation. For example, fast motoneurons retained their fast conduction velocities and short duration afterhyperpolarizations after reinnervation of a slow muscle. The influence appears to be in one direction, nerve on muscle and not muscle on nerve, in the matching of their characteristics.

The next logical question is, how does a nerve control the contraction speed of its muscle? Buller, Eccles, and Eccles in the same two studies discovered that if the spinal cord was isolated from all afferent input, if motoneurons were silenced, a nerve lost its influence over muscle contraction speed. Slow muscles, whether self reinnervated or cross reinnervated with a fast nerve, showed a characteristic increase in contraction speed (short of that of a fast muscle), while fast muscles under similar conditions of reinnervation showed little if any change in contraction speed. The simplest explanation was that the activity of each type of nerve was the factor in controlling contraction speed, and when shut off by the spinal cord

isolation, muscles converted to a contractile state of "no activity". Buller discussed the possibility that either the frequency or the pattern of discharge of fast and slow nerves might be the controlling factor over contraction speed. However, in the end he argued against both possibilities (and others) and, somewhat surprisingly, suggested that slow (and possibly fast) nerves release their own intrinsic trophic factor to exert control over muscle contraction speed. His argument for a trophic factor loses some impact when it is realized that nerve activity is necessary for release (i.e. the trophic factor must not be released with the silencing of spinal cord isolation, since control is lost). His argument against frequency of activity being the controlling factor is probably correct, but it is predicated on the idea that muscles influenced by "no activity" should develop contraction speeds even slower than muscles influenced by low-discharging slow nerves, when in actuality they tended to become fast. However, it may be that "no activity" is a special case with respect to frequency. Finally, and most importantly, his argument against the pattern of impulse activity (phasic versus tonic) being the controlling factor is not significantly supported by the data (see p. 432).

In light of many recent studies, it appears that the pattern of activity of a nerve controls the contraction speed of its muscle (refer to Figure 6). Salmons and Sreter

(1976), in an interesting study, opposed the putative chemotrophic effects of a nerve on its muscle's contraction speed with patterned electrical stimulation of the nerve. Whether a muscle was innervated by its intrinsic nerve or cross reinnervated by a nerve of opposite type, the contraction speed was determined by the pattern of electrical stimulation of the nerve irrespective of the nerve type. A trophic factor is not necessarily ruled out, since one might argue that fast and slow nerves contain the same trophic factor and the pattern of its release, established by the nerve activity, controls contraction speed. Occam's razor says, though, to forget a trophic factor, since activity pattern alone is a simpler explanation. Furthermore, it appears that the presence of a nerve and its supposed trophic factor is not even required, as long as a pattern of activity is available to control contraction speed. Lomo and Westgaard (1974) demonstrated that denervated soleus muscles, (i.e. without the supposed chemotrophic influence), differed in their contraction speeds following chronic electrical stimulation, in accordance with the pattern with which they were stimulated. It can be concluded from Lomo and Westgaard's studies, that under normal conditions the pattern of activity of a nerve sets the contraction speed of its muscle, but only as a result of the pattern of evoked muscular activity itself.

From the previous discussion, it is apparent that muscles, given sufficient time, are quite adaptable in their contraction speeds to efficiently accommodate different patterns of activity brought on by an artifical means, such as through cross reinnervation or electrical stimulation. Under normal conditions, however, the activity patterns and contraction speeds of muscles don't change to any noticeable extent. Even during a prolonged increase in the level (average frequency) of firing of motoneurons, as during repeated exercise, their tonic and phasic patterns must be minimally affected, since the contraction speeds of their respective muscles remain unchanged (Barnard, Edgerton, and Peter, 1970a,b).

The change in level of activity of motoneurons that naturally occurs poses a different problem for the muscles they innervate. Their muscles must be able to meet the increased energy demands placed upon them during high levels of activity, such as occurs during exercise. It is fortunate that muscles exhibit adaptability to not only patterns of activity but also to levels of activity, and can increase their fatigue resistance to prolonged high levels of activity (refer to Figure 6) by changing the efficiency with which they metabolize glucose: by changing from the less efficient anaerobic pathway of glycolysis to the considerably more efficient aerobic pathway of Krebs cycle. For example, fast-white muscles, such as some flexors of the

limbs, can become fast-red with exercise training. Although they remain fast, they become redder in appearance, due to an increase in their myoglobin content, indicating that they have become more dependent upon oxygen in the catabolism of glucose. Slow muscles, on the other hand, are always red and resistant to fatigue, because the high levels of activity they experience in their postural functions require that they catabolize glucose via the more efficient aerobic pathway (i.e. although slow motoneurons discharge at lower frequencies, they are tonically active and often have higher average frequencies or levels of activity than fast, rapidly discharging, phasic motoneurons). In conclusion, muscles, and the motor units that comprise them, tend to be one of three different types based on their contraction speed and fatigue resistance: fast-white, fast-red, and slow-red (refer to Figure 6). One might wonder, parenthetically, why fast-white motor units exist at all, given the inefficiency of their metabolic pathway. Fast-white motor units, however, don't require greater efficiency to adequately maintain tension, without fatigue, at their low levels of activity. Furthermore, using an anerobic pathway frees them from dependency upon the circulation. Finally, it must be remembered that inefficiency doesn't imply that energy is lost; less energy is derived from each molecule of glucose metabolized, but the rest is stored in the form of lactic acid for later reconversion.

So far the discussion has been limited to muscles composed of motor units which are all of the same type. However, most muscles are "mixed" in their motor unit types to provide for a greater diversity in the functions that can be performed. It must be acknowledged in this regard that the three types of motor units described are only "stereotypes". The actual number of types that comprise a muscle depends upon the number of motor units that experience significantly different patterns or levels of activity (see Brooke and Kaiser, 1974).

In view of the diversity of functions, both postural and phasic in nature, that each laryngeal muscle performs, they might be expected to contain a mixture of motor unit types. Although characterization of motor units in each muscle is best obtained through direct measurement of their contraction speeds and fatigue resistance, some appreciation of the contractile properties of motor units can be gained with knowledge of their muscle fiber histochemistry. It has been demonstrated in both motor unit studies (Burke, Levine, Tsairis, and Zajac, 1973) and whole muscle studies (Barany, 1967) that there is a direct correlation between the contraction speeds of muscle fibers and their (alkaline resistant) myofibrillar ATPase contents (refer to Figure 6). Muscle fibers viewed in cross section (e.g. Figure 13) which stain darkly for myofibrillar ATPase are classified as type II fibers (according to the nomenclature of Brooke and

Kaiser, 1970) and are presumed to be fast contracting, while those staining lightly are classified as type I and are presumed to be slow contracting. It has also been demonstrated at both the levels of the motor unit (Burke and Tsairis, 1974) and whole muscle that there is a direct correlation between the fatigue resistance of muscle fibers and their mitochondrial oxidative enzyme contents (refer to Figure 6). Muscle fibers that stain intensely for one of the oxidative enzymes are classified as either type IIA or I (red fibers) while those that stain lightly are classified as type IIB (white).

In Figures 13 and 14, the PCA and CT muscles have been stained for myofibrillar ATPase and appear to be quite mixed in their relative proportions of type I and type II muscle fibers. Type II (fast) fibers predominate, making up approximately 60% of the muscle fibers of these two muscles (The staining of individual PCA fibers is better appreciated at higher magnification, Figure 19). A considerably higher proportion (90%) of muscle fibers are type II in the LCA muscle (Figure 15). By using an acid instead of an alkaline preincubation medium, the relative paucity of type I fibers (which have acid resistant myofibrillar ATPase) in this muscle can be demonstrated (Figure 16, type I fibers are darkly stained). The relative proportions of type I and type II fibers in the TA and A muscles (not shown) are very similar to that observed for the LCA muscle (see Table 23).

All of the intrinsic laryngeal muscles exhibit intense staining for oxidative enzymes in comparison to most limb and extrinsic laryngeal muscles (e.g. sternothyroid), suggesting that they are quite fatigue resistant. It is not even certain that type IIB (white) muscle fibers are present in these muscles. Most certainly all type II fibers in the LCA and TA are type IIA (fast-red) as indicated by the intense staining for oxidative enzymes in the periphery of each fiber (Figure 18). Because of the relative paucity of type I fibers and the absence of type IIB fibers, these two muscles have a uniform "checkerboard" appearance (Figure 17). The PCA and CT, on the other hand, are more heterogenous in their appearance, not only because of a greater proportion of type I (slow-red) fibers but also because of greater variation in the oxidative enzyme content of type II fibers than is observed in the LCA and TA muscles. In Figures 19-22 serial cross sections of the PCA muscle are shown, stained for myofibrillar ATPase, an oxidative enzyme, and glycogen. The type II fibers indicated by arrows illustrate this variation in oxidative enzyme content from fiber to fiber. The lighter stained fiber might be called a type IIB. No attempt has been made, as yet, to determine how many subgroups (or "actual" types) there are of type II fibers in these muscles.

The high levels of oxidative enzymes and fatigue resistance (Edstrom, Linquist, and Martensson 1974), of the

laryngeal muscles, in general, is undoubtedly a reflection of the high levels of activity they experience as the generator of sound. The high oxidative enzyme content of the PCA can be attributed, as well, to the necessity of this muscle to open the glottis continually (even during sleep) with each breath.

In addition to being fatigue resistant and composed exclusively of red fibers, the LCA, TA and A muscles have been shown in the cat (Martensson and Skoglund, 1964) and most all species investigated (Hirose, et. al. 1969; Hast, 1969) to have fast contraction speeds. The large number of type IIA fibers relative to type I fibers in these muscles is consistent with those observations. In contrast to the CT and PCA, these three muscles have prime positions to quickly close the glottis and protect the airway, the most important function of these muscles and of the larynx, in general (see Pressman, 1953).

Both the CT and PCA muscles are relatively slower contracting muscles in the cat (Martensson and Skoglund, 1964) and in most animals studied. However, there is considerable variation among species and they are in many instances still considered fast muscles (Teig, 1978). Although still in the minority, the relatively greater percentage of type I fibers in these two muscles correlates well with their relatively slower contraction speeds. Type I fibers in the PCA probably provides a basis for its slow,

non-fatiguing movements during inspiration. It is to be expected that these PCA muscle fibers are innervated by small, highly excitable motoneurons which can become tonically active with each inspiration in a sleeping or barbiturate anesthetized animal. Similarly, the relatively large percentage of type I fibers in the CT muscle may reflect in part its own inspiratory capacity. Inspiratory, as well as expiratory, contractions of this muscle are commonly observed in the anesthetized animal (Zealear and Dedo, 1976, p. 517). Furthermore, many motor axons innervating this muscle fire tonically during inspiration, while others fire tonically during expiration (Suzuki, Kirchner, and Murakami, 1970). Since the CT lengthens and tenses the vocal cord, it acts synergistically with the abducting motion of the PCA to increase the glottal opening and reduce airway resistance during inspiration. Their combined contractions result in a larger glottal opening than occurs with vocal cord abduction by the PCA alone (Konrad and Rattenborg, 1966), so that the CT increases the inspiratory capacity of the larynx particularly during stressful situations. The main function of the CT, however, appears to be an expiratory one. With the adductors active during vocalization, the CT to a large extent determines the tension and length of the vocal cords and thus the pitch of the sound produced (Koyama, Harvey, and Ogura, 1971). Since maintenance of tension (and pitch) is of a postural nature,

the relatively high proportion of type I fibers in the CT could also reflect the phonatory capacity of this muscle. Teig, et. al. 1978 has emphasized that the proportion of type I fibers in laryngeal muscles, in general, might be an indication of the phonatory capacity of an animal, as suggested by the increase in this proportion in more evolved species. Man has a considerably higher proportion of these muscle fibers as shown in Table 23. In this regard, it should be interesting to find out if there is a relationship between the relatively high proportion of type I muscle fibers in the CT and PCA muscles and their relatively large representation in the retrofacial nucleus, with its smaller sized cell bodies. The complex arrangement of this nucleus suggests that it may be involved in a more complex function.

Finally, it is not to be implied that type II fibers do not play a role in vocalization. The high oxidative enzyme content of these fibers in each laryngeal muscle suggest that they participate in more than the infrequent event of airway protection. Intuitively, subtlety in vocalization, as in speech production, would require fast contacting motor units.

III. Conclusions

Hopefully a "picture" of what laryngeal motor units are like and some insight as to what they do has been gained from this chapter. However, many of the generalizations that have been made are based upon principles established for limb motor units. Since laryngeal muscles are unusual in some of their characteristics (egs. presence of multiple innervation: Rossi and Cortesina, 1965; Hunt and Kuffler, 1954; Rudolph, 1960; and absence of typical muscle spindles in some species: Baken and Noback, 1971; Tiegs, 1953), it must be demonstrated that these principles hold for laryngeal motor units as well. One of the goals of the single motor unit studies to be described was to determine if the principles outlined in Figure 6 apply to laryngeal motor units: Do laryngeal motor units obey the size principle? Is there a relationship between motoneuron activity and their muscle fiber contractile properties? Do motor unit contractile properties correlate with their muscle fiber histochemistries? What type of motor units originate from each of the brainstem nuclei described? All of these questions should be answered. Of course, the most important question posed is what functions do motor units mediate and how are their characteristics adapted to meet these functions. These questions will be addressed in Chapters 3 and 4, with respect to motor units of the

thyroarytenoid (TA) muscle. It should be recalled that the TA is composed of 90% type IIA fibers and receives most (if not all) of its innervation from the nucleus ambiguous. Before describing the motor units of this muscle, a description of the method used is warranted.

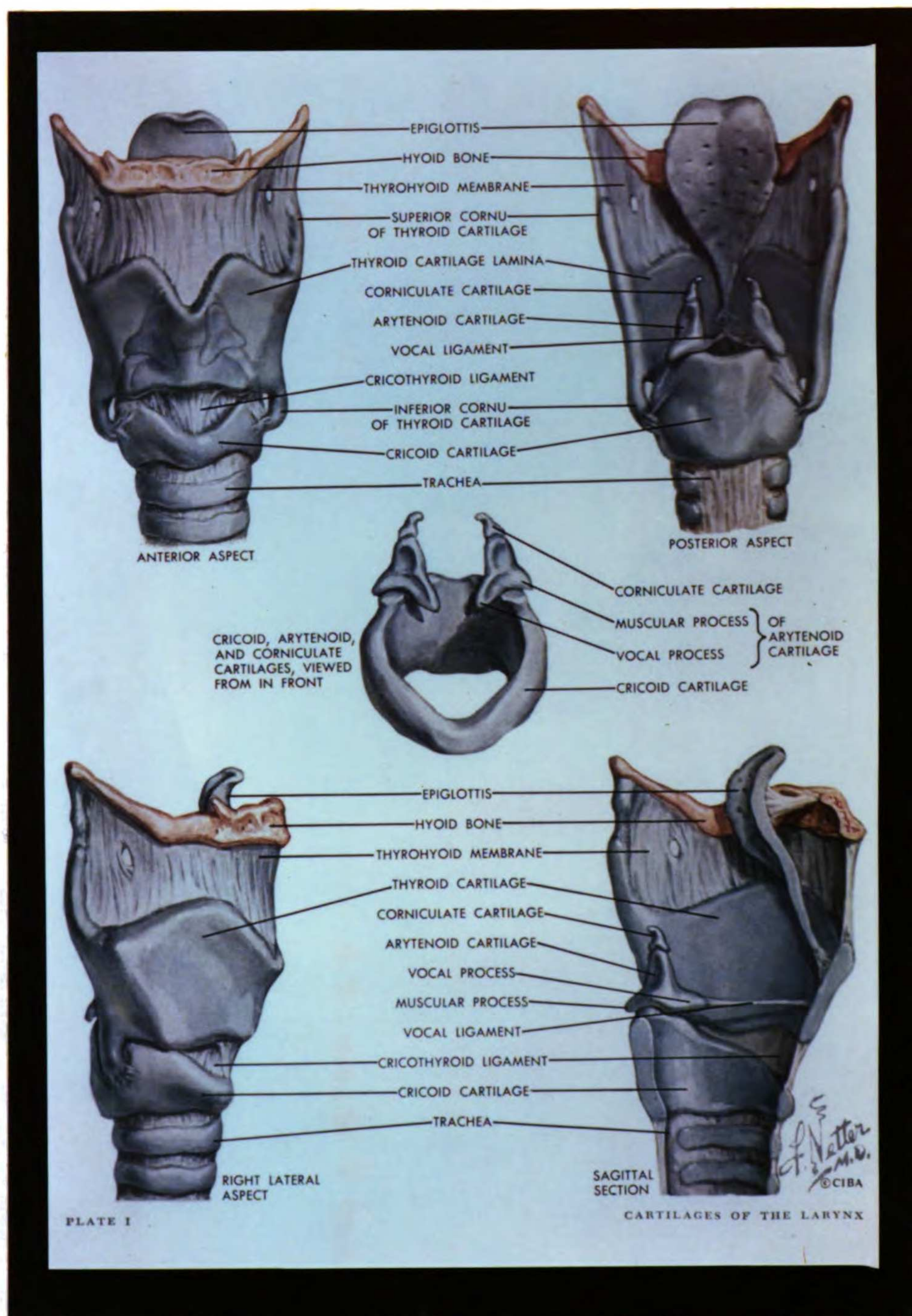

FIGURE 1

Illustration of the cartilages of the human larynx. Although the human and cat larynx do differ in some of their features, illustrations of the human larynx are shown in this figure and the following 4 figures to better explain the locations and actions of the intrinsic laryngeal muscles, in a general sense (illustration from Netter, 1964).

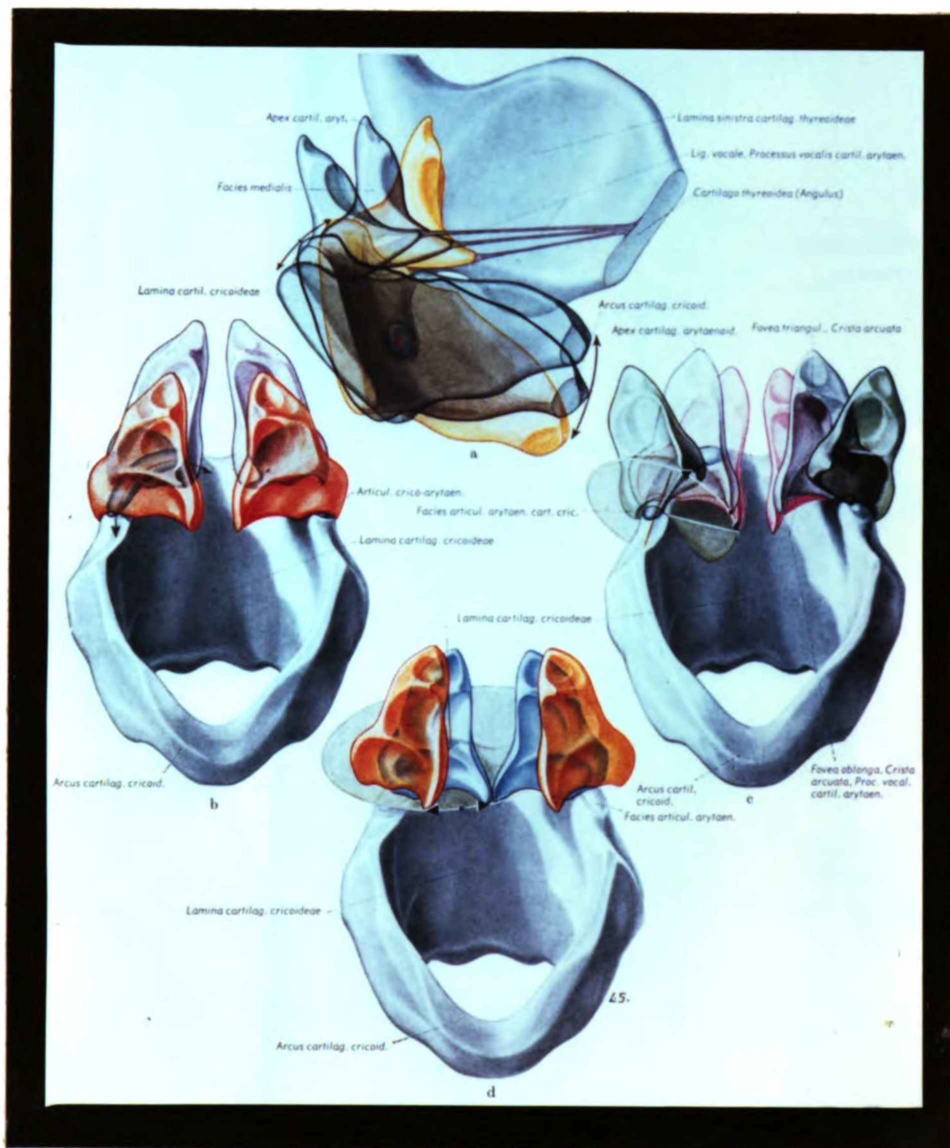

FIGURE 2

Illustration of the movements of the arytenoid cartilages (illustration from Pernkopf, 1952).

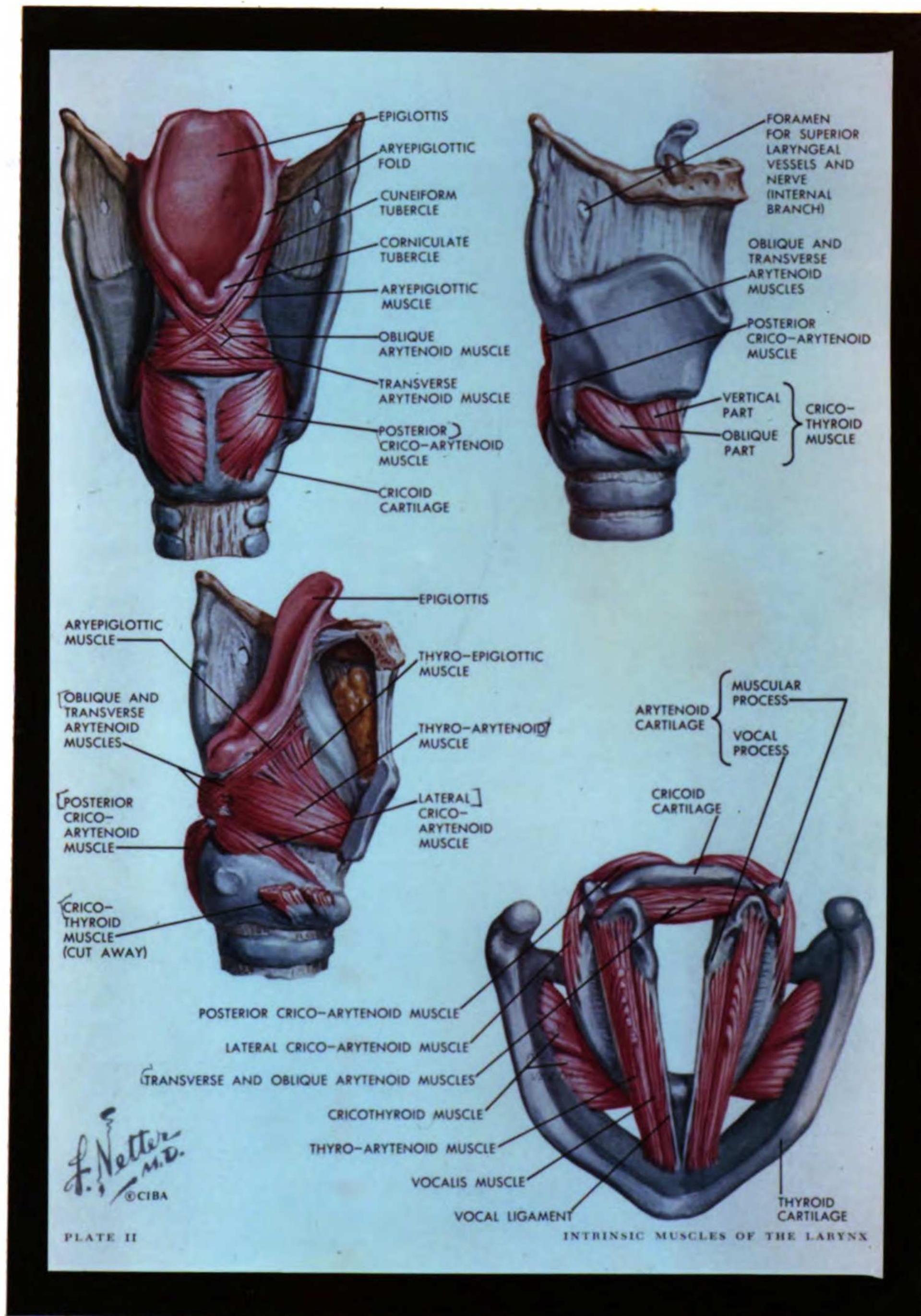

FIGURE 3

Illustration of the origins and insertions of the intrinsic laryngeal muscles (illustration from Netter, 1964).

