Section 1 Chapter 1

The scientific basis of muscle disease

Structure and function of muscle fibers and motor units

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Introduction

The term "motor unit" was introduced by Sir Charles Sherrington, a founder of modern neurophysiology, who observed that force occurred in discrete steps when a muscle contracted in the stretch reflex [1]. He postulated that each step was produced by the all-or-none action of a single motor neuron upon the muscle fibers it innervated. Sherrington's concept of the motor unit assumed that each muscle fiber receives innervation from only one motor neuron, and that the muscle fiber faithfully responds to every impulse of the motor neuron. These assumptions have subsequently been shown to be true in healthy adult skeletal muscles. The motor unit has become a fundamental concept in understanding the physiology of muscle and the control of movement.

A motor unit consists of one motor neuron and all the muscle fibers it innervates. The term muscle unit has been introduced to refer to the group of muscle fibers innervated by a given motor neuron [2]. The motor neuron and its muscle unit are inseparable in function because each action potential in the neuron activates all fibers of the muscle unit. Thus motor units are the indivisible quantal elements in all movements. The electrophysiological, metabolic, mechanical, and anatomical properties of the motor neuron and its muscle unit are coordinated in a manner that allows efficient muscle contraction over a wide range of motor behaviors. The coordinated expression of the proteins that govern these properties reflects the interplay between the trophic control that motor neurons exert over their muscle fibers through activity patterns and chemical trophic factors, as well as trophic feedback from the muscle fiber to the motor neuron. Although most of the properties of a given motor unit become specified during the early postnatal period of development, physical activity and disease processes can modify certain properties to a limited extent. In this chapter, the basic structural and physiological properties of motor units and muscle fibers will be introduced, with a particular emphasis on humans and other mammals.

Anatomy of motor units

Motor neurons

Motor neurons are the only central neurons with axons that leave the central nervous system (CNS) to innervate non-neuronal tissue. Their cell bodies are located in the anterior horn of the gray matter of the spinal cord (Figure 1.1). The motor neurons that innervate the same muscle cluster together in motor nuclei that form elongated columns that generally extend over several spinal cord segments [3]. The number of motor neurons innervating each muscles varies, ranging from the estimates of 30–40 motor neurons innervating the delicate tenuissimus muscle in the cat [4] to estimates of 100–200 motor neurons innervating human thenar muscles [5, 6]. In the lumbar and cervical enlargements of the spinal cord, the motor neurons that innervate distal limb muscles are located most laterally within the anterior horn, and motor neurons innervating proximal muscles lie more medially [7, 8]. The axons of motor neurons exit the spinal cord through the adjacent anterior roots. When motor axons innervating the same muscle exit from roots of several segments, they rejoin in a muscle nerve after traversing peripheral plexuses and nerve trunks. The muscle nerve contains motor axons innervating the muscle and the sensory axons arising from receptors within the muscle, such as the muscle spindles and tendon organs.

In mammals, there are three kinds of motor neurons in the motor nucleus. Alpha motor neurons are large cells [9, 10] that innervate the striated muscle fibers that make up the bulk of skeletal muscle tissue (extrafusal fibers). Gamma, or fusimotor, neurons are considerably smaller [11] and exclusively innervate one or more of the three types of specialized muscle fibers within the muscle spindle – stretch receptor organs that are present in virtually all somatic muscles [12, 13]. A third class of motor neuron, called skeleto-fusimotor or beta motor neurons, innervates both intra- and extrafusal muscle fibers [14]. Beta motor neurons have been found in higher primates [15] and probably also occur in humans. Because beta motor

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