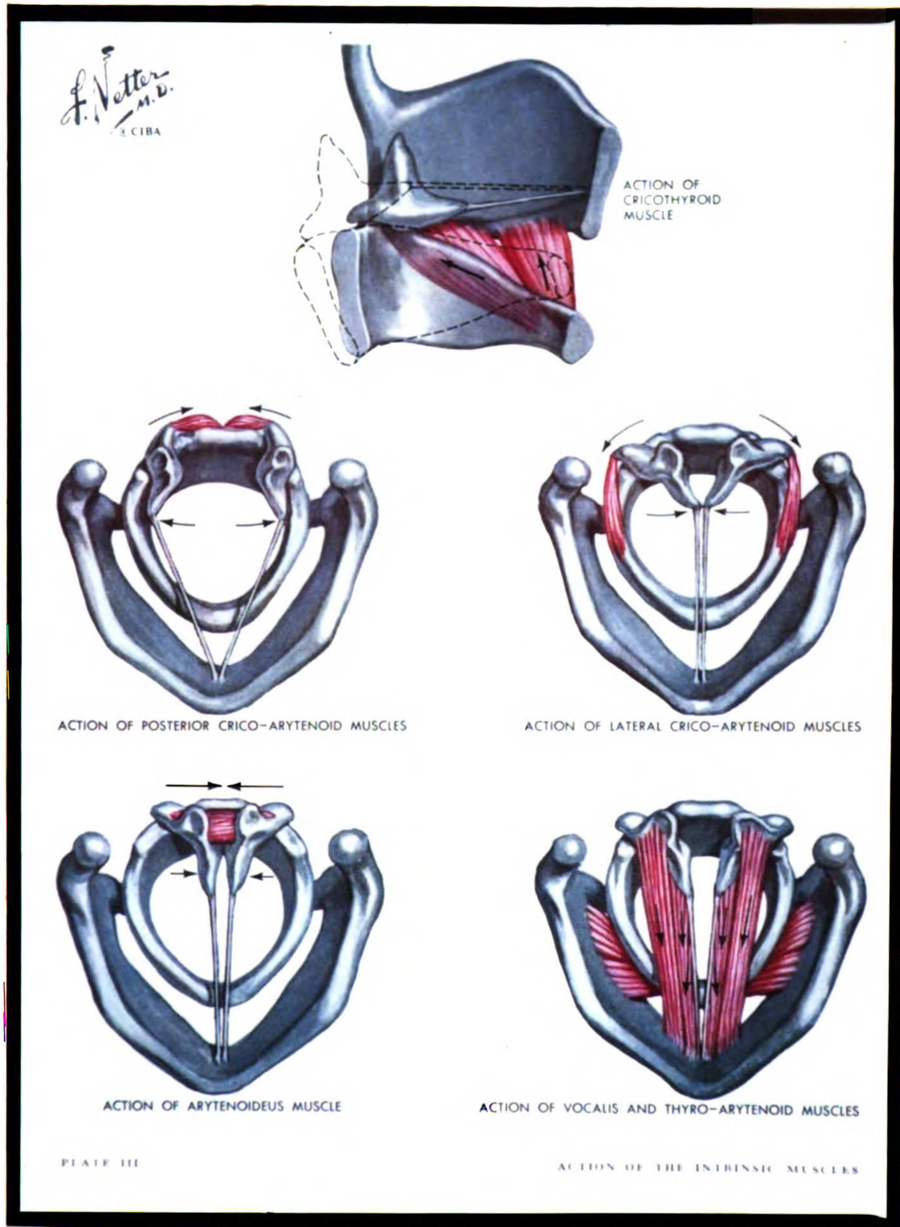

FIGURE 4

Illustration of the actions of the intrinsic laryngeal muscles (illustration from Netter, 1964).

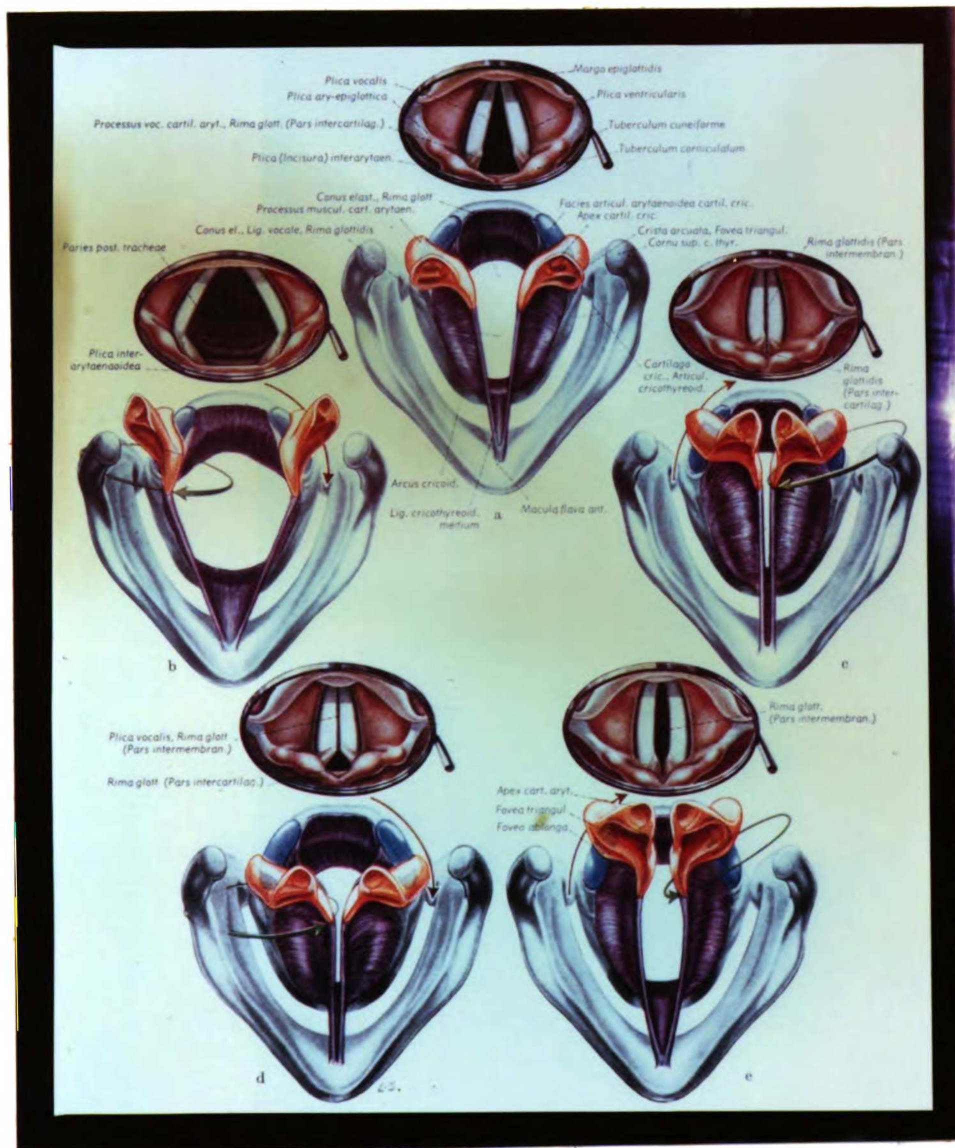

FIGURE 5

Illustration of the movements of the arytenoids from resting position (a) to abducted position (b) during inspiration. The corresponding vocal cord positions (when viewed from above) are depicted above each of the figures (from Pernkopf, 1952).

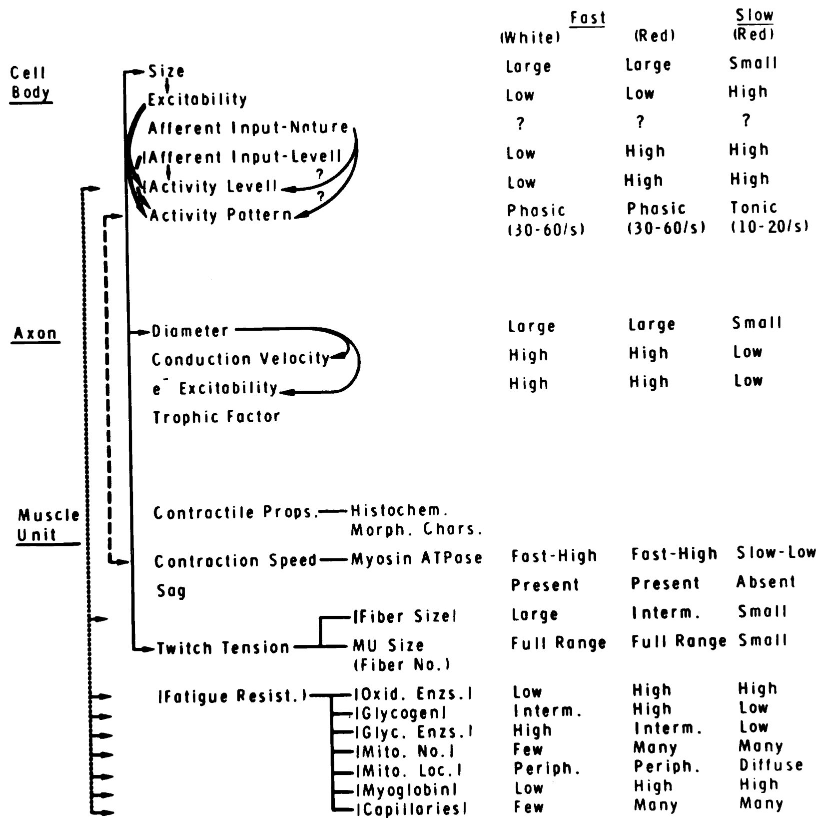

FIGURE 6

FIGURE 6

Some of the fundamental characteristics of the three basic types of motor units are shown at the level of cell body, axon, and muscle unit (i.e. the motor unit's muscle fibers). Characteristics within each level which are interrelated are indicated by connecting solid lines (with arrows when there is an apparent causal relationship). Characteristics at different levels which are interrelated are indicated by solid, dashed, and broken lines. The solid line signifies characteristics that don't ordinarily change, the dashed line characteristics that can change under certain conditions (e.g. electrical stimulation), and the broken line characteristics that do change in a normal animal (e.g. during exercise) without manipulation by an external force. Qualifications: 1) Arrow at twitch tension indicates component of twitch tension related to MU size; twitch tensions can vary with changes in fiber diameter. 2) Fast-white and fast-red motor units have considerably broader ranges of MU sizes than slow units, but not necessarily full ranges as shown; fast-white are usually intermediate to large in size, fast-red small to intermediate in size, and slow small in size. 3) Fiber diameters are not always as indicated. 4) Fast-red fibers containing a considerable number of mitochondria can have them spread diffusely throughout the sarcoplasm like slow muscle fibers. 5) Myosin ATPase refers to myofibrillar ATPase. 6) Trophic factor was included in the diagram to indicate its possible role in the control of muscle unit properties. See Close, 1972 (p. 132) particularly, and text for references. See also: Barnard et. al., 1971; Varnauskas et. al., 1970; Bergstrom and Hultman, 1967; Dohm et. al., 1973; Gollnick and King, 1969; Bergstrom and Hultman, 1972; Holloszy, 1967; Mole et. al., 1971; Folkow and Halicka, 1968; Henneman and Olson, 1965; Nishiyama, 1965; and Pande and Blanchaer, 1971,for further references.

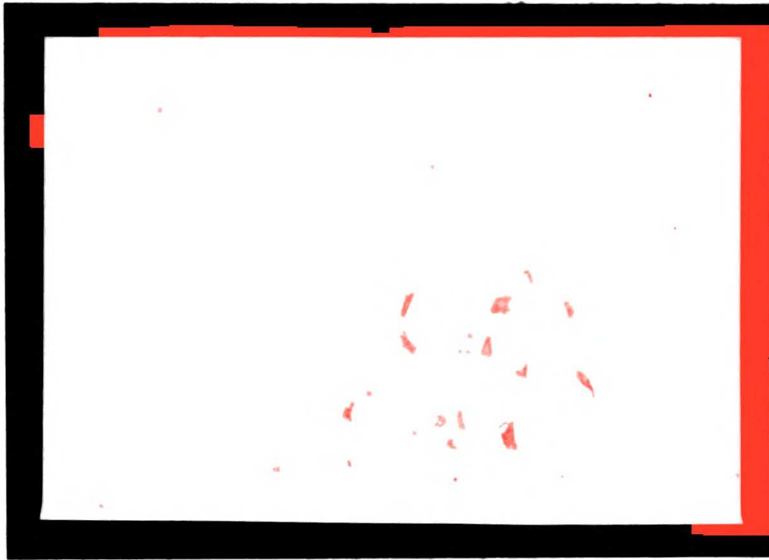

FIGURE 7

Nucleus ambiguous PCA motoneuron cell bodies containing HRP in the cat. Histochemical technique was a modification of Grahams and Karnovsky's (1966). Section not counterstained, x150.

FIGURE 8

Retrofacial nucleus PCA motoneurons containing HRP in the cat. Same magnification as in figure 7.

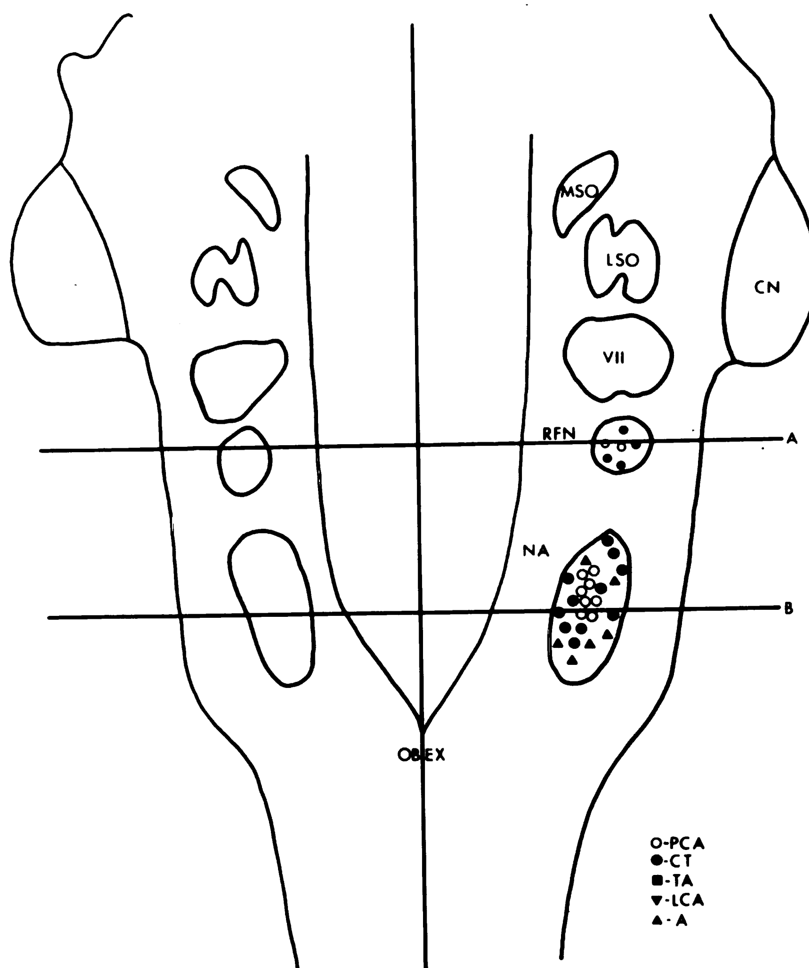

FIGURE 9

Outline drawing summarizing location of labelled neurons in those kittens where the brain was evaluated in a horizontal plane (taken from Gacek, 1975, p. 11).

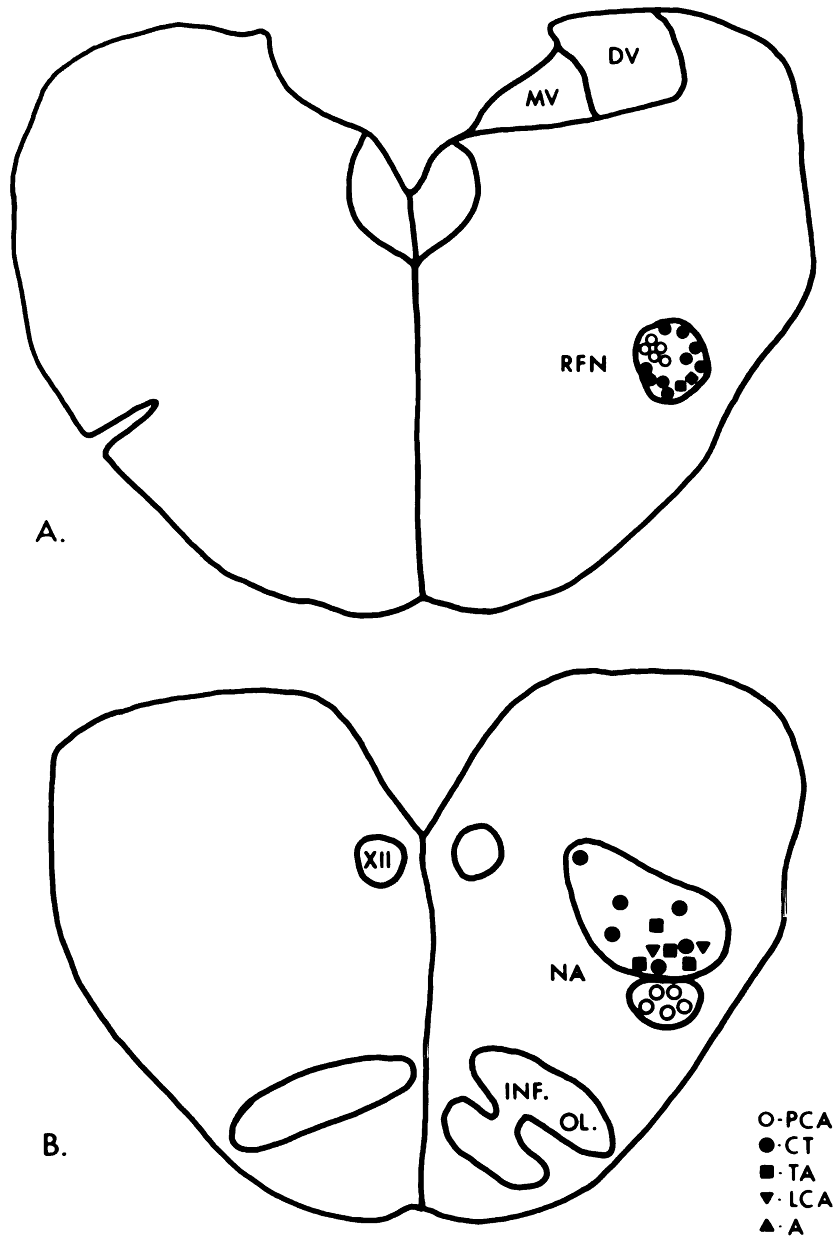

FIGURE 10

Drawing of transverse sections of the brain stem taken through levels A and B in figure 9. "A" summarizes the location of labelled neurons in the retrofacial nucleus and "B" the location of labelled neurons in the nucleus ambiguous in kittens (taken from Gacek, 1975, p. 12).

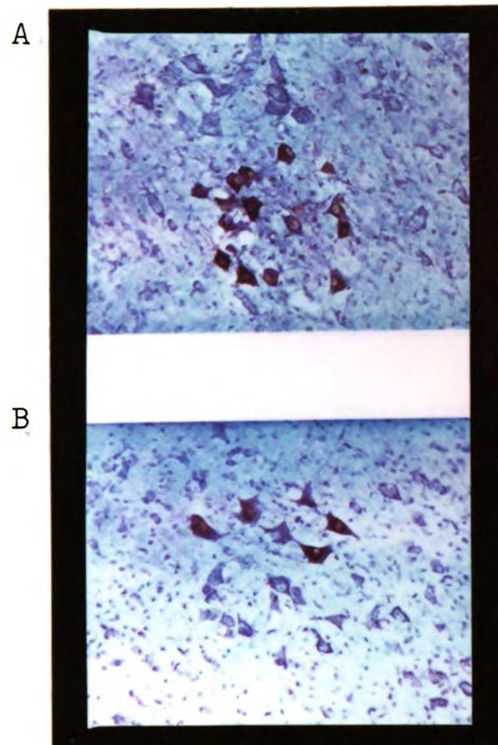

FIGURE 11

Transverse sections through the nucleus ambiguous in two kittens, following injections of the PCA muscle (A) and the CT muscle (B). PCA motoneurons are smaller and more compactly arranged in a ventral subdivision as shown. Nissl counterstained, x150 (taken from Gacek, 1975, p. 3).

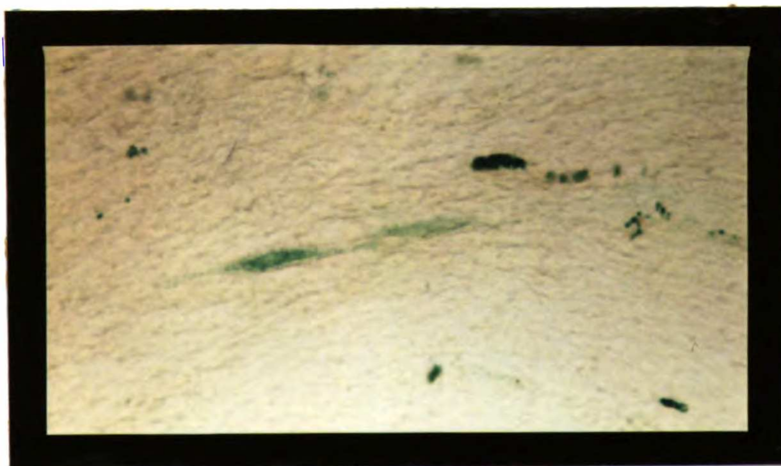

FIGURE 12

PCA motoneurons containing HRP in the lateral division of the nucleus solitarius. Section treated with dianisidine according to the protocol of de Olmos, 1976. Uncounterstained, x200.

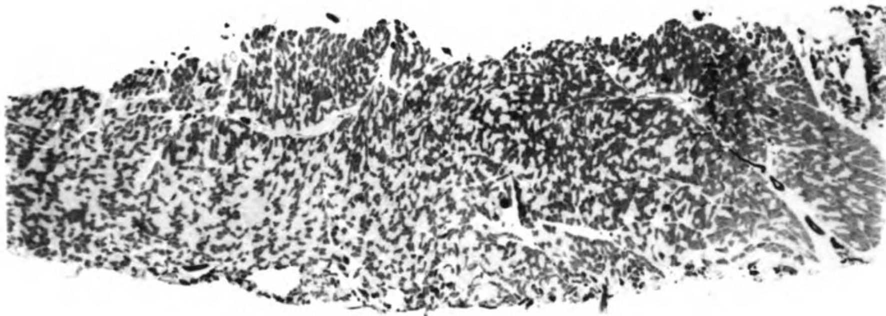

FIGURE 13

Cross section of cat PCA muscle stained histochemically for myofibrillar ATPase using a modification of the technique of Guth and Samaha, 1970. Alkaline preincubation. x13.5

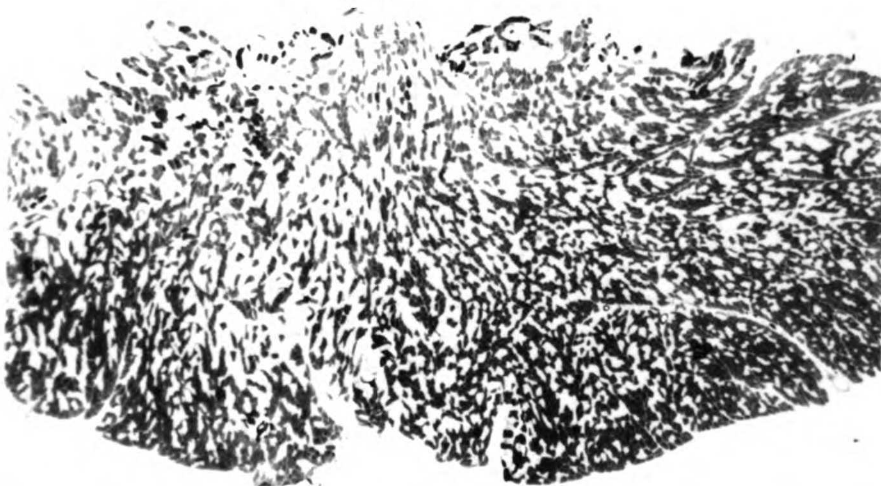

FIGURE 14

Cross section of cat CT muscle treated as above. x13.5

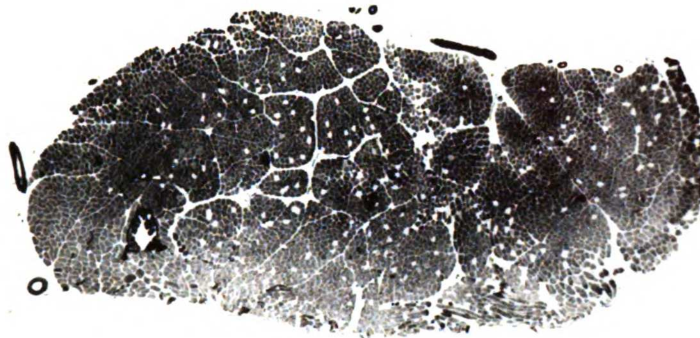

FIGURE 15

Cross section of cat LCA muscle treated histochemically for myofibrillar ATPase as in figure 13. Alkaline preincubation. x13.5

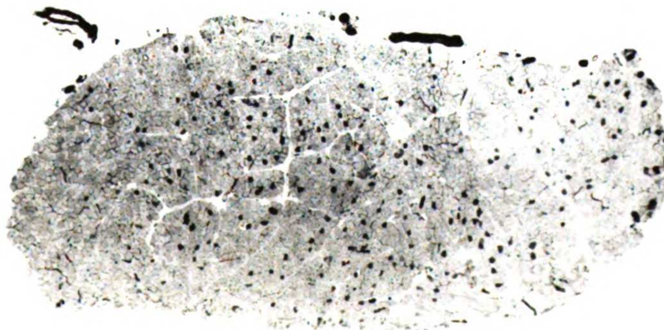

FIGURE 16

Non contiguous serial cross section of same LCA muscle as above treated histochemically for myofibrillar ATPase with acid preincubation. Dark staining fibers are type I. x13.5

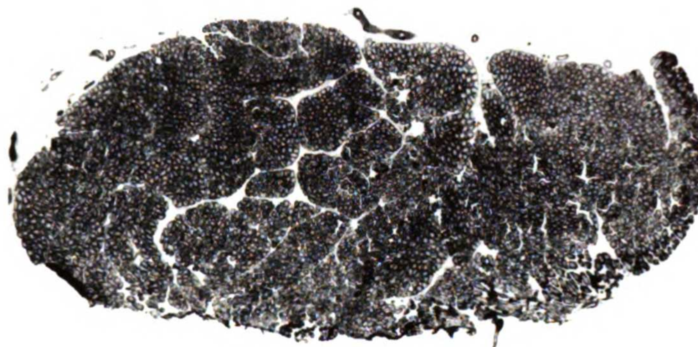

FIGURE 17

Non-contiguous serial cross section of the same LCA muscle as in figure 15, treated histochemically for NADH diaphorase, an oxidative enzyme, according to the technique of Novikoff et. al., 1961. x13.5

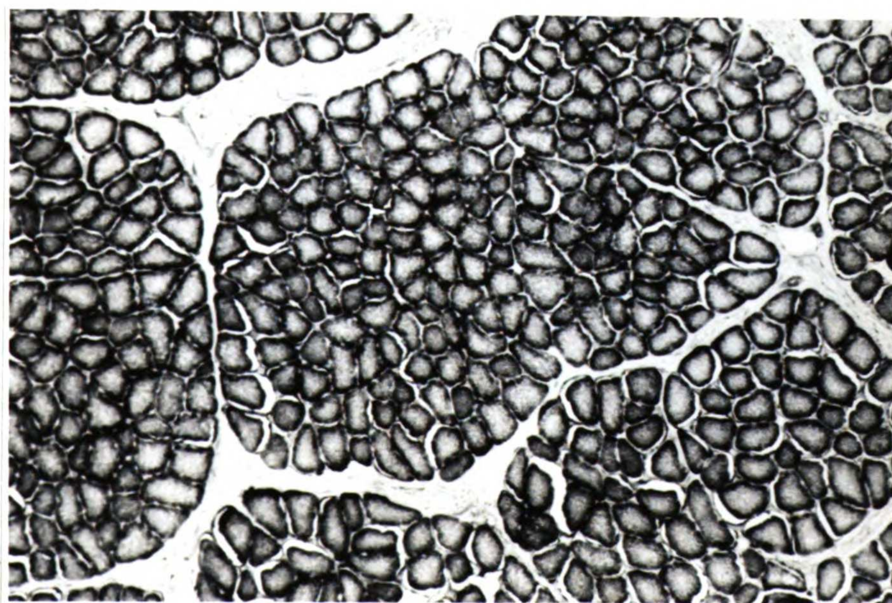

FIGURE 18

Higher magnification of same section in figure 17. x102

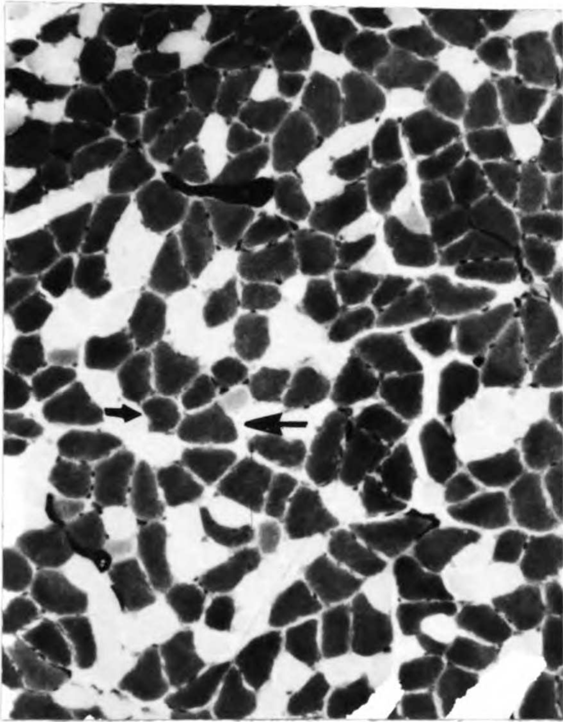

FIGURE 19

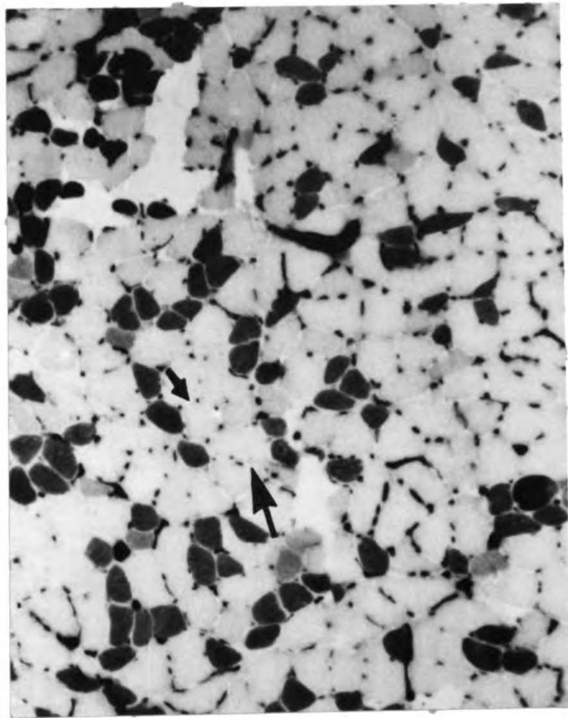

FIGURE 20

FIGURE 21

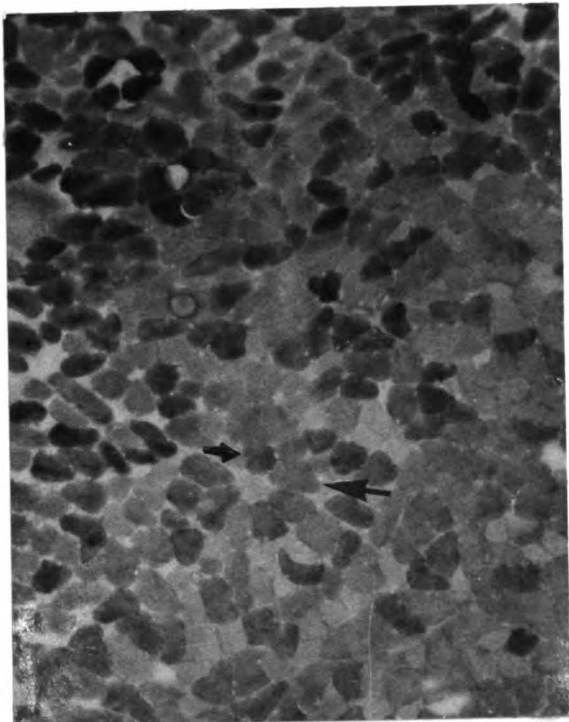

FIGURE 22

FIGURES 19-22

Non-contiguous serial cross sections of the PCA muscle stained histochemically for: alkaline resistant myofibrillar ATPase (figure 19), acid resistant myofibrillar ATPase (figure 20), the oxidative enzyme NADH diaphorase (figure 21), and glycogen (figure 22). Two type II fibers are indicated by the arrows, as shown by their dark staining for alkaline resistant myofibrillar ATPase and their light staining for acid resistant myofibrillar ATPase. Contrast these two fibers with the one immediately **below**, a type I fiber, staining just the opposite for myofibrillar ATPase. The type II fiber indicated by the small arrow is a type IIA, as shown by its high content of NADH diaphorase, close to the type I fiber in staining intensity. However, notice that the staining is still mostly in the periphery (refer to figure 6). The type II fiber indicated by the large arrow might be considered type IIB, since it stains fairly lightly for NADH diaphorase. Note that the type IIA stains highest for glycogen, while the type I stains lowest for glycogen (refer to figure 6). **x135**

	PCA	CT	A	LCA	TA
Human	67	47	46	40	35
Monkey	40	30	?	12	19
Cat	39	37	?	8	11
Rabbit	40	35	?	?	0

TABLE 23

Percentage type I fibers in each of the intrinsic laryngeal muscles for 4 different species. Notice the tendency for a general increase in the proportion of type I fibers in each laryngeal muscle the more evolved the species (see Tieg, 1978). Values taken from: Teig, 1978 (human), Sahgal and Hast, 1974 (monkey), investigator's studies (cat), and Asmussen and Wohlrab, 1972 (rabbit).

Chapter 2: QUASI-INTAAXONAL STIMULATION AND RECORDING FROM THE RECURRENT LARYNGEAL NERVE: A STABLE AND NON-DAMAGING METHOD FOR STUDYING SINGLE MOTOR UNITS

I. Technical Approach

The contractile properties of limb motor units are commonly determined by hanging cut isolated axon filaments on a pair of electrodes for stimulation (see discussion). The spiking sensitivities of motoneurons can likewise be determined by recording centrally from isolated filaments. Unfortunately, this technique can not provide for a description of both contractile properties and functions of single motor units. Even if axons are not cut, but rather are stimulated and recorded from extracellularly with microelectrodes, it can not be known for certain that only a single unit is involved. Stimulation and recording from the same motoneuron can be achieved, on the other hand, with intracellular penetrations. Cell body penetrations, furthermore, have been successfully used in the spinal cord to study limb motor units (Burke, 1967). Attempts were made accordingly to penetrate thyroarytenoid (TA) motoneuron cell bodies in the brainstems of spontaneoulsy breathing cats. Unfortunately, cells could not be "held" with a micropipette for sufficient periods of time for their complete description, in face of respiratory and circulatory movements of the brain. Paralyzing with flaxedil and artificially respirating the animal to reduce brainstem

movements was not a solution to the stability problem, of course, since motor unit contractions would be precluded by the paralytic drug. In view of the difficulties associated with the brainstem technique, a completely different approach was taken, that of penetrating axons of TA motoneurons in the recurrent laryngeal nerve (RLN), and found to be a surprisingly stable technique for studying these motor units. Axons could be held with a micropipette for a few hours using this approach. Upon stimulation of an axon, the inherent contractile properties of its muscle fibers (including their fatigue characteristics during an hour's stimulation) were determined, after which recording from the axon during physiological maneuvers revealed the spiking sensitivity of the axon and hence the functional role(s) of the motor unit in TA movements.

II. Animal Preparation

A barbiturate anesthetized cat, illustrated diagramatically in Figure 24, was positioned supinely in a Kopf stereotaxic apparatus and a midline incision made from sternum to mandibular process. Neck skin was drawn up and secured to an immobolized wire loop to support a pool of saline which bathed the internal structures of the neck and was maintained at 37°C by a servo-controlled d.c. heater. The strap muscles were transected, the trachea hemisected, and a stainless steel tracheal cannula inserted and

immobolized to prevent tracheal movement during respiration. The larynx was then isolated by transecting the pharynx rostral to the thyroid cartilage and suspending it above the prevertebral muscles and carotid sheaths by clamping the cricoid cartilage. Suspension of the pharynx above the carotids was necessary so that tensions of the smaller motor units could be monitored free of circulatory artifact arising from pulsation of the arteries. Isometric tensions of a quasi-isolated TA muscle were monitored via a force transducer linked to the origin of the muscle, a longitudinal piece of the thyroid cartilage, while the insertion of the muscle, the arytenoid cartilage, was immobolized. Fixation of the insertion rather than the origin of the muscle was preferred since other muscles insert upon the arytenoid cartilage. Finally, whole nerve electrodes were placed upon the vagus nerve for orthodromic activation of TA motoneuron axons penetrated with the micropipette in the RLN (i.e. axons innervating the TA muscles as well as most other intrinsic laryngeal muscles descend with the vagus into the chest, then branch off and recur back into the neck as the RLN to innervate their respective target muscles).

III. Factors Affecting Successful Penetration and Holding of an Axon With the Micropipette

Several factors were considered of importance in successfully penetrating and holding axons with a micropipette for the extensive time required for these studies.

1. Stabilization of the nerve on the dissection plate.

After a few centimeters of the RLN was freed from surrounding fascia, the animal's torso was positioned near its head to produce slack in the nerve. Immobolization of the stainless steel tracheal cannula minimized movement of the nerve during the "tracheal tug" of inspiration, but further stabilization of the nerve was required for successful holding of axons with the micropipette. Thus, the nerve was pinned* via its connective tissue, without disrupting its circulation, to a layer of sticky wax** covering the dissection plate in such a mannner as to leave the slack in the nerve caudal to the plate. This slack was taken up during the nerve's descention during inspiration without movement of the nerve on the plate.

2. Exposure of the endoneurium with minimal loss of extraaxonal pressure and collagen support.

After exposing the funiculus in the nerve for a few millimeters and pinning the epineurial vessels aside, the

---
* Minutiae pins (Clay-Adams)
** Whipmix Company

perineurium of the funiculus was picked with two jewelers forceps until evulsation of the endoneurium occurred through a hole. Desheathing the funiculus or extending the perineurial opening resulted in poor penetration of axons with the micropipette, presumably because of substantial loss of endoneurial pressure required to support the axons during hydraulic advancement of the micropipette. Likewise, use of proteolytic enzymes such as collagenase in an attempt to expose the endoneurium resulted in inadequate penetration of axons apparently because of partial loss of extraaxonal collagen support. On the other hand, insufficient picking of the perineurium and inadequate exposure of the endoneurium left a residual barrier too tough to penetrate with the micropipettes used in these studies.

3. Angle of attack.

The micropipettes were hydraulically advanced along the axons' axis at an oblique angle of approximately 45° C. Dimpling was more prominent if the angle of attack was normal to the endoneurial surface.

4. Saline bathing medium.

Mineral oil would be preferred as a bathing medium to minimize the stimulus artifact recorded by the micropipette and the general spread of current during vagal stimulation. However, the integrity of the exposed endoneurial surface was better maintained in a pool of saline, presumably because of less protein agglutination at the surface.

5. Type of micropipettes used.

Short tapered micropipettes pulled on a recently introduced "air puff" puller (Brown and Flaming, 1977) were used in these experiments. Tip diameters averaged .05 micron with impedances ranging from 60 to 150 megohms when filled with 5M potassium acetate depending upon the length of the taper. During hydraulic advancement without a stepping motor, micropipettes glided instead of popping into axons, suggesting that the micropipettes were sharp and that axons didn't budge or dimple when confronted with the advancing tip. The smallest TA axons penetrated were estimated to be no larger than 4.28 microns in diameter (as indicated by conduction velocity measurements).

The conduction velocities of TA axons penetrated in one experiment were determined by the conduction latencies to the micropipette from stimulation at a nearby site on the RLN. Assuming that axon diameter is linearly related to conduction velocity by the proportionality factor 1/6 u/m/s (Hursh, 1939), the slowest conducting axon penetrated in this experiment (25.7 m/s) has a calculated diameter of 4.28 microns. In other experiments the vagus was stimulated and conduction velocities determined more centrally. The conduction velocities were considerably higher on the average, probably because of peripheral branching of axons (see Shin and Rabuzzi, 1971), and can not give an accurate estimate of axon diameters at the site of penetration. Histologically, motoneuron axons in the RLN have been reported to range between 4 and 9 microns in diameter (Gacek and Lyon, 1976).

IV. Quasi-intraaxonal recording and stimulation with the micropipette

Upon touchdown of the micropipette tip and advancement into the RLN endoneurium, negative field potentials were not detected during orthodromic activation of TA axons, presumably because of the high impedance of the micropipette. Nor was it possible to activate TA axons extracellularly with maximum current injection (i.e. 150 nA) of either polarity through the micropipette. Penetration of the myelin sheath of an axon was first indicated by the appearance of a small positive (vagus initiated) action potential which was accompanied by little if any d.c. shift. With further advancement through the sheath, the action potential grew in magnitude to a maximum, again with little associated d.c. decrease (Figure 25A, upper traces). In some instances a true axon penetration occurred as signified by a sudden d.c. drop and increase in the size of the evoked action potential (Figure 25A, lower trace). However, no attempt was made to achieve this end, since activation of single axons could be obtained through anodal stimulation within their myelin sheaths. Although physically outside, myelin sheath penetrations resembled actual axon penetrations functionally, with respect to recording and stimulation.

Quasi-intraaxonal recording and simulation as such has been described previously in studies of isolated axons by Tasaki,

1952 and others (Huxley and Stampfli, 1949; Huxley and Stampfli, 1951; Woodbury, 1952). Little if any recorded resting membrane potential in face of graded, positive action potentials have been explained as arising from the extremely high resistance of the myelin sheath to passing d.c. current but its rather low impedance to passing transient current of the duration of the action potential (Tasaki, 1952).

The parallel resistance and capacitance of each of the Schwann cell lamellae when wrapping an axon are added in series and thus increase the total transverse resistance but lower the total transverse capacitance of the axis cylinder of the internode (Figure 26A). The high transverse resistance minimizes shunting of current arising at the adjacent nodes during their maintenance of the resting membrane potential (see Huxley and Stampfli, 1951) and their generation of action potentials (see Huxley and Stampfli, 1949). The low transverse capacitance minimizes time for charging of the internode cylinder to a level sufficient for firing of the next node during saltatory conduction of the action potential.

If the internode cylinder is viewed as a passive (parallel) RC cable in this manner loaded by the potential of its nodes, a successive decrease in d.c. voltage must exist inwards across the internode cylinder at rest (with capacitance charged) as the resting membrane potential is serially divided among the resistances of the myelin lamellae. That is, as the resistance between micropipette tip and extracellular ground increases and the resistance between tip and axoplasm decreases during inward movement of the micropipette across the sheath, the recorded d.c. potential should decrease. Possibly, insertion of the micropipette itself into the sheath partially disrupts the resistance of the lamellae and partially shorts out the tip to ground voltage. On the other hand, it may be that little d.c. voltage gradient actually exists across the sheath in the unperturbed state: There is an indication that the internode axolemma is not totally passive but exhibits conductance selectivity to potassium and sodium (Huxley and Stampfli, 1951). Hence, it may aid the nodes in maintaining the resting membrane potential. The extent of its involvement will determine the magnitude of an internodal

emf in series with and opposing that at the nodes, effectively reducing the d.c. current and voltage drop across the myelin sheath (see Frank and Fuortes, 1955, p. 635). The capability of the internode axolemma in generating a resting emf is questionable, however. The gap separating axolemma and first myelin lamella is not in communication with extracellular space, and is largely occluded at segments by the joining of these two membranes to form an "external compound membrane" (Robertson, 1960, p. 358). Both limit the accessibility of the axolemma to extracellular ions.

Recording of small positive action potentials from within a myelin sheath undoubtedly occurs because of the lowered (passive) impedance of the sheath during passage of an action potential. As the sheath capacitance is charging by the action potential, some capacity current will be shunted through this lowered impedance pathway to ground (see Huxley and Stampfli, 1949) and will drop a potential across the tip to ground impedance. The size of the recorded potential will depend upon the tip to ground impedance relative to the tip to axoplasm impedance. With inward movement of the tip, the recorded action potential will grow in size, as observed in these experiments, as the former increases and the latter decreases. One might expect, furthermore, that near full sized action potentials would be recorded from within the sheath during deep penetrations just short of the axolemma. However, the largest potentials recorded (Figure 26B) were less than half the size of those recorded intraaxonally (e.g. Figure 25A). Their small size might possibly be explained by the observation that the capacity current and the associated low sheath impedance are short-lived. Both return to resting levels before the peak of the action potential is reached at any given point along the internode (Huxley and Stampfli, 1949, Figure 12). Under such conditions, recording the peak of the action potential from within the sheath may present the same problem suggested in recording the resting membrane potential: Any shunt pathway alongside the micropipette created upon insertion may become important as the sheath capacitance acquires full charge. A relatively low tip to ground impedance may be present in face of the rising tip to axoplasm impedance across intact myelin.

Since transient current (arising from an action potential) is passed preferentially to d. c. current in an outward direction across the sheath, it is also passed more easily in an inward direction during stimulation within the

sheath. Thus, the make and break of a square wave current pulse are effectively passed to axoplasm for activation of a nearby node. As might be expected, the make of an anodal pulse and the break of a cathodal pulse were observed to activate TA axons as demonstrated in Figure 27. The latency of the twitch response following the make of an anode stimulus was invariable with changes in stimulus duration (Figure 27A). With switch in polarity , the twitch latency following the break of a cathode pulse was also invariable with changes in stimulus duration (Figure 27B). The latency following the cathode break was identical to the latency following the anode make, as would be predicted.

Although levels sufficient for cathodal activation were usually 5 to 10 times higher than those for anodal activation, threshold levels for both decreased with inward movement across the sheath, as the recorded action potential grew in size. It is likely that thresholds decreased for the same reason mentioned previously that recorded action potentials grew in size. As the tip to ground impedance increased and tip to axoplasm impedance decreased with inward movement, a larger proportion of the injected current would reach the axoplasm and depolarize nearby nodes.

V. Criteria Ensuring Successful Activation of and Recording from a Single Axon with the Micropipette

Since axons could neither be activated nor recorded from extracellularly, it was doubtful that neighboring axons were activated or recorded from secondarily to one penetrated. Criteria were used during the course of an experiment, nevertheless, to confirm successful activation of and recording from a single TA axon. Once the sheath (or axolemma) of an axon had been penetrated as shown in Figure 25A, the axon was identified as a TA motoneuron by evoking a twitch response from its muscle fibers with stimulation of the axon (Figure 25B, middle trace). Three observations were then made to confirm that only a single TA axon was activated and recorded from. First, square wave stimulation of a single axon should be followed by near synchronous discharge of its muscle fibers and was recorded as a characteristic diphasic or triphasic EMG response (Figure 25B, upper trace). Second, no variation in either the EMG or twitch response (Figure 25C, lower trace) occurred with variation of stimulus strength, suggesting that the stimulus did not spread to recruit nearby TA axons. That is, both responses were all-or-none in nature. Third, during suprathreshold square wave stimulation through the micropipette, a <u>single</u> resulting action potential was simultaneously recorded (Figure 25D, upper trace, arrow). The action potential was identical to the one evoked by the

vagus electrode pair (Figure 25D, lower trace, arrow head), assuming no shifts in micropipette position occurred between the two events. That is, the orthodromically activated axon was the same axon stimulated by the micropipette. This latter observation guaranteees that spiking activity delineating the motor unit's function was recorded from the same axon stimulated to determine its muscle fibers' contractile properties.

VI. Absence of Axon Damage with Sheath Penetrations

It is significant that quasi-intraaxonal penetrations are "functionally inside" axons and thus provide a method for stimulating and recording from single motoneurons. Quasi-intraaxonal penetrations are nonetheless physically outside axons, and therefore do not damage them as intraaxonal penetrations can. Tasaki, 1952 and Woodbury, 1952 concurred that myelin sheath penetrations resulted in no apparent injury, while intraaxonal penetrations at either nodes or internodes invariably met with axon damage and conduction block. Soon after intraaxonal penetration at an internode, they noticed that a recorded action potential developed a notch, splitting it into two components. Within minutes the second component disappeared. Analysis of action currents (i.e. extraaxonal axial current) indicated that the first and second components were proximal and distal nodes firing, respectively, so that the notch

represented the slowing of conduction between them, across the leaky internode. Eventually, the distal node fell out and conduction blocked as the second component disappeared.

Since most axons weren't entered in these studies, there was little apparent damage done to them. The conductive capacity across the site of penetration was not impaired during the course of an experiment, in general, as demonstrated by equivalent whole muscle twitches elicited by stimulation on either side of the site. Furthermore, only several of a few hundred recorded action potentials were notched, and disappearance of their second components didn't necessarily follow. Perhaps the small tips of these micropipettes (one-tenth the diameter of Tasaki's) were less injurious to those axons that were penetrated. Penetration of axons, in any case, had the risk of damage so that sheath penetrations were preferred.

VII. Discussion

A quasi-intraaxonal approach to studying TA motor units provides a very stable method for stimulating and recording "functionally inside" single motoneurons without actually entering and thus risking injury to them.

This approach is preferred to extraaxonal stimulation with microelectrodes or by hanging cut isolated axon filaments on a pair of stimulus electrodes, because the possibility exists that more than one axon is recruited by

the stimulus or that activation switches from one motoneuron to another during the course of stimulation. Quasi-intraaxonal stimulation assures activation of a single axon, because current sufficient to activate the axon is too small to also activate adjacent axons by an extracellular route. For similar reasons, inadvertent movement of the micropipette (possibly dislodging the tip from the sheath) will not switch activation to another axon as can happen during extraaxonal cathodal stimulation. Similar observations have been made on intra versus extrasomatic stimulation of motoneurons in the spinal cord (Burke, et. al. 1970, footnote #4). This limitation of extracellular techniques may be of minor importance, particularly if a motor unit's EMG and twitch response is tested for consistency and an all-or-none nature over the course of its study. However, there are additional limitations to both extraaxonal methods. Stimulating and recording from the same axon as was done in these experiments, to correlate target organ properties with spiking sensitivity, is not guaranteed with extraaxonal microelectrodes and is not possible when axons are cut. Cutting and isolating filaments, besides being a tedious process, may also compromise the circulation and viability of axons. Finally, in contrast to both extraaxonal approaches, this technique offers the possibility of labelling cell bodies of physiologically described motor

units. By horseradish peroxidase iontophoresis into the axoplasm of an axon near its cell body, the cell body can be backfilled and identified at the conclusion of an experiment.

A quasi-intraaxonal approach is also preferred to a brainstem intrasomatic approach to studying these motor units. TA cell bodies were difficult to find with a micropipette and, once found, impossible to hold for reasonable lengths of time in a spontaneously breathing animal. On the other hand, TA axons could be found in the nerve with relative ease and usually held for more than an hour, sufficient time to complete the investigation of a unit. A dorsal approach to the brainstem also made it difficult to bathe and monitor tensions from the ventrally located TA muscle. A ventral approach to the brainstem, also tried several times, was found to be a better arrangement for monitoring tensions but even less stable for holding cells than the dorsal brainstem technique.

It is suggested that the major application of the quasi-intraaxonal technique is in studying single motor units of head and neck muscles where sufficient extracranial length of nerve is available for stabilization and penetration (egs. facial, laryngeal muscles).

In some instances the technique may also offer an alternative method for studying motor units of limb muscles. The predominant technique used to investigate these motor

units is ventral root filament isolation and stimulation (McPhedran, Wuerker, and Henneman, 1965; Wuerker, McPhedran, and Henneman, 1965; Edstrom and Kugelberg, 1968; Reinking, Stephens, and Stuart, 1975) with only a few studies conducted intrasomatically in the spinal cord (Burke, Levine, Zajac, Tsairis, and Engel, 1971; Burke, Rudomin, and Zajac, 1976). The former technique may be preferred to the latter for the same reasons the quasi-intraaxonal technique was favored over the brainstem technique: It is easier to find and hold axons than their cell bodies. A quasi-intraaxonal nerve approach to studying limb motor units should have the same advantages as ventral root filament isolation without the limitations discussed above. More specifically, the technique could combine the position advantages of ventral root filament isolation with the functional advantages of cell body penetration, with one further advantage: motoneurons wouldn't be violated during stimulation and recording.

Finally, a quasi-intraaxonal approach may also be useful in studies of non-motor systems (e.g.s. autonomic, sensory) where there is a desire to determine the spiking sensitivities and target organ properties of single neurons.

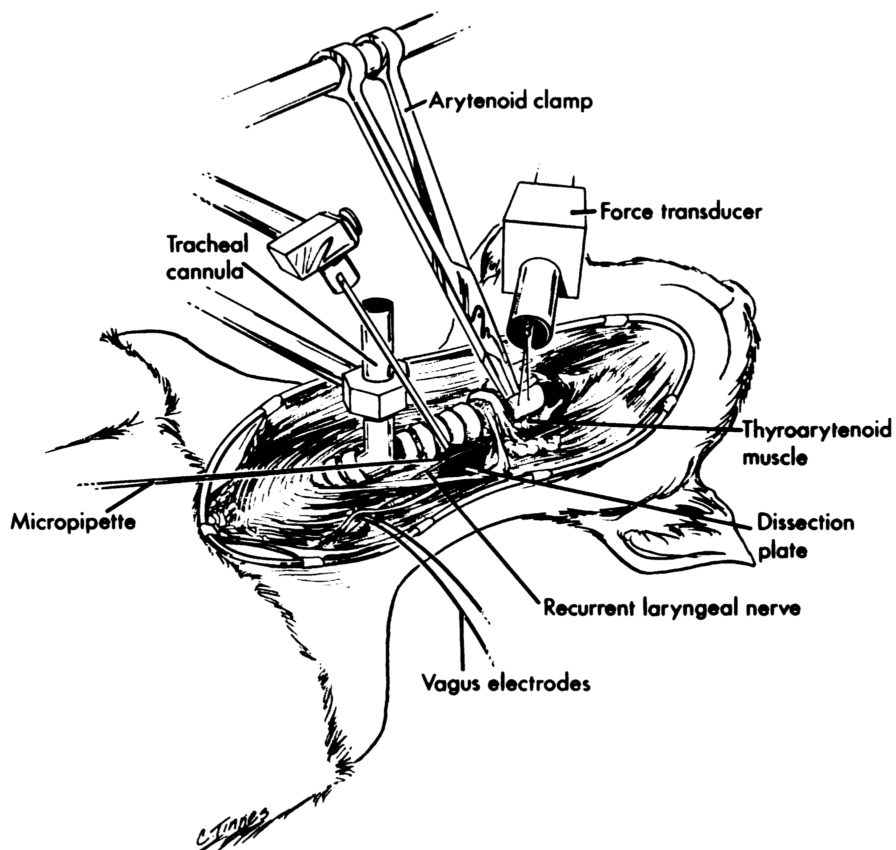

FIGURE 24

Animal Preparation.

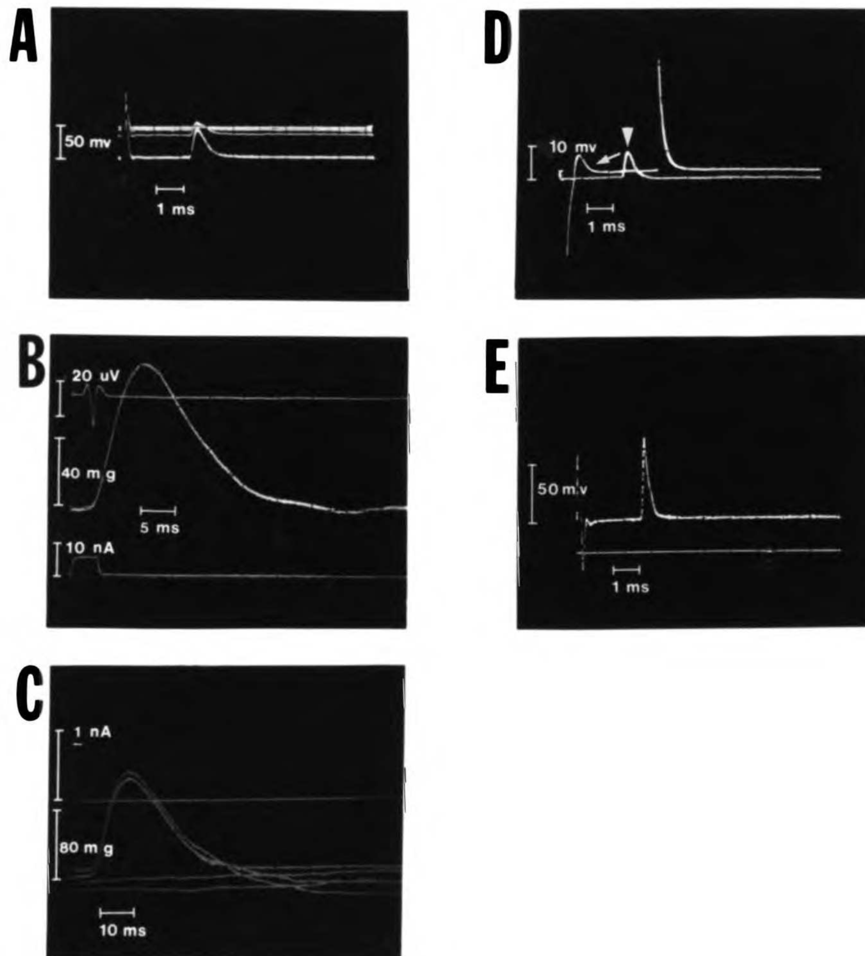

FIGURE 25

FIGURE 25

A. Repeated traces of recordings from micropipette during vagal stimulation. Stimulus artifacts were recorded at the beginning of each trace. As the micropipette was advanced through the myelin sheath of an axon, slight d.c. decreases (top traces) occurred from zero reference and reached approximately 5 mv (middle trace) before the sudden drop of 50 mv (bottom trace) as the tip penetrated the axolemma. Negative evoked responses were not recorded extraaxonally in these studies. Not until the micropipette tip entered the sheath of an axon was an evoked action potential recorded, which was positive in polarity. As the micropipette was advanced through the sheath, the recorded action potential was larger than its associated d.c. shift at any given position, and reached approximately 10 mv in size (middle trace) before a 50 mv action potential was recorded (bottom trace) upon entry into the axon. In most cases recorded action potentials were attenuated due to incomplete RC compensation. It was usually not possible to fully compensate for capacitative shunting across the micropipette glass wall into the surrounding saline pool. B. EMG response (top trace) and twitch response (middle trace) to square wave current injection (bottom trace) into the axon. In general, current pulses used were of shorter duration (approximately .5 msec) and ranged in magnitude from a few nanoamps to as high as 50 nanoamps, depending upon the threshold. C. Repeated sweeps (lower traces) of single motor unit all-or-none twitch responses at threshold (.9 nA) and high (15 nA) current levels (upper trace). Two failures and one success occurred at threshold level. D. Quasi-intraaxonal action potentials initiated by micropipette (upper trace, arrow) and vagus electrodes (lower trace, arrowhead). E. A fully RC compensated, intraaxonally recorded, action potential was obtained in this instance by draining the saline pool around the nerve and micropipette. Quasi-intraaxonally recorded action potentials still retained rounded peaks under such conditions. B and D were taken from unit TA94, A and C from TA7, and E from TA4.

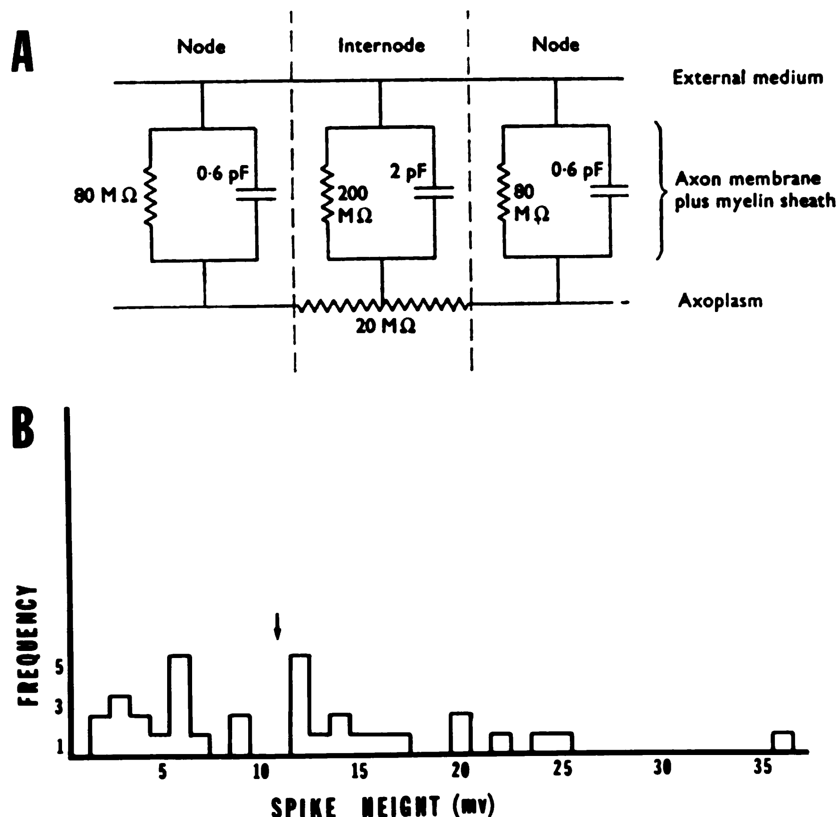

FIGURE 26

A. Representation of the passive electrical properties of a short length of a 15 micron myelinated nerve fiber. The myelin sheath increases the transverse resistance and decreases the transverse capacitance of the internode, so that its component values are comparable to those of the adjacent nodes, despite its considerably larger surface area (diagram from Aidley, 1971; component values from Stampfli, 1954). B. Sizes of quasi-intraaxonally recorded action potentials. The action potentials were probably slightly larger than shown, since they weren't fully RC compensated. All action potentials (except possibly the 36 mv potential) were recorded within myelin sheaths based on several criteria: their small size, variation in their size with micropipette movement, and the absence of sudden d.c. shifts. Arrow=average spike height.

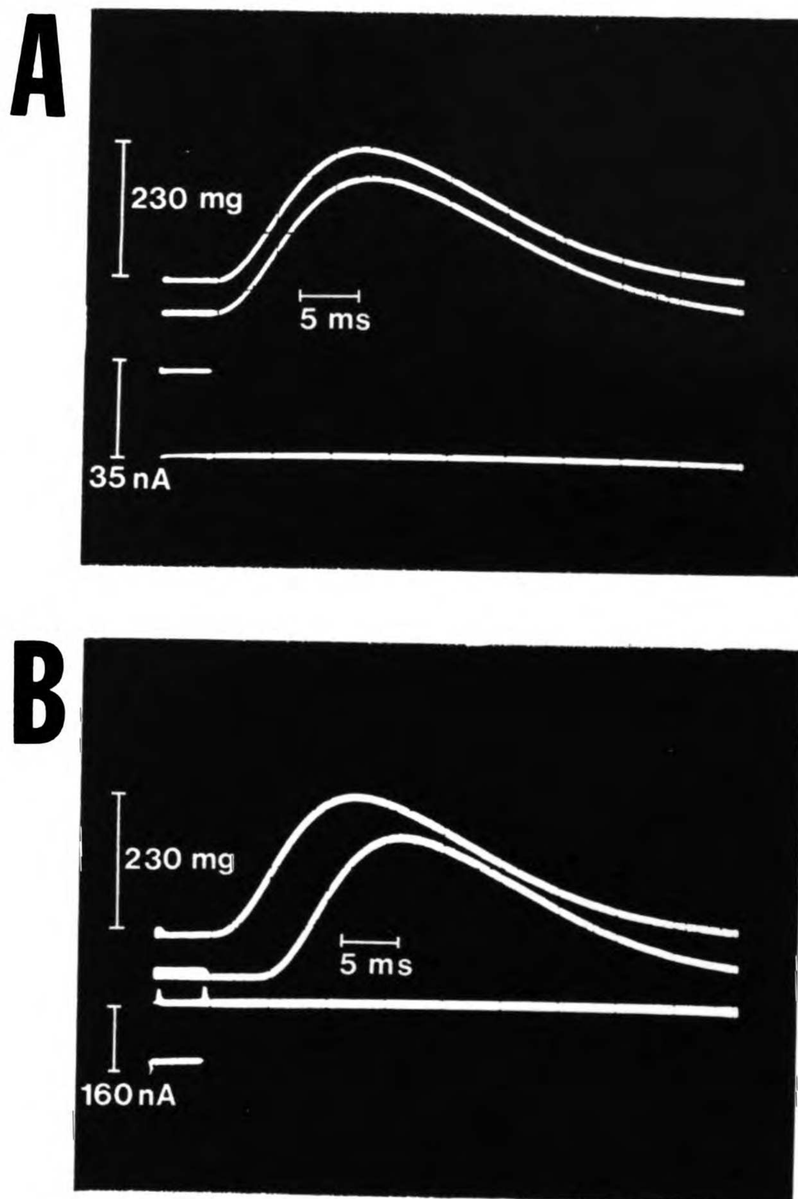

FIGURE 27

A. Quasi-intraaxonal anodal stimulation. Two current pulses equal in magnitude of .45 msec and 4.5 msec duration (lower trace) were used to evoke twitches (upper 2 traces) with identical latencies from the makes of the stimuli. B. Two cathodal current pulses of .45 msec and 4.5 msec durations (lower trace) evoked twitches (upper and middle traces, respectively) with identical latencies from the breaks of the stimuli. The duration of the stimulus used to evoke each twitch is shown on each twitch record. The magnitudes of the stimuli used in A and B were set at threshold levels for a .45 msec duration stimulus.

Chapter 3: THE CONTRACTILE PROPERTIES OF SINGLE TA MOTOR UNITS

I. Protocol

Upon penetrating a TA axon in the recurrent laryngeal nerve with a micropipette, the protocol shown in Figure 28 was used to investigate various characteristics of the motor unit. The conduction velocity (A) of the axon was first determined by observing the conduction latency of an orthodromic spike from its site of initiation at the vagus electrodes to its site of recording at the micropipette tip (see Figure 24).

With injection of square-wave current pulses through the micropipette into the axon, while monitoring tension development of its muscle fibers, the contractile properties of the motor unit were next determined (Figure 28B). Injection of a single current pulse resulted in an unpotentiated twitch response of the motor unit's muscle fibers. The contraction speed (B1) and size (B2) of the motor unit was obtained from knowledge of the contraction time (latency to peak) and magnitude of this twitch response. MU size was expressed as percent of whole muscle twitch tension, the latter evoked by stimulation of the vagus or recurrent laryngeal nerve. Since fast and slow limb motor units usually exhibit considerable overlap in their contraction speeds and it was anticipated that both

these types would be encountered in these studies, the "sag" test described by Burke, 1971, was adopted to better distinguish between these two types of motor units (B3). The tendency for motor units to exhibit a decline or sag in their plateau tensions when undergoing tetani, unfused in each instance to the same degree, was ascertained. More specifically, each motor unit was stimulated at a frequency corresponding to an interpulse interval of 125% of its contraction time to assure that each motor unit underwent a similar tetanus. Stimulus trains of the determined frequency were repeated at durations of 200 msec, 500 msec, 1 second, 3 seconds, 5 seconds, 7 seconds, and 10 seconds, with rest periods between each train. The fatigue resistance of the motor unit was finally determined by stimulation of its axon for prolonged periods of time, in some instances as long as 2 hours (B4). Two types of stimuli were used: either 50 Hz, 333 msec in duration, repeated every second, or 10 Hz continuously. Higher frequencies were also used on some occasions. Both the tetanus/twitch ratio and the frequency response of each motor unit were also determined but will not be described.

After the contractile properties of the motor unit were determined, the extent to which it participated in gag and expiratory contractions of the TA muscle was studied by recording spike activity within its axon when these two types of contractile functions were initiated (Figure 28C).

Gag contractions of the TA muscle were initiated by stimulation of the sensory branch of the internal laryngeal nerve. Stimulation of the mucosal afferents in this nerve provided for fine control over the intensity of afferent input to the brainstem and thus the magnitude of the reflex gag contraction. Repeated stimulation of the nerve also served as an arousal stimulus to the animal to induce expiratory contractions of the TA muscle, presumed preparatory to vocalization. The participation of motor units in these two types of functions will be described in the following chapter.

In some instances two further steps were added to the protocol for further characterization of TA motor units. If a micropipette was filled with HRP, the axon of the motor unit being studied could be iontophoresed and its cell body backfilled with HRP in the brainstem for later identification (Figure 28D). In cases when this step was added, TA axons were penetrated in the vagus nerve two to three centimeters from its exit from the skull to shorten the distance required for HRP diffusion (i.e. HRP is probably not incorporated into the axoplasmic transport system when injected intraaxonally). In a few experiments, attempts were also made to determine the muscle fiber histochemistry of motor units studied (Figure 28E). With prolonged stimulation of a motor unit, glycogen contained in its muscle fibers could be depleted as described by Edstrom

and Kugelberg, 1968. With appropriate treatment and sectioning of the TA muscle at the conclusion of an experiment, the motor unit's muscle fibers, having been depleted of glycogen, could be identified in cross section using the PAS method. In adjacent cross sections, these same muscle fibers could also be recognized and stained for various metabolites including myofibrillar ATPase, glycolytic enzymes, and oxidative enzymes. In this manner the muscle fibers could be "typed" according to their histochemical profile and correlated with their previously observed contractile properties. Insufficient data has been accumulated with respect to these last two steps, so only the first three characteristics of motor units will be described in this and the following chapter.

II. Results

In a total of 12 cats, 120 TA motor units were studied. Not unexpectedly, all motor units were observed to be of one of two types: fast-red or slow. The contractile properties and functions of two motor units (TA54 and TA55 from the same experiment) representative of these two types will be described in detail in this chapter and the next, along with their respective population characteristics where warranted.

A. Conduction velocities and twitch contractions

The fast contracting unit in Figure 29B (TA54, middle trace) had a contraction speed approximately five times faster than that of the slow unit illustrated in Figure 29E (TA55, middle trace). Fast units often had larger twitch tensions (i.e. were larger motor units) than slow units, as was the case for these two units. However, many small fast motor units and several large slow motor units were observed. The conduction velocities (and axon diameters) of fast units were almost always greater than those of slow units. The fast unit in Figure 29A had a conduction latency of only 2 msec, while the slow unit had a conduction latency of 4 msec (Figure 29D).

B. Sag

The tendency of the two units to exhibit sag when undergoing unfused tetani are shown in Figure 30 (fast unit on the left, slow unit on the right). The fast unit displayed considerable sag, particularly with increases in duration of unfused tetani (from top to bottom), while the slow unit displayed no sag, even for long duration tetani as in I.

It can be noticed in Figure 30B,C, and D that there was a decrease in the EMG response (top traces) associated with the sudden change in tension at the intial peak and its decay to a gradual sloping plateau (see D). There was also a slight gradual decrease in the EMG with the plateau

decrease. The decrease in the EMG level apparently resulted from failure of activation of some of the motor unit's muscle fibers because of a reduction in epp size to subthreshold levels in these fibers. Successive decreases in epp magnitude are commonly observed at <u>normal</u> amphibian and mammalian neuromuscular junctions during repetitive stimulation, and are attributed to decreases in the quantal content (i.e. number of quanta) of released transmitter from presynaptic terminals. This is known as depression and is believed to arise because of partial depletion and thus decreased availability of presynaptic transmitter (Del Castillo and Katz, 1954a,b; Thies, 1965). No change in postsynaptic ACh sensitivity occurs, so that the epp depression arises strictly from presynaptic decrease in vesicle availability. If depletion is not allowed, facilitation or a successive increase in quantal release and epp magnitude during repetitive stimulation can be observed. For example, if the frequency of stimulation used is low, facilitation will be unmasked because of the absence of transmitter depletion and depression. Facilitation has been commonly described and studied in low calcium and/or high magnesium preparations, where depletion is prevented by partially blocking transmitter release (Magleby, 1973a,b). Even with the high frequency of stimulation of fast units to study their sag characteristics, facilitation was often observed before partial transmitter depletion and depression

set in. For example, unlike TA54 and the fast unit shown in Figure 31A where EMG potentials began diminishing immediately, the fast unit shown in Figure 31B exhibited an initial increase in EMG magnitude or facilitation with the onset of tension before depression ensued.

All of the fast units studied had some depression (EMG decline) associated with the decline in tension following the intial peak and the more gradual subsequent decline of plateau tension, as shown in Figure 31A. However, in all cases the most significant decrease in EMG magnitude occurred during the rise in tension to the initial peak with only a slight EMG decrease with the subsequent sag (see Figures 30D and 31A). Sag, therefore, may only partially reflect depression of synaptic transmission, with a more significant contribution arising from decreases in the active states of fast muscle fibers themselves. Furthermore, Burke observed no synaptic component to the sag of fast limb motor units and attributed sag entirely to decreases in their muscle fiber active states.

It can be noticed in Figure 30D that the initial EMG response and tension peak and their subsequent decay were more pronounced in comparison to the shorter duration tetani in the previous frames. Since tetanus duration has no effect on EMG and tension onset, there must have been another explanation for the increased initial responses. Fast units, in general, varied in a consistent manner in

their initial EMG and tension responses with variation in the recovery time preceding a tetanus. Only 10 seconds recovery time from previous sags was allowed for the three shorter duration tetani in Figure 30A,B, and C, while 30 seconds was allowed for the longer duration tetanus in D. The relationship between recovery time and peak tension and sag is more clearly demonstrated in Figure 32A,B. If sufficient time was allotted for recovery from a previous sag, a tetanus resulted (arrow) with greater peak tension and sag (compare this tetanus with one at arrowhead where recovery time was insufficient). Between 15 and 20 seconds were usually required for full recovery of peak tension. A similar time course for recovery of epp size following transmitter depletion and depression has been demonstrated (Thies, 1965, Figure 6), supporting the notion that peak tension and sag of fast units in these studies may have been in part a reflection of presynaptic transmitter availability.

In Figure 33 it can be seen that TA motor units were quite clearly distinguished on the basis of their tendencies to exhibit sag. All fast units (open symbols) displayed pronounced sag, while slow units (closed symbols) did not. Although sufficient time was allotted for complete recovery and peaking of these units, fast motor units could still be distinguished from slow units by their presence of sag with shorter rest times, although less definitively. Slow units

exhibited no initial peaking or sag regardless of the recovery time, excepting two motor units whose tetani have been added to the diagram in Figure 34 (closed stars). Although these two motor units exhibited initial peaking, they were distinguished from fast units and similar to slow units with respect to their retention of plateau tension following peak decline. It was interesting to note that these two units were more than twice the size of any other slow unit except one (inverted triangles), so that MU size may effect the susceptibility of slow units to experience initial peaking and decline with sag. Fast units, as well, appeared to experience greater peak declines the larger their MU size, as shown in Figure 35. The percentage of peak tension of fast motor unit tetani is plotted as a function of their MU size at 4 time periods following the stimulus onset. At .5 seconds, declines from peak tension became apparent for all motor units, but the declines were more significant for the larger motor units. If peak declines occurred, in part, because of synaptic depression, possibly the terminals of larger motor units were more susceptible to transmitter depletion during stimulation than those of smaller units. The greater peak declines (and greater depression) of larger motor units could not have arisen from stimulation at higher frequencies, since they were not necessarily stimulated at higher frequencies, (i.e.

larger fast motor units were not necessarily faster contracting (see Figure 35 legend and Figure 39).

C. Fatigue.

The fatigue characteristics of TA motor units when stimulated with the 50 Hz pattern are demonstrated by using the fast and slow units as examples. Since the fast unit's fusion frequency was higher than that of the slow unit, its tetanus was unfused and smaller in magnitude (Figure 36A) than that of the slow unit (Figure 36C), despite its larger MU size. The two units differed in their fatigue profiles as shown in B and D. The fast unit (B) showed a gradual rise in tension or potentiation over the first 15 seconds of fatigue, while the slow unit (D) showed an initial slight tension decrease with no subsequent potentiation. Since there were no apparent EMG changes, the potentiation was probably not effected through modulation of synaptic transmission but rather through changes in the active state of muscle fibers themselves, as discussed by Close, 1972 in describing post-tetanic potentiation (PTP)*.

It is not certain whether the quick initial tension decline of the slow unit was also due to a changes in muscle

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*It is unfortunate that the term PTP is used to describe changes in muscle fiber contractile characteristics following repetitive stimulation, when it was originally intended to signify presynaptic modulation enhancing transmitter release (and by a different mechanism than facilitation: see Granit, 1956b and Magleby, 1973a,b).

fiber active state, since EMG responses of this unit were almost undetectable. In any case, fast units could exhibit quite pronounced initial tension decreases as well, when fatigued at higher stimulus frequencies, as demonstrated by TA54 in Figure 37A,B. After a minute's rest, raising the stimulus frequency to 75 Hz resulted in a quick drop in tension (middle frame, Figure 37A). After another minute's rest, stimulation at even higher frequency (80 Hz, right frame, Figure 37A) resulted in a more pronounced initial drop, and with continued stimulation, a slight gradual potentiation as shown in Figure 37B. The initial tension drop shown here during fatigue and the peak decline observed during sag, at even higher stimulus frequency, probably have a common basis. It is not certain whether the associated EMG decreases in each case can sufficiently account for the tension declines observed.

Similar fatigue profiles to those shown for each type of unit have been observed for fast and slow limb motor units (Close, 1972; Burke, Levine, Tsairis, and Zajac, 1973). Fast TA motor units, however, appeared to be more fatigue resistant than typical fast-red limb motor units. As shown in Figure 36E, the fast unit maintained tension during prolonged stimulation just as easily as the slow unit shown in Figure 36F.

Since failure of synaptic transmission can occur at stimulus frequencies of greater than 10/second (Krnjevic and

Miledi, 1958), the fatigue characteristics of most motor units were studied at the lower stimulus frequency of 10/second (continously). The fatigue profiles of 4 fast units stimulated at this frequency are shown in Figure 38. There was in every case a more gradual rise, over the first 45 seconds, to a relatively larger potentiated tension than was observed during 50 Hz stimulation. Again the units displayed considerable fatigue resistance, with maintenance of twitch tensions after an hour's stimulation above their initial tensions but below their peak potentiated tensions. In Figure 38B* the potentiation of individual twitches during the first 45 seconds can be appreciated. It is apparent that there was no change in the contraction speed with the increase in twitch tension. However, there was a slight reduction in twitch relaxation time during the first few seconds of fatigue. The twitches of every other unit displayed in this manner showed indications of similar changes over the course of potentiation.

III. Discussion.

As shown in Figure 39, a large majority of the motor units studied were fast-red. Using sag as a criterium for distinguishing the two types, 79% were fast-red, the slowest contracting having a contraction time of 12.5 msec (rightmost arrow), while 21% were slow with the fastest contracting having a contraction time of 9 msec (leftmost arrow). Thus, there was some overlap in the contraction times of these two populations.

As was established by Eccles (1958) for motor units of whole limb muscles and by Burke (1967), Wuerker et al. (1965), and McPhedran et al. (1965) for individual limb motor units, fast TA motor units had faster conducting, larger axons than slow units (refer to Figure 6). The average conduction velocity for fast units was 64 m/s, while the average for slow units was only 45.6 m/s (The average for each contraction time is shown as filled squares in the middle graph of Figure 39).

The proportion of fast motor units observed physiologically was similar to the proportion of type IIA muscle fibers (90%) observed histochemically, providing indirect evidence for the notion that fast TA motoneurons innervated muscle fibers high in myofibrillar ATPase (refer to Figure 6). The small discrepancy could be attributed to a statistical bias favoring slow motor units in the sample

of motor units studied. Another possible explanation for the discrepancy might be that fast motoneurons tended to innervate more muscle fibers, i.e. were slightly larger motor units on the average than slow motor units.

Looking at the distribution of sizes of fast and slow motor units in the upper graph of Figure 39, fast units were apparently undistinguished from slow units in their motor units sizes, in contradistinction to fast-red and slow limb motor units which are intermediate - large and small in size, respectively (refer to Figure 6 and burke et al., 1973, Figure 5). It must be acknowledged, however, that fast and slow motor unit initial twitch tensions may not have necessarily provided an accurate estimate of their respective motor unit sizes, particularly if they were in different states of potentiation during their twitches. As with fast limb motor units, fast TA units were unpotentiated without immediate prior activation and exhibited two fold increases in twitch tension, on the average, with repeated stimulation, as shown by the examples in Figure 38. Slow units, on the other hand, may have been in a state of greater potentiation initially, since they exhibited less potentiation of tetani at repeated 50 Hz stimulation as mentioned. Unfortunately, no data is available on the potentiation of their twitch responses. There is some disagreement on the extent to which slow limb motor units exhibit potentiation of their twitch responses. Burke et

al. (1973, Figure 4) and Edstrom and Kugelberg (1968, Figure 6) found little differences in the potentiation of fast and slow unit twitches while Close (1972) suggested that slow motor units are in a higher state of potentiation at rest and therefore exhibit considerably less further potentiation with repeated stimulation. Even if it is assumed that fast motor unit sizes were underestimated by half (relative to slow unit sizes) many of the slow units observed were unusually large.

Slow units were also unusual in that they had just detectable or undetectable EMG responses, irrespective of their motor unit size, as shown in Figure 40. It is possible that their muscle fibers didn't conduct action potentials, suggesting that they might be similar to non-twitch, slow, tonic type muscle fibers found in amphibian muscles and mammalian extraocular muscles (Hess and Pilar, 1963). However, they did twitch. Furthermore, tonic contractions of the cat TA muscle are not observed following intravenous injections of decamethonium, which elicits tonic contractions of non-twitch slow muscle fibers (Edstrom, Linquist, and Martensson, 1974). Another possibility is that the muscle fibers of these slow motor units were all contained within the vocalis muscle, the medial division of the TA muscle, and were considerably removed from the EMG electrodes positioned on the lateral aspect of the TA muscle. Although no systematic investigation of the whole

TA muscle including its medial division has been made by this investigator, it stands to reason that the vocalis muscle, the tensing portion of the TA muscle, would contain a higher percentage of slow fibers than the lateral division, the adducting portion. This might partially account for the large proportion of type II fibers observed histochemically.

The larger slow motor units appeared to be unusual in a third respect, as shown in Firuge 41. Although small slow units (filled circles and possibly open squares) and fast units (open Figures) exhibited a loose correlation between MU size and conduction velocity like limb motor units (refer to Figure 6 and Burke, 1967, Figure 8), the larger slow motor units did not fit in this correlation. They appeared to have greater twitch tensions for the size of their axons in comparison to fast and smaller slow units. Although more data on slow motor units is required, it might be suggested that slow units are actually comprised of two subpopulations of large and small motor units, based on the observations that: 1) the larger slow motor units were quite large in comparision to fast motor units and there was a paucity of intermediate sized slow units, as shown in Figure 39. 2) the EMG responses of large slow units were no larger than those of small slow units (Figure 40). 3) The larger units did not fit in the apparent correlation between C.V. and MU size observed for small slow units and fast units.

Fast-red motor units were more conventional than slow units in their contractile properties. However, they did exhibit considerable fatigue resistance and sag in comparison to most fast-red limb units. Although the underlying mechanism involved in sag in unclear, the prominent sag of fast TA units may have been related to their tendencies to exhibit synaptic depression, as well as their tendencies to exhibit muscle fiber active state decreases like fast limb motor units. Because they were faster contracting, they were stimulated at higher frequencies than fast limb motor units and may have experienced partial transmitter depletion particularly during the initial peak declines of sag. They may also have been more susceptible to transmitter depletion, because of smaller terminals and less available pool of releaseable vesicles. There are several indications that this might be the case: First, there is considerable peripheral branching of parent axons within the RLN, as mentioned previously, providing a possible basis for fast axons being "spread thin" before they reach their muscle fibers. Second, it might be expected that the larger fast units, which displayed greater peak declines during sag, had smaller axon terminals. A relationship between peak decline and terminal size is more clearly demonstrated in Figure 42, in which the extent of branching of a parent axon of a given size is expressed as the ratio of MU size to conduction velocity and

plotted as a function of peak decline. Note that larger motor units with parent axons of a given size experienced greater peak declines. Third, multiple innervation of most of the muscle fibers in the medial portion of the TA muscle has been demonstrated in man (Rossi and Cortesina, 1965) and probably also occurs in the cat, so that further dividing of fast parent axons may be present for the establishment of redundant synapses.

Although explicit tests for multiple and polyneuronal innervation of muscle fibers were not performed in these studies, there was an indication that the muscle fibers of a few fast motor units were multiply innervated. During the course of 50 Hz stimulation to test fatigue resistance, a few fast motor units showed repeated partial drop outs of their EMG responses near the ends of tetani, without any drops in tetanus tension, suggesting that some redundant synapses had become depressed. It might be wondered if there is any functional advantage to multiple innervation of fast motor unit muscle fibers, particularly if it compounds the problem of synaptic depression. However, multiple innervation probably minimizes the time required for excitation-contraction coupling (see Hunt and Kuffler, 1954), a considerable teleological advantage to these units in mediating quick gag contractions. Furthermore, even with phasic spiking of fast motoneurons at rates as high as 350-450/second (see next chapter) during

the gag reflex, the activity is so shortlived that depression of their synapses probably does not occur. If fast motoneurons are also involved in tonic (expiratory) activity, maximum axon spiking frequencies observed for this function are considerably less (35/second), so again synaptic depression is probably avoided.

PROTOCOL

A. Conduction Velocity

B. Contractile Properties

  1. Twitch Contraction Time
  2. Twitch Tension--MU Size
  3. Sag
  4. Fatigue Resistance

C. Function--Gag versus Expiratory Contractions

D. Cell Body Location--HRP Iontophoresis

E. Muscle Fiber Histochemistry--Glycogen Depletion

FIGURE 28

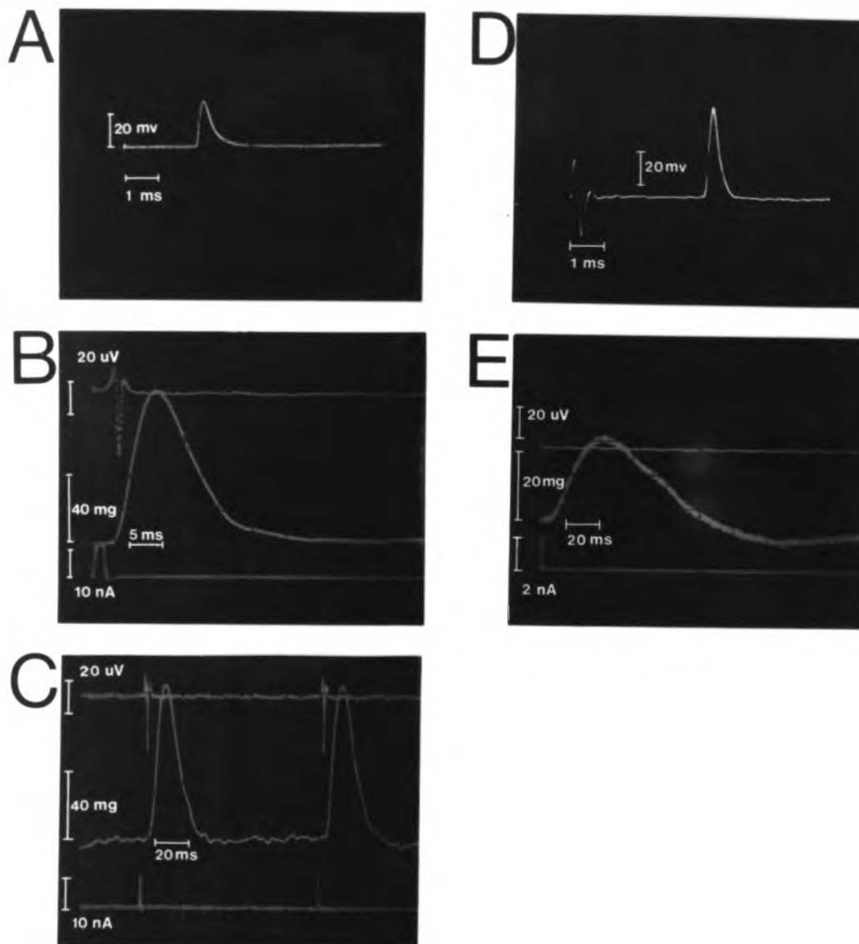

FIGURE 29

Conduction latencies of fast (A) and slow (D) unit axons and twitch contractions of fast (middle traces, B and C) and slow (middle trace, E) units with current injection (lower traces in B,C, and E) into their respective axons. EMG responses are shown in the upper traces of B,C, and E. Instantaneous current was monitored in these studies by observing the voltage drop across a known resistance in series with the micropipette. Contraction time of the fast unit was 6 msec, while that of the slow unit was 34 msec. The differences in their twitch contraction times can be appreciated at the same sweep speed, as shown in C and E.

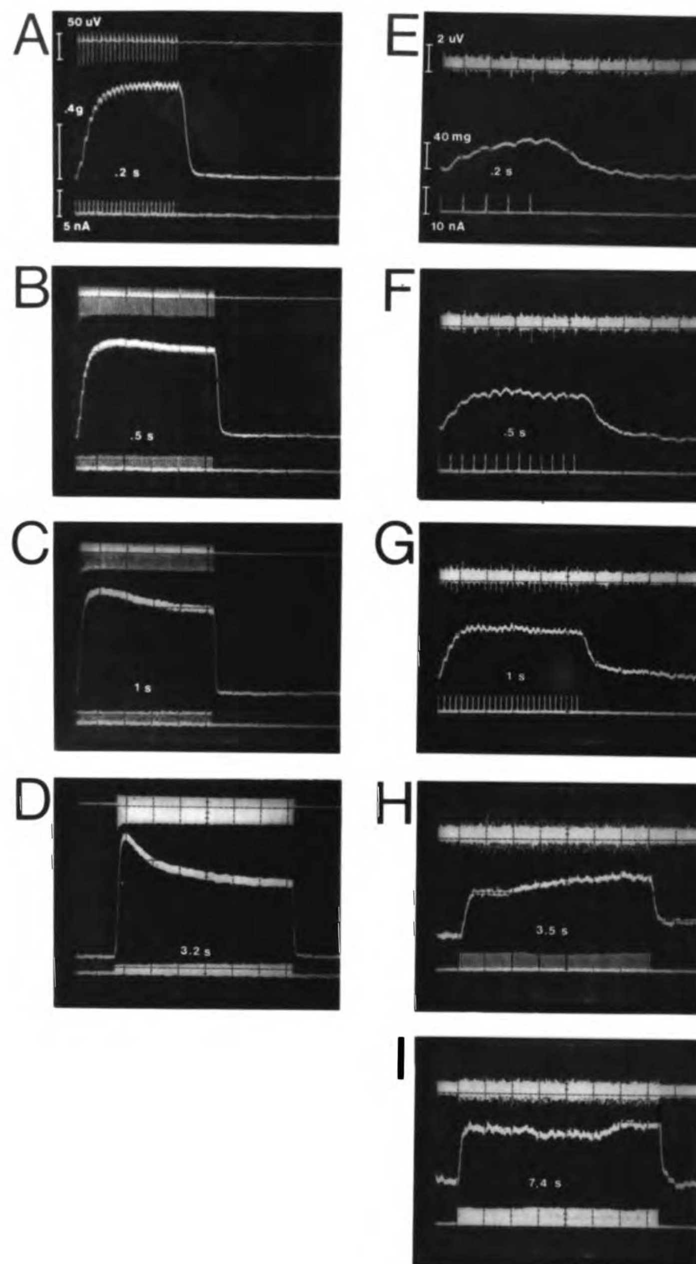

FIGURE 30

Sag characteristics of the fast (A,B,C, and D) and slow (E,F,G,H, and I) unit with increasing duration of unfused tetani (from top to bottom). The duration of tetani are indicated in each frame. EMG top traces and current bottom traces.

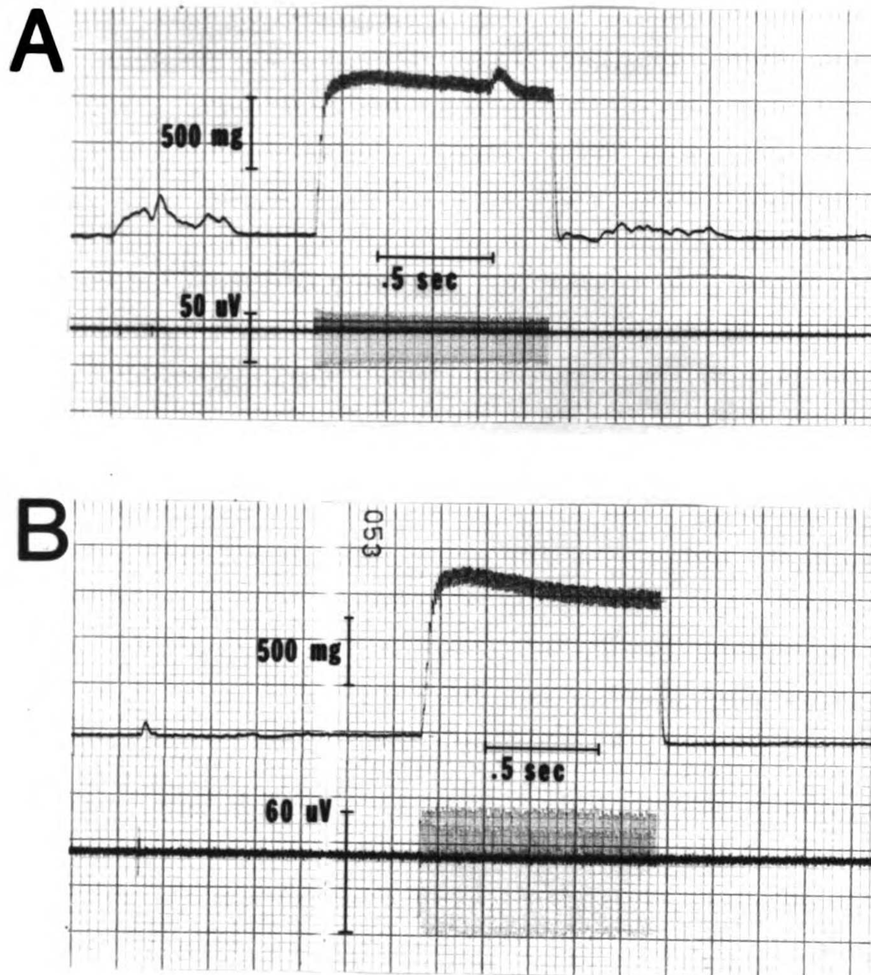

FIGURE 31

Sag characteristics of two other fast units (TA94 in A, and TA93 in B) when undergoing unfused tetani 1 second in duration. Note the differences in their EMG responses (lower traces). TA94 exhibits only a decrease while TA93 exhibits an initial rise (facilitation) before a slight decrease with sag. Notice also the spontaneous contractile activity, particularly in A, most of which has no detectable EMG response associated.

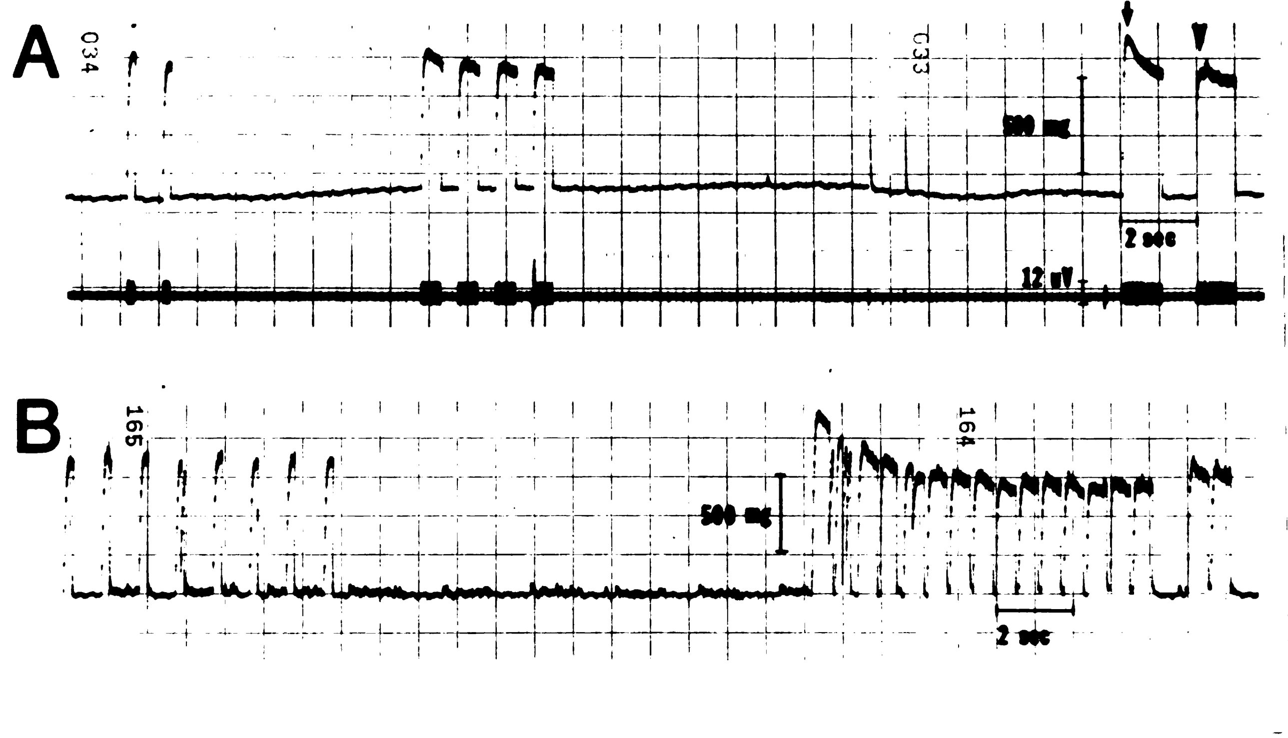

FIGURE 32

Variation in the sags of two fast units (TA75 in A, and TA50 in B) with variation of the recovery time preceding their unfused tetani. Fifteen seconds recovery time was allowed before the unfused tetanus at arrow, while only one second was allowed before the tetanus at arrowhead in A. EMG response are shown in lower trace of A.

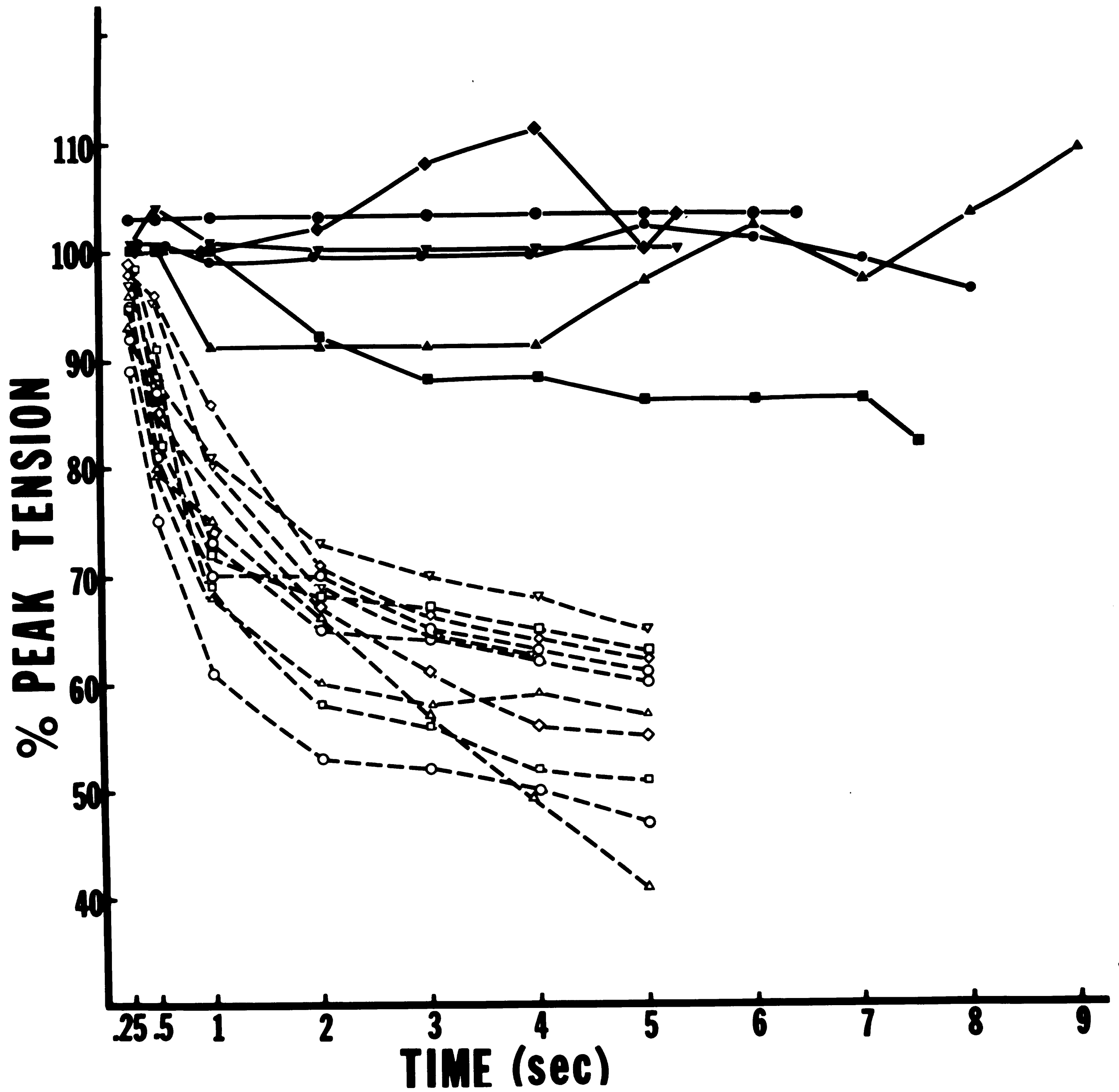

FIGURE 33

FIGURE 33

Sag properties of fast (open symbols) and slow (closed symbols) units with each unit's tension normalized to its maximum tension developed during the first 250 msec. Fast units are clearly distinguished from slow units by their pronounced sag, in part because of initial tension peaking. Recovery time preceding each unit's unfused tetanus was 45 seconds to 1 minute. Peaking and sag of fast units could be due to recovery of partially depleted transmitter followed by depression, or post-tetanic potentiation followed by muscle fiber active state decreases. See text for further details.

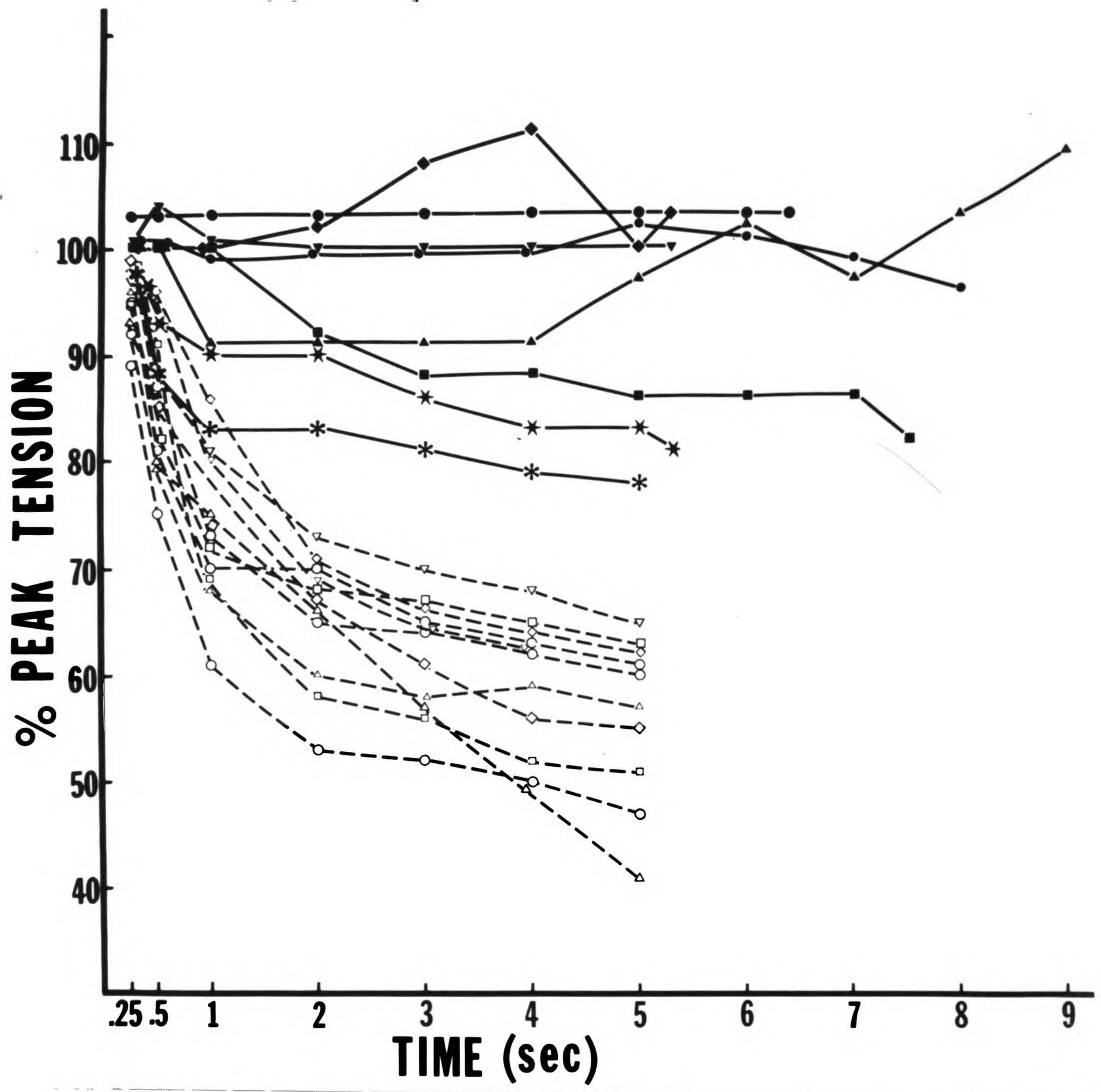

FIGURE 34

FIGURE 34

The tetani of two slow units (closed stars) have been added to the diagram from the previous figure to show their initial tension peaking and decline. Since their initial peaking was less pronounced than fast units, they were more like slow units in retention of their plateau tensions. The diagram includes all of the slow units whose sag properties were studied. Three were large units, the two which exhibited peaking and a third which did not (closed inverted triangles). The diagram includes approximately half of the fast units whose sag properties were studied. Those not included in the diagram displayed sag within the range shown.

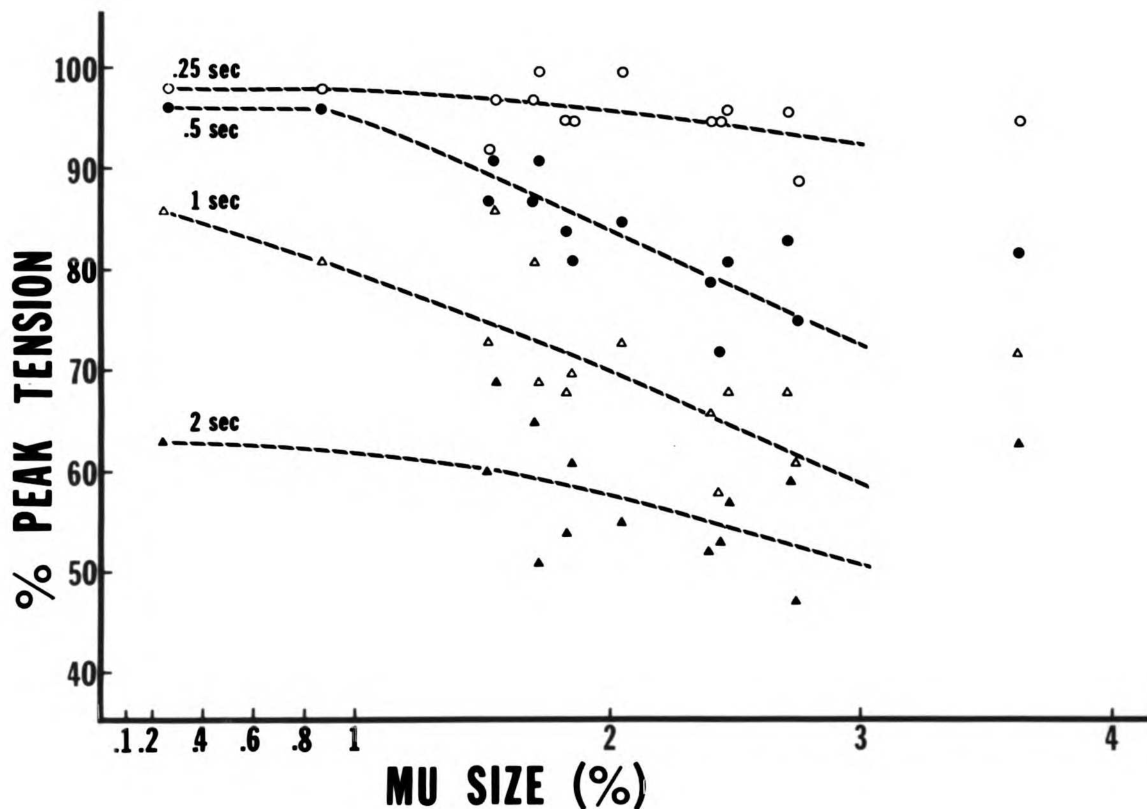

FIGURE 35

Normalized tension of fast units from figure 34 are plotted as a function of their motor unit sizes, at 4 different time periods following the stimulus onset. The larger motor units exhibited greater initial tension peaking and decline, which is particularly noticeable at .5 and 1 second. Larger motor units were not necessarily stimulated at higher frequencies. Stimulus frequencies were set at 125% of their contraction time and contraction times from the smallest to the largest motor unit (from left to right) were 5.0, 7.5, 7.0, 5.5, 6.5, 7.5, 6.0, 7.5, 6.5, 6.0, 6.0, 6.5, 7.0, 6.2, and 6.0 msec. The largest motor unit deviated considerably from the other units in this relationship and was not used in the establishment of best fit lines (by eye).

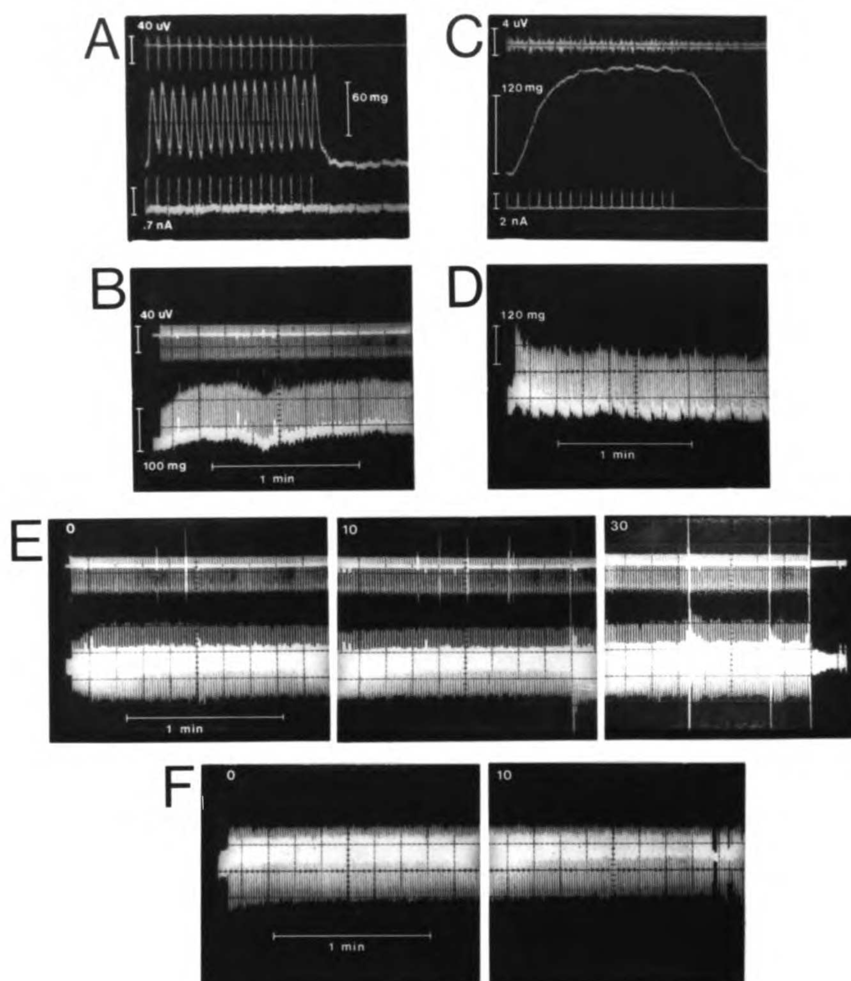

FIGURE 36

The fatigue characteristics of the fast (A,B, and E) and the slow (C,D, and F) units when stimulated with the 50 Hz pattern. EMG responses are shown in the upper traces of A, B,C, and E. Current is shown in the lower traces of A and C. The tetani were 333 msec in duration, one of which is shown at fast sweep speed for each unit in A and C. Tension was filtered in E and F, with a lower cut-off frequency of 1 Hz, to better appreciate the fatigue resistance of each unit. Samples of tension development are shown at 10 minutes (second frames in E and F) and at 30 minutes for the fast unit.

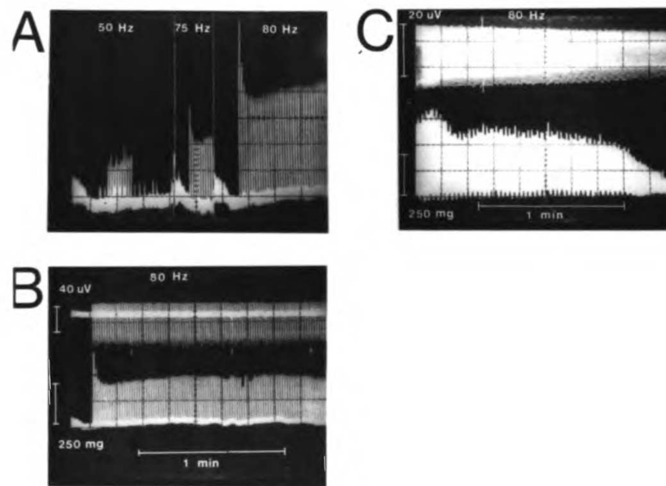

FIGURE 37

The fatigue profiles of the fast unit when stimulated at higher frequencies are shown in A and B. With stimulation at 50 Hz, the unit displayed a gradual potentiation (left-most section in A) as before. With stimulation at 75 Hz (middle section), an intitial tension drop occurred and with stimulation at even higher frequency (80 Hz, right-most section) the drop became quite pronounced. A gradual potentiation still occurred following the initial drop as shown in B with continued stimulation.
In C the only unit that exhibited fatigue is shown. Both synaptic fatigue, as shown by the EMG decrease, and muscle fiber fatigue occurred at this high stimulus frequency.

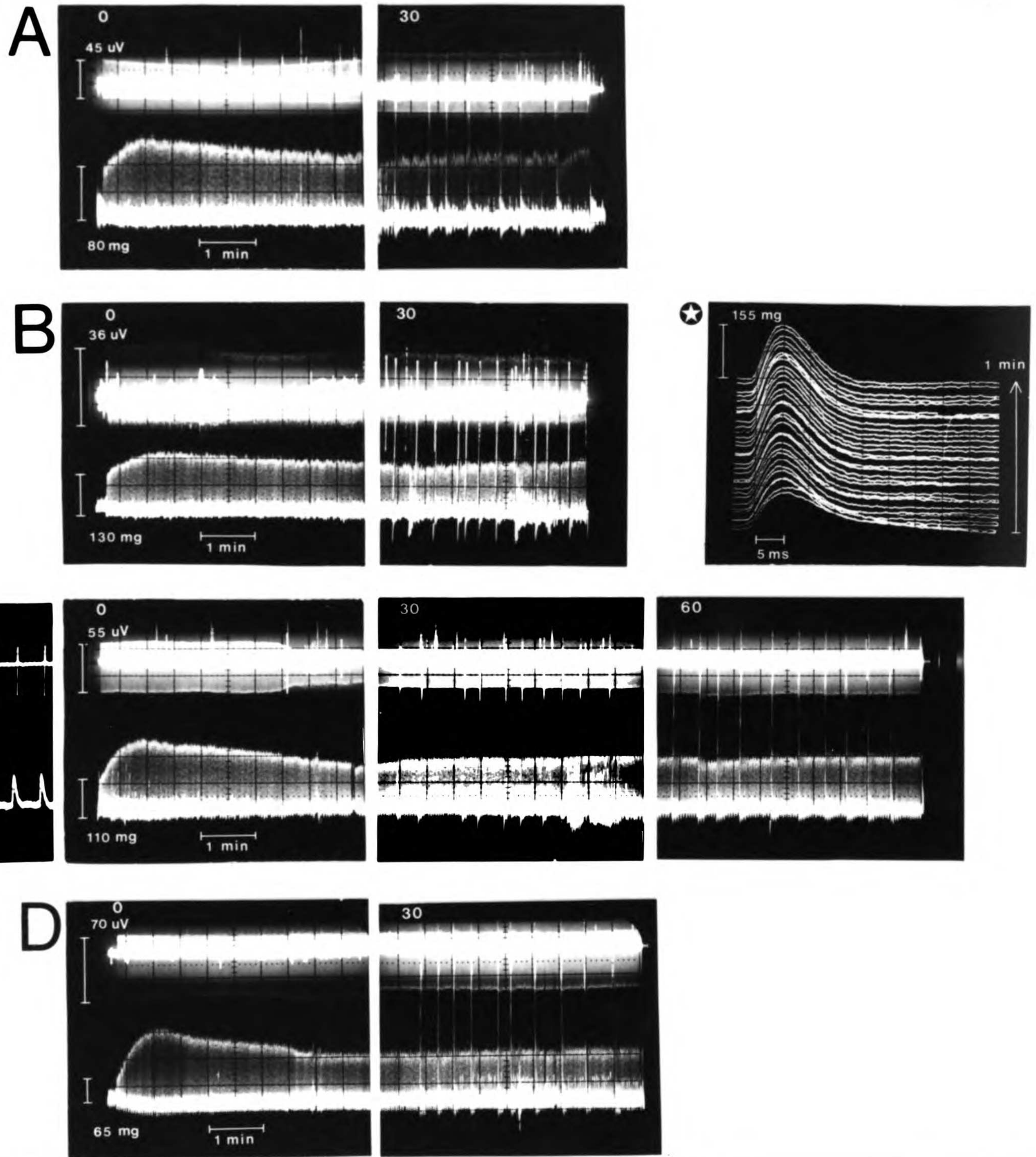

FIGURE 38

FIGURE 38

The fatigue profiles of 4 fast units stimulated at 10/second continuously are demonstrated. EMG responses are in upper traces; filtered tension, with lower cut-off frequency of 1 Hz, in lower traces. Cut-off frequencies of filters were always set so that no modification of waveforms resulted. A gradual potentiation of twitch tension occurred for each unit as shown, reaching a maximum in approximately 45 seconds. EMG responses often decreased following potentiation within the first 5 minutes, presumably because of partial synaptic depression. Changes in the individual twitch responses over the course of potentiation can be appreciated in B* for the unit in B. Twitch contraction times were unaffected by the potentiation, but a slight initial decrease in relaxation time was observed. The individual twitch responses of all units displayed in this manner exhibited similar changes. All of the units studied were extremely fatigue resistant as shown by these examples, with retention of twitch tensions after 30 minutes of stimulation (second frames) and after 1 hour for the unit in C. A fast sweep of the individual twitch and EMG responses at the start of fatigue is shown in the leftmost insert at C. Vertical lines in second and third frames of A-D are tape recorder stops and starts.

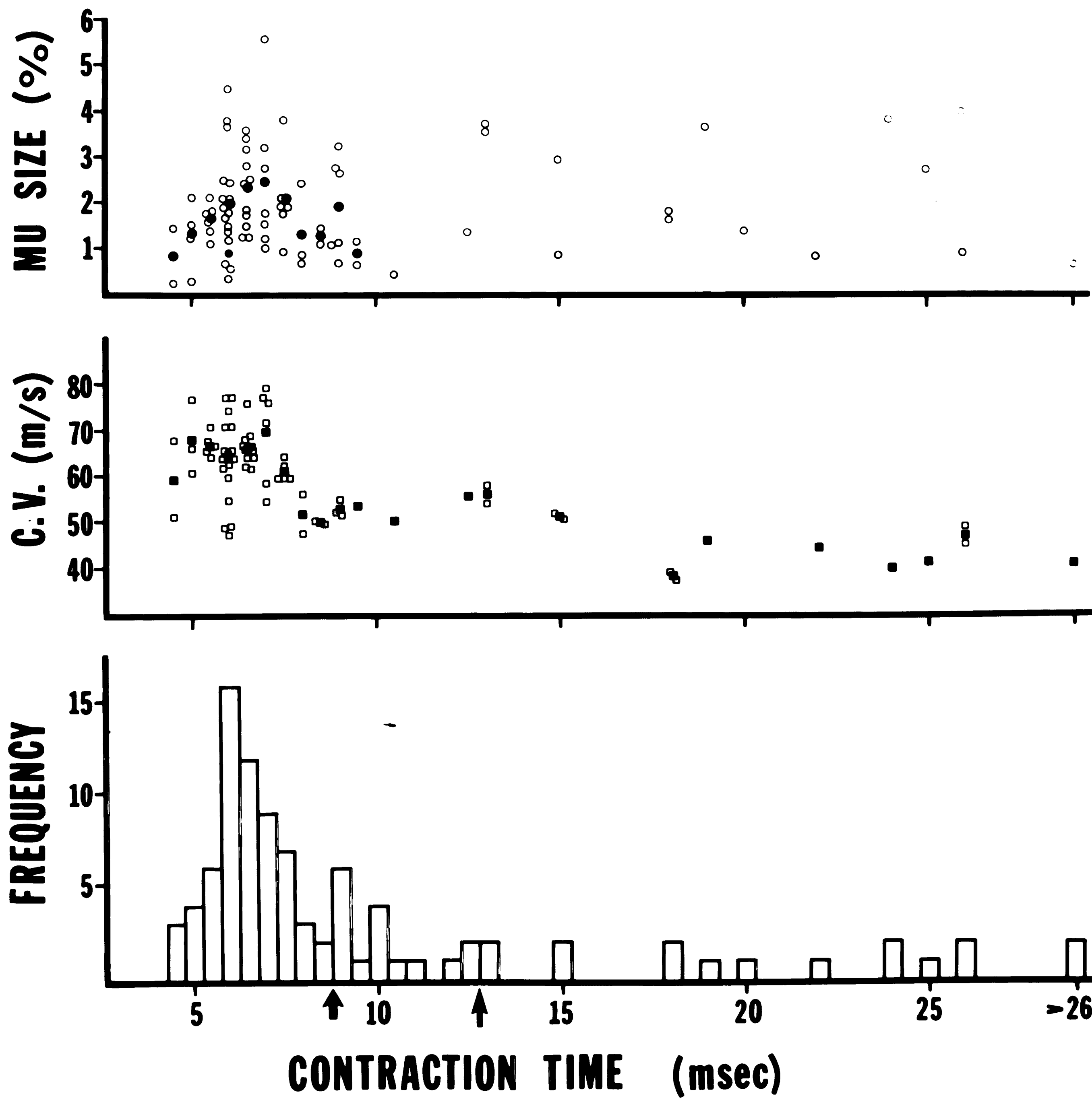

FIGURE 39

FIGURE 39

Summary diagram of the contraction times, axon conduction velocities, and sizes of the motor units studied. Since fast and slow units were clearly distinguished in their sag properties (figure 34), they can be considered as two distinct populations. Based on sag as a criterium, the fastest contracting slow unit had a contraction time of 9 msec (leftmost arrow), while the slowest contracting fast unit had a contraction time of 12.5 msec (rightmost arrow). The region between the two arrows in the figure, the overlap region, contained two fast units and one slow unit. The sag properties of the rest of the units in the region weren't studied. Thirty-five fast units and eight slow units were tested for the presence of sag. Closed symbols in the upper two graphs represent the average conduction velocity and motor unit size at a given contraction time.

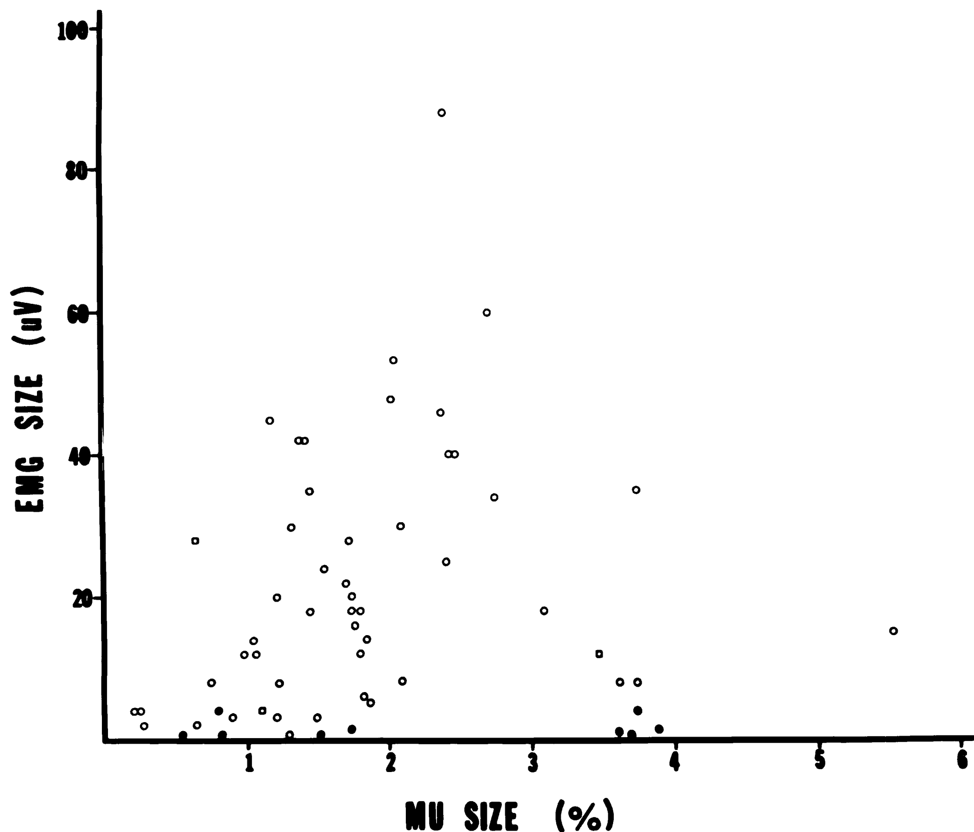

FIGURE 40

Relationship of EMG size and MU size of motor units. All of the slow units (closed circles) had quite small EMG responses, irrespective of their motor unit sizes. EMG responses of units indicated by circles touching the abscissa were undetectable. The three open squares may have been slow units as well, since they had contraction times within the overlap region and their sag properties weren't studied. There was a general trend for an increase in EMG size with increase in MU size for fast units (open circles and possibly open squares), but with wide scatter. The scatter is probably a reflection of variation of distances of muscle units from the EMG electrodes located on the lateral surface of the muscle in each experiment.

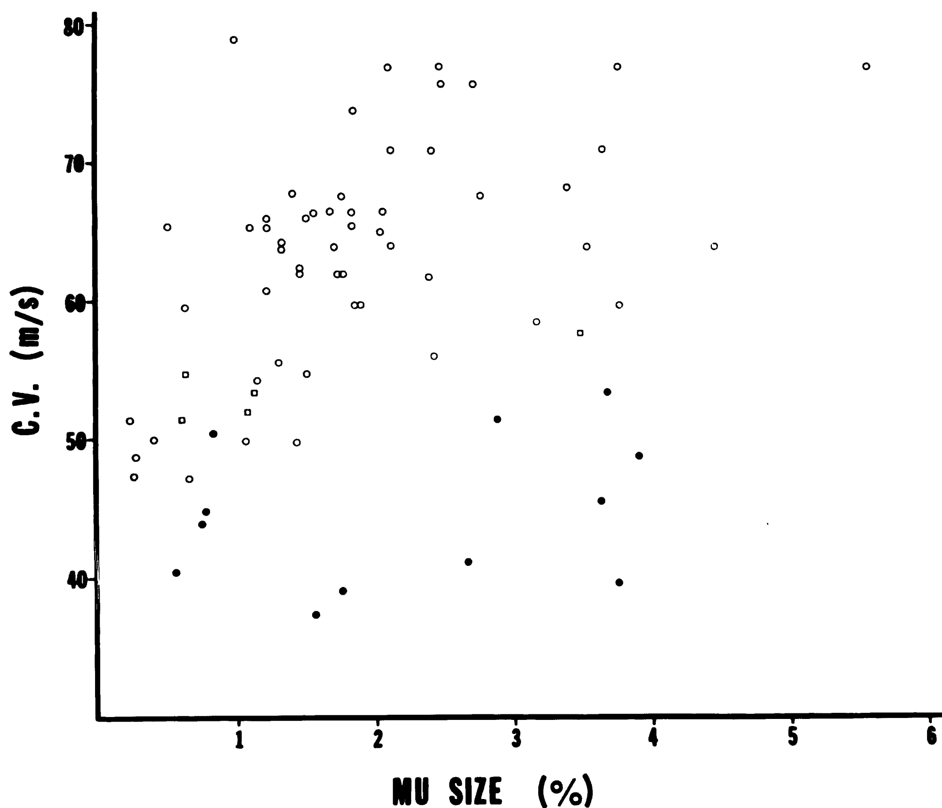

FIGURE 41

Relationship between conduction velocity and MU size of motor units. Small slow units (closed circles) and fast units (open circles) exhibited a general trend for an increase in conduction velocity with MU size. Large slow units deviated from this relationship as shown, with slower conducting axons at any given MU size. Again, open squares were units with contraction times within the overlap region whose sag properties were unknown, so they could have been either fast or slow.

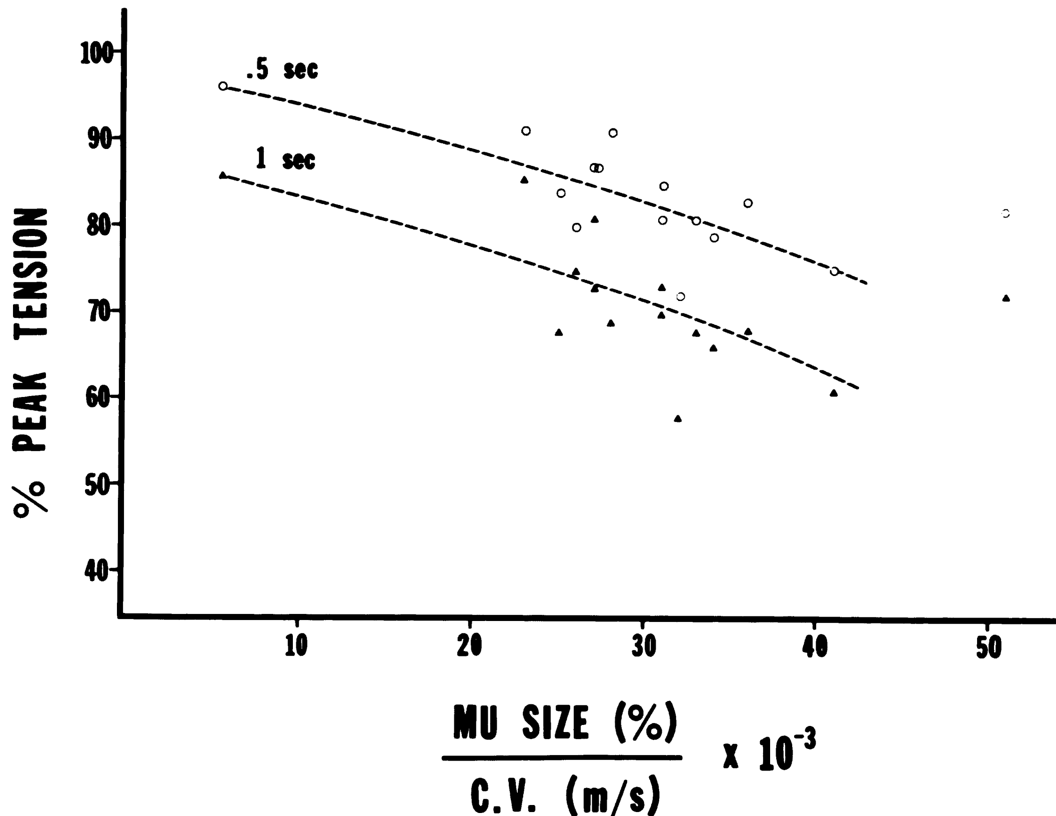

FIGURE 42

Relationship between the peak decline of fast units during sag and the relative size of their axon terminals. The values of percent peak tension at .5 and 1 second were taken from figure 33 for each fast unit and plotted against the ratio of their motor unit size to their axon conduction velocity. If it is assumed that each of the muscle fibers of fast units develop the same twitch tension (i.e. that fibers are equal in size; see figure 18), then the relative twitch tension (MU size) of these units is an indication of the number of muscle fibers they each have. If each muscle fiber is innervated by the same number of axon terminals, MU size is also an indication of the number of terminals they each have. Since conduction velocity is proportional to axon diameter (Hursh, 1939), the ratio of MU size to conduction velocity indicates the number of terminals or the extent of branching of parent axons of a given size. The greater this ratio, the more likely that axon terminals are smaller. Notice in the figure that motor units with smaller terminals exhibited greater peak declines.

Chapter 4: THE FUNCTIONS MEDIATED BY SINGLE TA MOTOR UNITS

I. Experimental Procedure:

Usually before or instead of studying a motor unit's fatigue characteristics, the spiking sensitivities of its axon were determined by recording with the micropipette during stimulation of the internal laryngeal nerve. As mentioned previously, the internal laryngeal nerve is purely a sensory nerve containing mostly mucosal afferents but also IA afferents from laryngeal muscles and proprioceptive fibers from joints. Stimulation of the nerve initiated a reflex gag contraction of the TA muscle and with repeated stimulation sufficient arousal of the animal for induction of expiratory activity of the muscle. The presence of spike activity in an axon during these two types of activities was used as an indication of the motor unit's participation in these functions. The manner in which fast and slow units were distinguished in mediating these two functions will be described by presenting details of the spiking sensitivities of the fast and slow units introduced in the previous chapter as examples, along with the functional characteristics of their respective populations where warranted.

II. Results:

A. Fast Motor Unit Functions:

When a low voltage square-wave stimulus was applied to the internal laryngeal nerve, a spike was recorded in the fast unit's axon (lower trace, Figure 43A), which was followed by an EMG response (upper trace) and gag contraction (middle trace) of the TA muscle after a short latency for conduction of spikes in the motoneuron axons mediating the reflex.* With an increase in the strength of the sensory stimulus, an increase in the number of spikes was observed, as shown in Figure 43B and C. There was also an increase in the magnitude and duration of the associated EMG responses and gag contractions of the TA muscle. Distinct peaks in the EMG response were added with the sequential increase in stimulus strength, as shown by comparing frames A, B, and C in Figure 43, suggesting that motoneurons recruited with the stimulus increase fired almost synchronously. That is, at a stimulus strength of 11 volts, some motoneurons mediating the reflex fired three times, as indicated by the three peaks in the EMG response, while the one under investigation fired only twice. The maximum number of spikes exhibited by motor unit axons with

*The peak of the gag contraction cannot be seen in this figure, nor in others, since the myograph amplifier gain was set fairly high so that small spontaneous motor unit contractions could be observed.

maximum sensory stimulation ranged between four and seven. The unit in Figure 43D showed a maximum of four spikes. There was also a decrease in the latency for the first gag spike and the interspike intervals of subsequent spikes with increase in the strength of sensory stimulation (Figure 4D). Minimum interspike intervals of fast motor units ranged between 2.2 and 2.8 msec during maximum sensory stimulation, corresponding to a maximum gag spiking rate of 350 to 450/second.

Without changes in the strength of sensory stimulation, there was also variation in the number of spikes exhibited by a fast motoneuron axon with changes in phase of respiration (Figure 44E). Three spikes of the fast axon were always evoked reflexly by maximum sensory stimulation, as shown by the repeated sweeps in the figure, but occasionally a fourth spike was added when the stimulus was applied during expiration. The excitability of the cell apparently varied with the phase of respiration, presumably because of inhibitory input from brainstem inspiratory neurons and/or excitatory input from brainstem expiratory neurons. The latency of the first spike and the interspike intervals of subsequent spikes also decreased if the sensory stimulus was applied when the motoneuron was most excitable, during the expiratory phase of respiration.

With close examination of the EMG response shown in Figure 43F and G, it can be noticed that there was a

quiescent period of approximately 40 msec following the gag activity, when maximum sensory stimulation was applied during expiration. This quiescent period, in turn, was followed by low level, tonic EMG activity. Unlike the gag activity, this tonic EMG activity always occurred during expiration, even when the sensory stimulus was applied during inspiration. Thus, the latency between the gag and tonic expiratory activity could be greater than 40 msec (i.e. with stimulation during inspiration), but it was never observed to be less then 40 msec in any experiment. Expiratory activity was also distinguished from gag activity of the TA muscle by the fact that it continued for several respiratory cycles after cessation of the sensory stimulus, as shown in Figure 43H. Although only gag EMG activity (upper trace) evoked by the last sensory stimulus can be observed at the low amplifier gain, expiratory contractions of the TA muscle (middle trace) were observed for at least two respiratory cycles following cessation of the stimulus. Since tonic activity occurred only during expiration, had a longer latency following the sensory stimulus, and recurred over the course of several respiratory cycles after cessation of the stimulus, it can be concluded that the activity was a second type of function, which arose indirectly out of the arousal nature of the sensory stimulus, and had a longer CNS delay than the gag reflex.

Although the fast unit exhibited spiking and participated in the gag reflex, it did not exhibit spiking with expiratory activity of the TA muscle. In Figure 43H, the fast axon spiked (lower trace) with gag activity evoked by the last sensory stimulus, but it did not spike with the subsequent expiratory activity of the muscle. Similarly, all of the fast units studied participated in the gag reflex, but none of them participated in expiratory activity of the TA muscle.

B. Slow Motor Unit Functions:

All of the slow motor units studied participated in the gag reflex and were undistinguished from fast units in most respects with regard to this function. Like fast axons, slow unit axons exhibited an increase in the number of gag spikes, a decrease in latency for the first spike, and a decrease in interspike intervals of subsequent spikes if the strength of the sensory stimulus was increased or if it was placed closer to the expiratory phase of respiration. An attempt was made to determine which of the two unit types, fast or slow, displayed a greater number of gag spikes and shorter interspike intervals at a given level of sensory stimulation. When the two types of units were compared within each animal, so that the comparisons were made under similar states of anesthesia, no significant differences between the two types of units with respect to these two

characteristics could be found. In some experiments, slow units exhibited a greater number of gag spikes and shorter interspike intervals, but in the majority of experiments fast units superseded. Slow units were distinguished from fast units, on the other hand, in having longer latencies for appearance of the first gag spike with a given sensory stimulus, undoubtedly because of the slower conduction velocities of their axons (refer to Figure 39).

In Figure 44, the spiking characteristics (lower traces) of the slow unit are demonstrated when a constant strength sensory stimulus was timed with different points in the respiratory cycle. The beginning of inspiration is indicated by the peak of the waveform (upper traces) in each frame, while the beginning of expiration is indicated by the trough.* The frames are ordered sequentially (A through M) with respect to the timing of the sensory stimulus relative to the phase of respiration. The stimulus in frame A was applied after the beginning of inspiration, and stimuli in subsequent frames approached the expiratory phase in sequence. The actual order in which the stimuli were applied is indicated in the upper right hand corner of each

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*Since the respiratory cycle was monitored in a rather coarse manner, by sensing the temperature changes of the inspired and expired air in the tracheal cannula, inspiration probably started slightly before the peak and expiration slightly before the trough.

frame. Stimuli were spaced approximately 8 seconds apart, about every 2 or 3 respiratory cycles.

When the stimulus was applied, a stimulus artifact was recorded by the micropipette (e.g. upper arrow in Figure 44A) which was followed by spiking of the axon (e.g. lower arrow in 44A). It can be seen in Figure 45A at faster sweep speeds that 2 to 3 gag spikes followed the stimulus artifacts at the beginning of each trace, the number of spikes depending upon when during the respiratory cycle the stimulus was applied. Each trace in Figure 45A (top to bottom) corresponds to Frames A through M in the previous figure. When the stimulus was applied during expiration (e.g. traces 8 and 9 and frames H and I), the number of gag spikes was maximum, and the latency of the first spike and the subsequent interspike intervals were at a minimum, demonstrating changes in the excitability of the motoneuron to "gag" afferent input with variation in the phase of respiration.

As before, the sensory stimuli aroused expiratory activity of the TA muscle (middle traces in Figure 44), but it also aroused expiratory spiking of the slow axon as shown. The arousal nature of the stimulus was much more effective when timed during expiration, as shown by the increase in the number of expiratory spikes and the increase in expiratory activity of the TA muscle. The increased effectiveness of the stimulus when placed closer to

expiration, as shown in the first 4 frames, was also signified by the earlier onset of expiratory spiking and contractile activity, and the decrease in latency between the stimulus and this activity. With faster sweeps of the spike activity in Figure 45A, it can be noted that there was in addition to a decrease in latency for the first spike, decreases in the interspike intervals of subsequent spikes as the stimulus was applied closer to the expiratory phase.

The pattern of expiratory spiking was much more influenced by the phase of respiration than the pattern of gag spiking, presumably because expiratory spiking arose directly from afferent input of excitatory expiratory neurons in the brainstem, while gag spiking arose from afferent input from the periphery. The pattern of gag spiking was to a greater extent determined by the level of the afferent stimulus. However, the level of sensory stimulation did influence the pattern of expiratory spiking in that it contributed to arousal. That is, the history of stimuli preceding a given stimulus (i.e. the number, frequency, and strength of previous stimuli) affected the expiratory spiking pattern. For example, in comparing frames D and E in Figure 44, the slow axon exhibited less spiking in frame E, even though the stimulus was applied closer to expiration, because there had been no stimuli preceeding the one in the frame.

As was concluded previously from observations of whole muscle EMG activity, the expiratory spiking of the slow axon occurred via a different mechanism than the gag spiking. There was always a quiescent period observed between gag spikes and expiratory spikes of at least 40 msec, as demonstrated by the repeated sweeps in Figure 45B following each stimulus. The stimuli were maximum and applied randomly with respect to the phase of respiration. The expiratory spiking and contractile activity also continued for several respiratory cycles after the last stimulus had been applied (arrow in Figure 45C).

Discussion.

It has been demonstrated that sensory stimulation can evoke two distinct types of activity of TA motor units, one phasic in nature involving reflex gag contractions and a second tonic in nature involving expiratory contractions. The CNS mechanisms involved in these two types of functions are quite different, as demonstrated by the considerably longer CNS delay following sensory stimulation for expiratory activity, suggesting that more synapses are involved for this function. The gag reflex, on the other hand, is probably a monosynaptic reflex. The source of afferent input for these two functions is also different, as shown by the continued expiratory activity after cessation of afferent input from the periphery. The source of

expiratory afferent input to TA motoneurons is probably brainstem excitatory expiratory neurons, while the source of gag afferent input is probably directly from the periphery.

Referring to Figure 6, the pattern of gag spiking (i.e. number of spikes and interspike intervals) is largely determined by the level of afferent input, but is also effected by the variation in excitability of motoneurons (irrespective of their size) with the influence of excitatory and inhibitory input of respiratory neurons. The pattern of expiratory spiking is also determined by the level of afferent input, the source of this input being brainstem expiratory neurons. The level of afferent input, in turn, is dependent upon the effectiveness of the sensory stimulation to arouse. The timing of the sensory stimulus with the phase of respiration, as well as the history of stimuli preceding it (i.e. number, frequency, and strength of stimuli) are important factors contributing to this arousal.

All of the motor units studied, whether fast or slow, exhibited gag spiking, as indicated by the triangles in the top graph of Figure 46 (after "G"). Those units which in addition exhibited expiratory spiking are indicated by the closed stars in the Figure (after "E"), while those that did not are indicated by open stars. Only slow units exhibited expiratory spiking as shown. The rest of the units which exhibited gag spiking were not studied in sufficient detail

to know for certain whether they did or did not, in addition, exhibit expiratory spiking.

Since slow units are the first, in fact the only in these studies, to exhibit the tonic firing pattern of expiratory activity, they are undoubtedly more excitable than fast motoneurons. Since they also have slower conducting, smaller axons and therefore smaller cell bodies, it can be concluded that the size principle holds when comparing fast and slow TA motoneurons (refer to Figure 6). It is not certain, however, whether the size principle also holds for individual motoneurons within each population. In addition to differing in excitability, fast and slow TA motoneurons may also differ in the nature of their synaptic input from expiratory neurons, particulary in view of the fact that no fast units exhibited this type of activity. However, fast units undoubtedly receive synaptic input from expiratory neurons and are involved in this type of activity to some extent for several reasons. First, it is unlikely that only 10% of TA muscle fibers are involved in expiratory activity. Second, the high oxidative enzyme content of fast muscle fibers suggests that they are involved in more prolonged types of activities than the gag reflex. Third, there is direct evidence implicating fast units in this type of activity, which will be discussed. It is interesting, nevertheless, that in a barbiturate anesthetized animal only slow units tend to be recruited for expiratory activity.

The large range of sizes of slow motor units and their deviation from fast units in the relationship of EMG size and conduction velocity to MU size (refer to figures 39-41) suggest that they may have some autonomy as a population. They might have a sufficient range of sizes, so that with sequential recruitment they could provide for the most primitive kind of vocalization: the monotone whine or groan of an anesthetized or sleeping animal. The second population of fast units might only be recruited in a more alert animal for vocalization more sophisticated and complicated in nature. This is, of course, speculation, since it cannot be stated emphatically that the expiratory activity observed in these studies represents the preliminary stages of vocalization (i.e. animals didn't actually vocalize).

Although only slow units exhibit expiratory activity under conditions of barbiturate anesthesia, both types of units exhibit gag activity under the same conditions. Based on the relative number of spikes and interspike intervals of fast and slow units, the two types of units are either undistinguished or fast units are slightly more responsive in this function to a given sensory stimulus. In either case, since fast units have less excitable motoneurons, they must receive stronger synaptic input from the periphery to exhibit the same gag spiking pattern as slow motor units. Since Henneman ascribed the differences in firing patterns

of fast and slow limb motor units strictly to differences in their excitability and dismissed the possibility that they might also differ in the nature of their synaptic inputs (see Henneman, 1965a, p. 574), fast and slow TA motor units may be unique in having different synaptic inputs. On the other hand, there is recent evidence indicating that different types of afferent input to limb motor units may be selectively directed to different motoneurons of the same pool (Clamman and Hgai, 1978).

A satellite study to those described also supports the notion that fast and slow TA motor units differ in their excitability and synaptic inputs with respect to gag and expiratory activity. In two animals that were lightly anesthetized, considerable spontaneous activity of motor unit muscle fibers (i.e. muscle units) could be observed by the presence of non-evoked EMG and contractile activity of the TA muscle. Most of the spontaneous contractions occurred during expiration, and had no associated EMG potentials. The contraction times of most of these units were greater than 12.5 msec, but a few had contraction times as short as 7.5 msec. Thus, slow units again were more excitable, but it was interesting to find that a few fast units also participated in this function under conditions of lighter anesthesia. Several other spontaneous expiratory units had detectable EMG responses associated with their contractions. Again most of them were slow units (i.e. one

of seven was presumably fast with a contraction time of 7.5 msec.). Two units were also observed with quite large, single, EMG responses occurring during both phases of respiration. Both of these units were fast with contraction times of 4.75 and 5 msec. Probably slight irritation of the mucosa reflexly evoked this gag activity. These observations further support the notion that although slow TA motor units have more excitable cell bodies, fast TA motor units probably receive stronger direct input from the periphery.

It must be stated, finally, that an important finding in these studies is the apparent importance of the nature of synaptic input to TA motor unit activity (refer to Figure 6). Granit found that limb motoneurons fired either phasically or tonically, irrespective of the source of the afferent input initiating the activity (Granit, 1957). Furthermore, although Henneman found that motoneurons could fire with either pattern, he ascribed the particular firing pattern of a motoneuron at any given moment to a combination of its excitability and the level of afferent input. These studies indicate that the nature of the synaptic input to a TA motoneuron not only establishes the type of function its motor unit mediates, but also contributes in the establishment of its firing pattern. In this regard, slow units could exhibit phasic gag spiking at rates as high as 350-450/second and then 40 msec later tonic expiratory

spiking at a much lower rate of 35/second. It should be interesting to determine if they would also exhibit phasic gag activity with more prolonged sensory stimulation. In any case, the question arises, if they can spike at such high rates, what is the duration of their afterhyperpolarization? If spiking rates are determined by the duration of the afterhyperpolarization as suggested by Eccles (1958), then slow TA motoneurons should have short afterhyperpolarizations, and are actually fast motoneurons. Even if this is the case, they still fire tonically most of the time because of the nature of their tonic expiratory input and their high excitability, and "convince" the muscle fibers they innervate (i.e. Type I) that they are slow motoneurons.

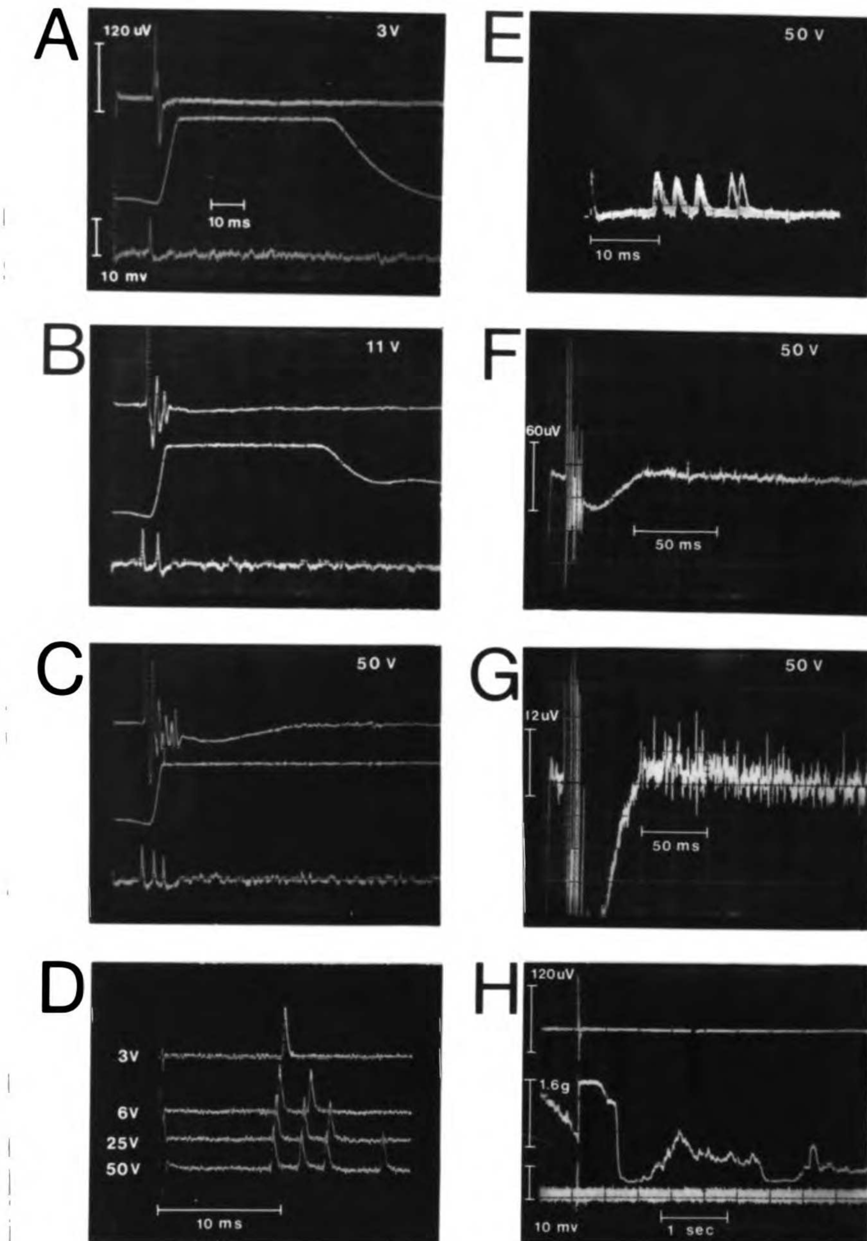
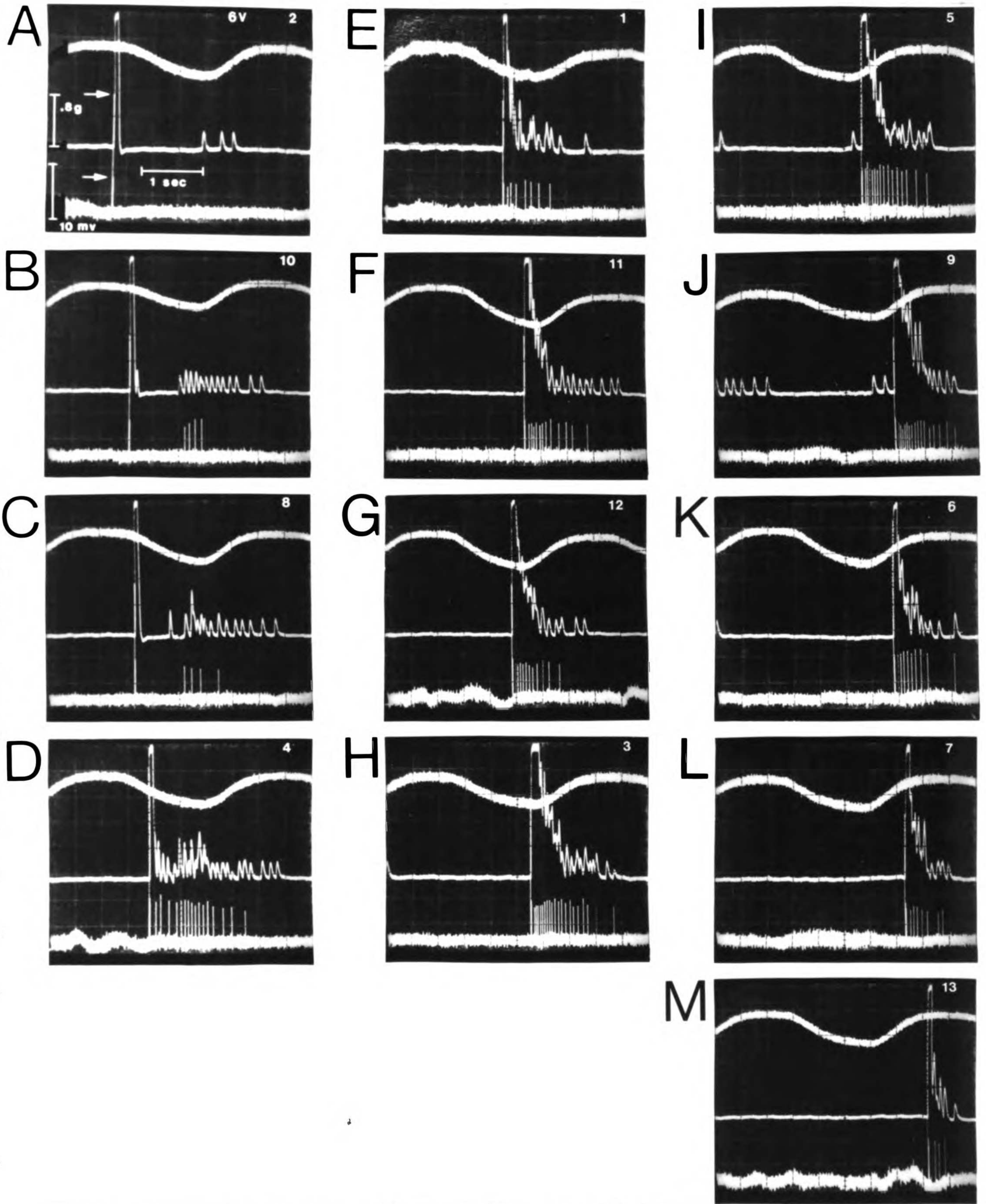


FIGURE 43

Fast motor unit axon spiking sensitivity to internal laryngeal nerve stimulation. See text for detail.


A
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2
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K
6
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7
M
13

FIGURE 44

Slow motor unit spiking sensitivity to internal laryngeal nerve stimulation. Voltage of each of the 13 stimuli was 6 volts. The order of the stimuli is indicated in the upper right hand corner of each frame. Respiratory cycle is indicated by the upper traces in each frame (start of inspiration at the peak, and start of expiration at the trough of the waveform), TA contractile activity by the middle traces, and recordings from slow axon by the lower traces. See text for details.

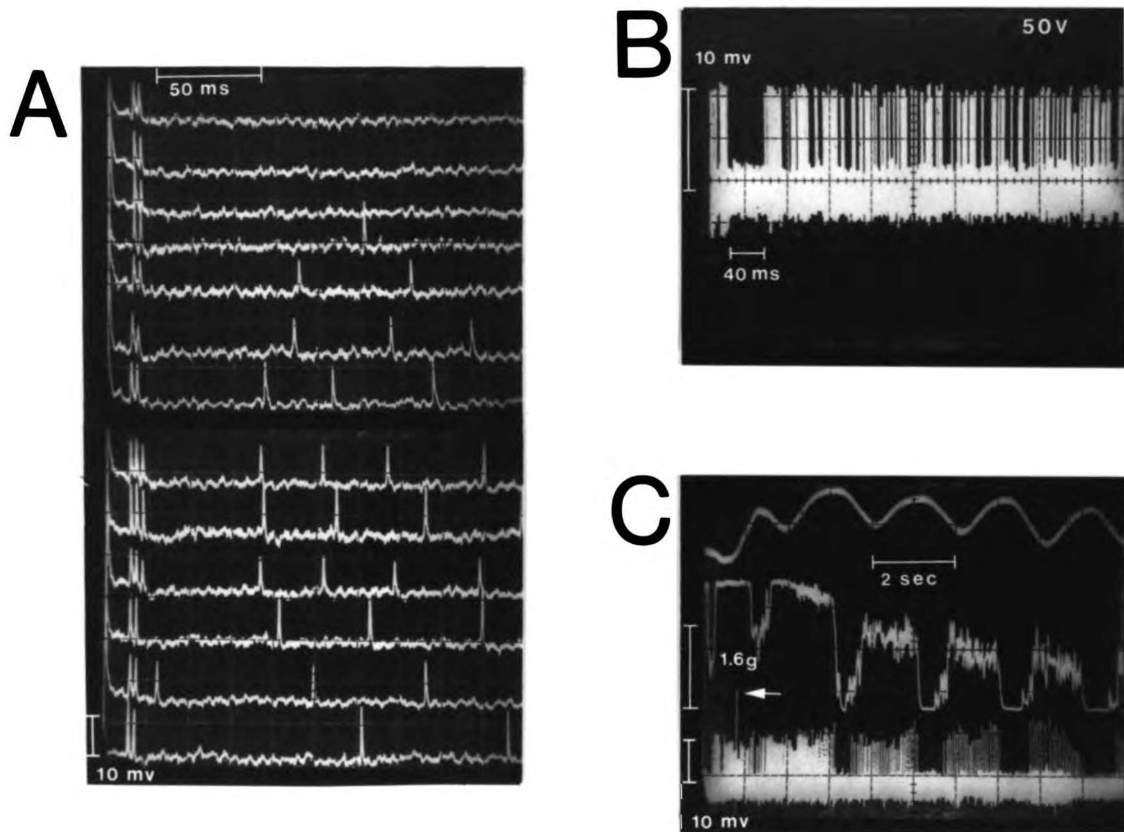

FIGURE 45

A. Faster sweeps (top to bottom) of the lower traces (A through M) in the previous figure. Two or three gag spikes follow the stimulus artifact at the beginning of each trace. Expiratory spikes are also present in most of the traces. B. Superimposed traces of slow axon spiking following sensory stimulation at 50 volts and timed randomly with respect to the respiratory cycle. Each sweep was triggered by the stimulus. C. Respiratory cycle (upper trace), TA expiratory activity (middle trace) and slow axon expiratory spiking (lower trace) following the last of many stimuli. The last sensory stimulus is indicated by the arrow. See text for details.

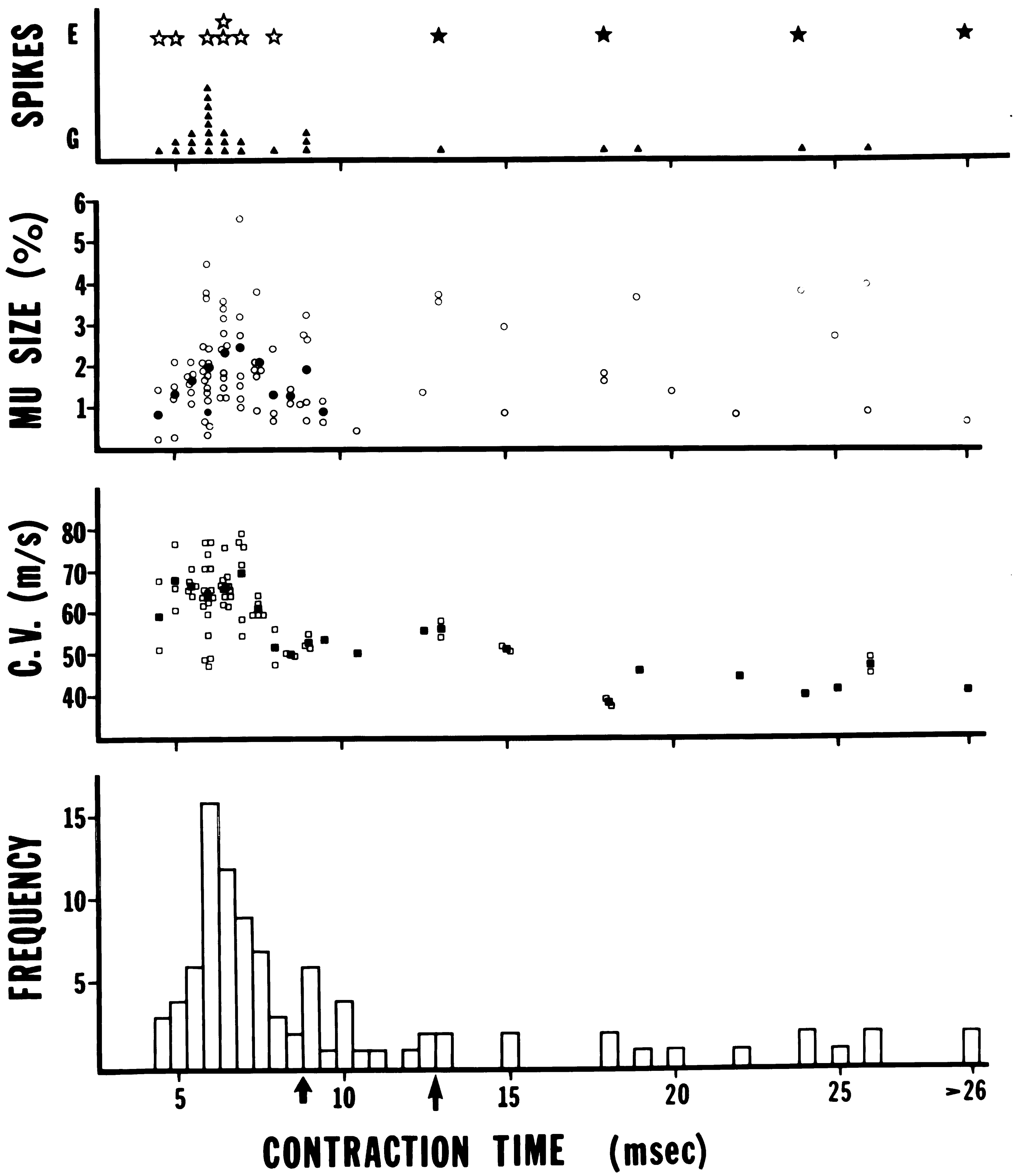

FIGURE 46

FIGURE 46

Summary diagram relating the contractile properties and functions of TA motor units. All of the units studied exhibited gag spiking (triangles after "G" in the upper graph). Units that were tested for the presence of expiratory spiking with repeated sensory stimulation are indicated by stars (after "E"). The four slow units indicated by closed stars exhibited expiratory spiking, while the fast units indicated by open stars did not.

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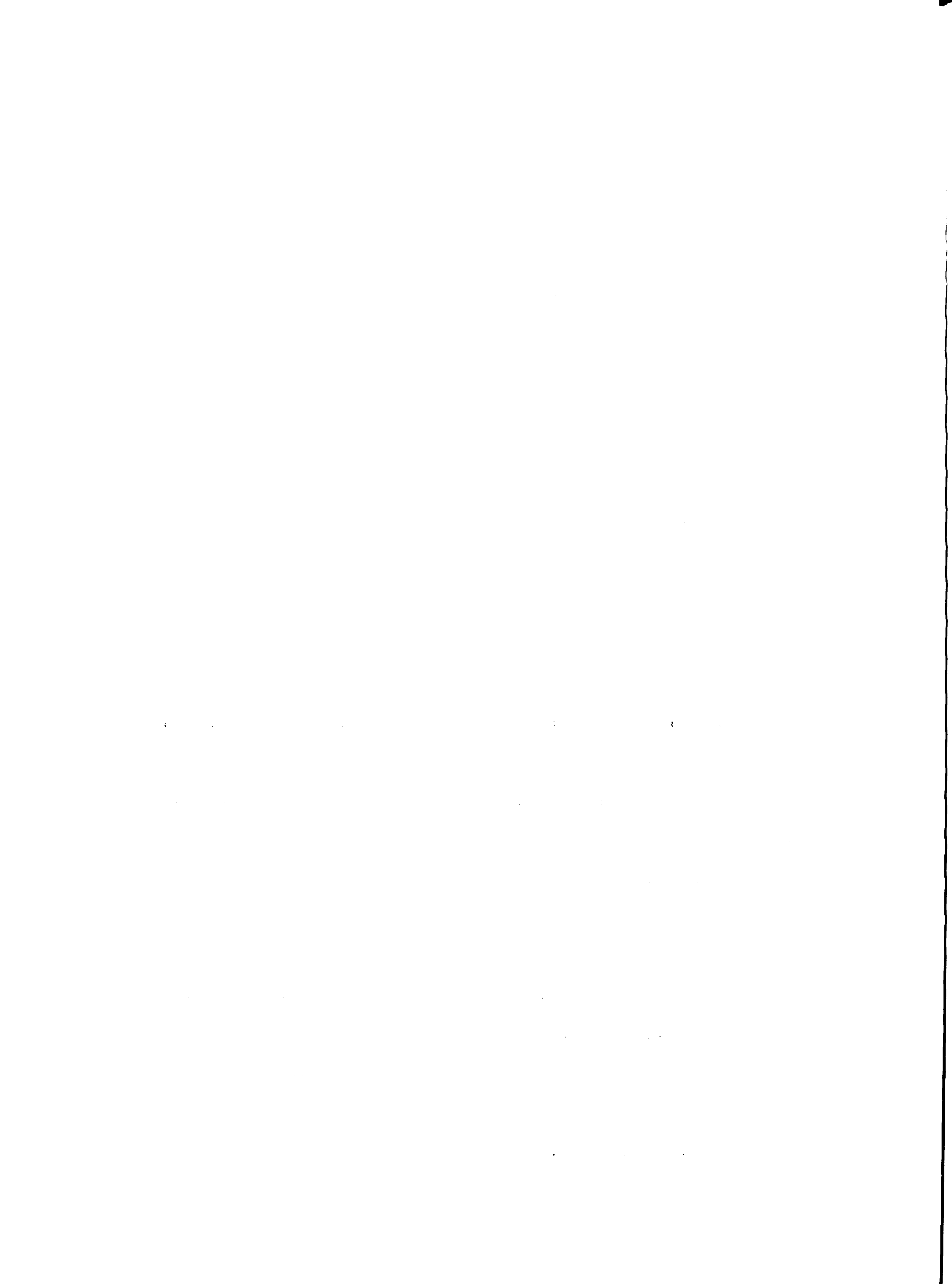

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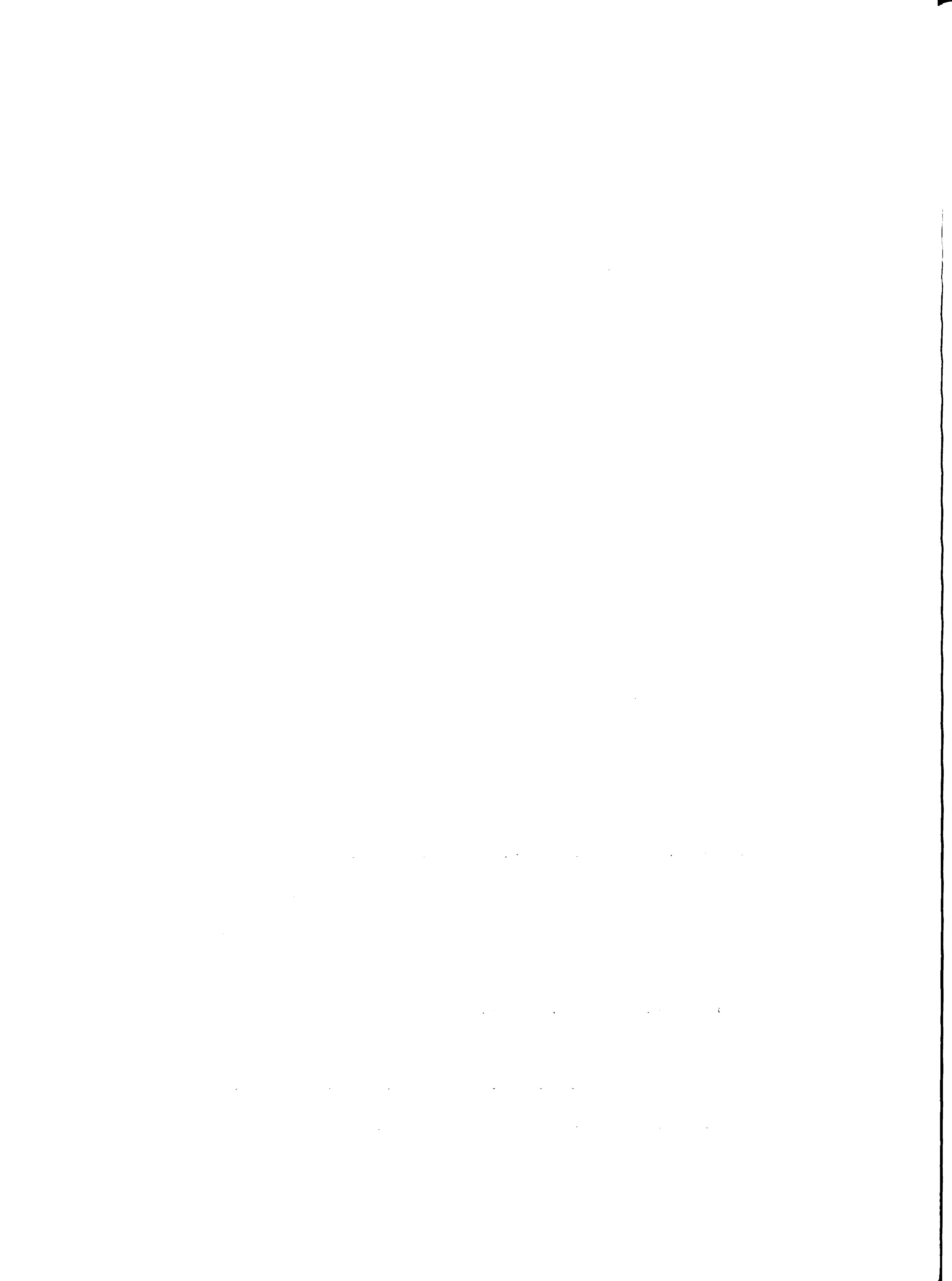

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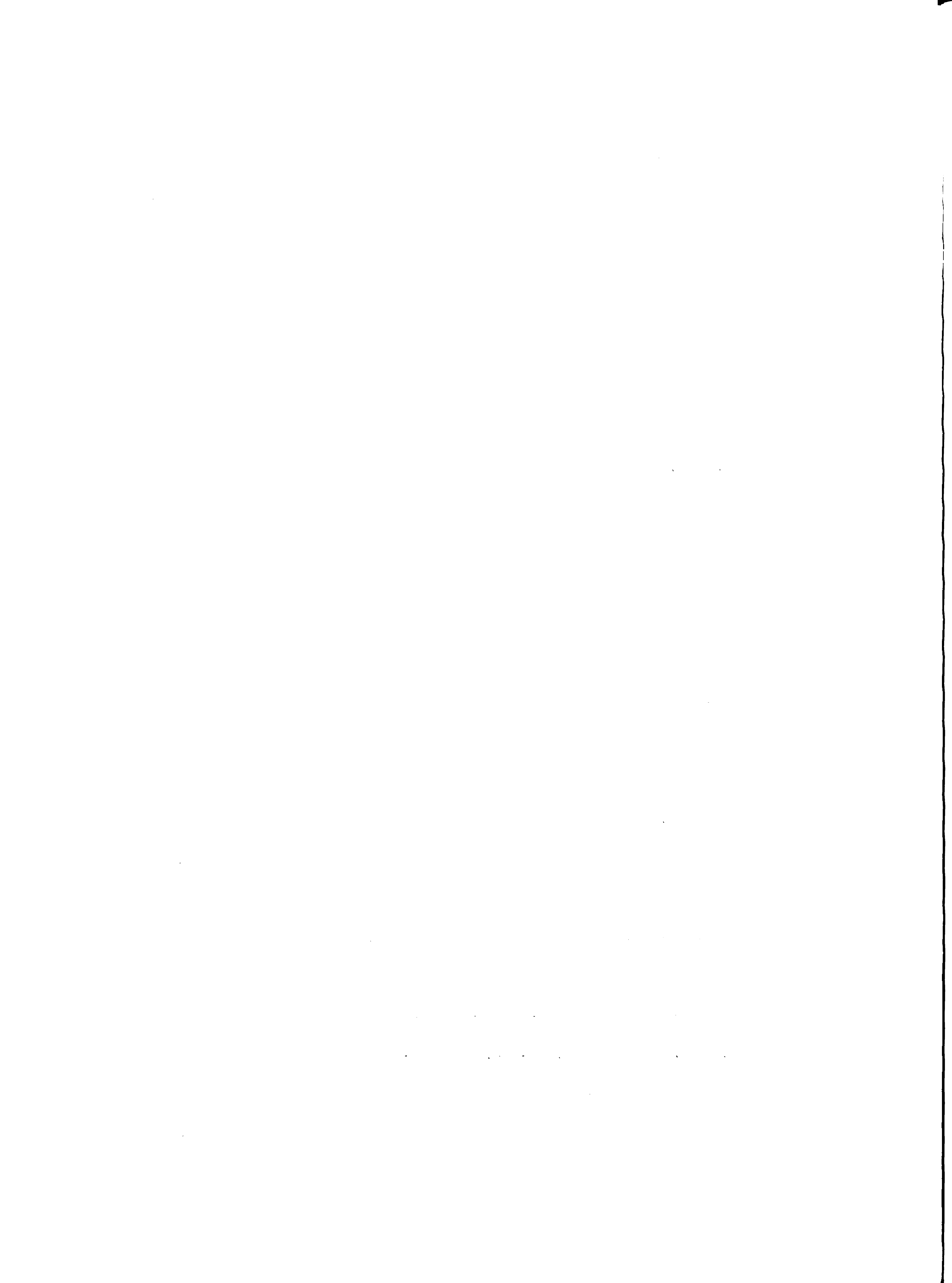

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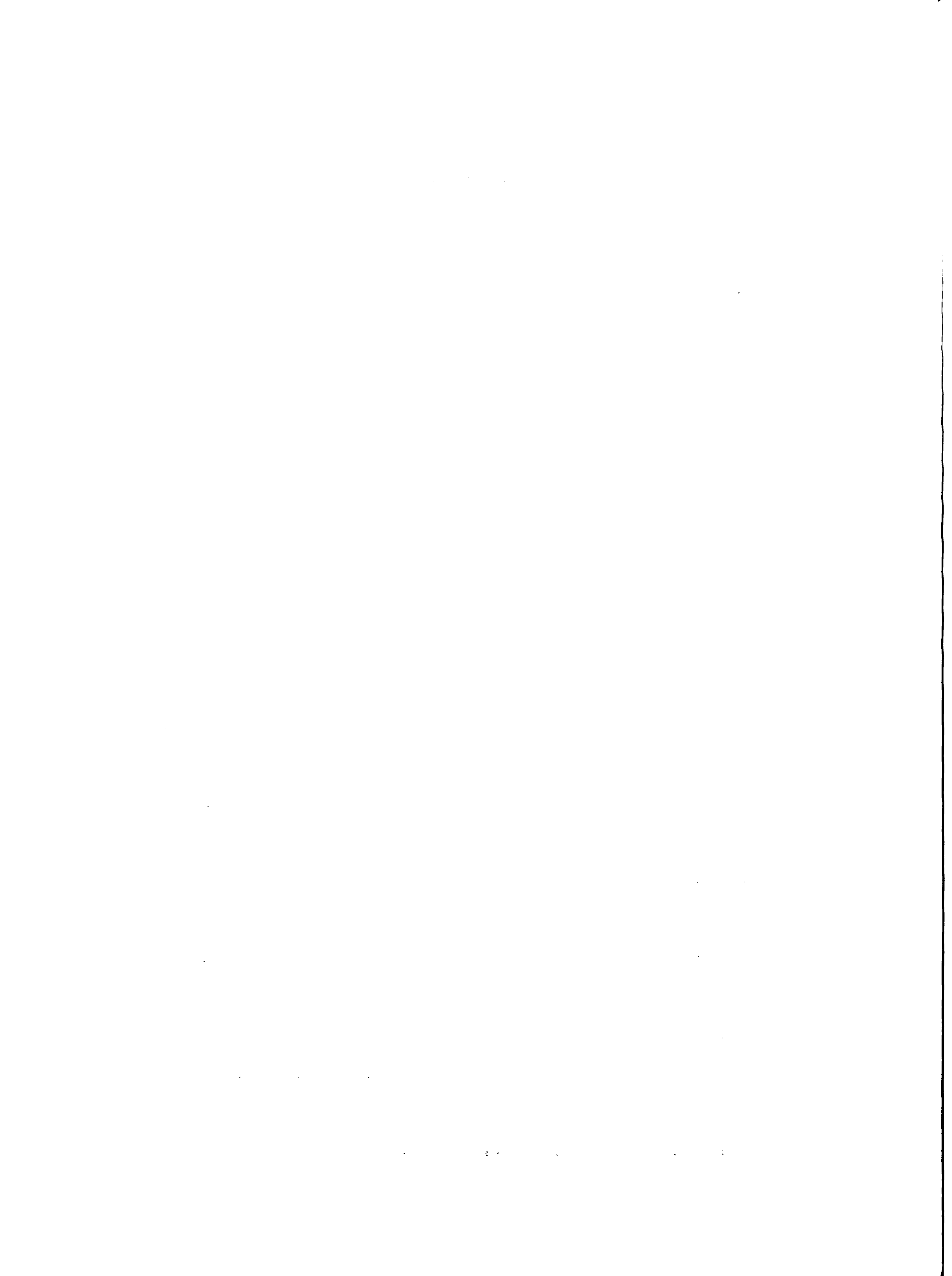

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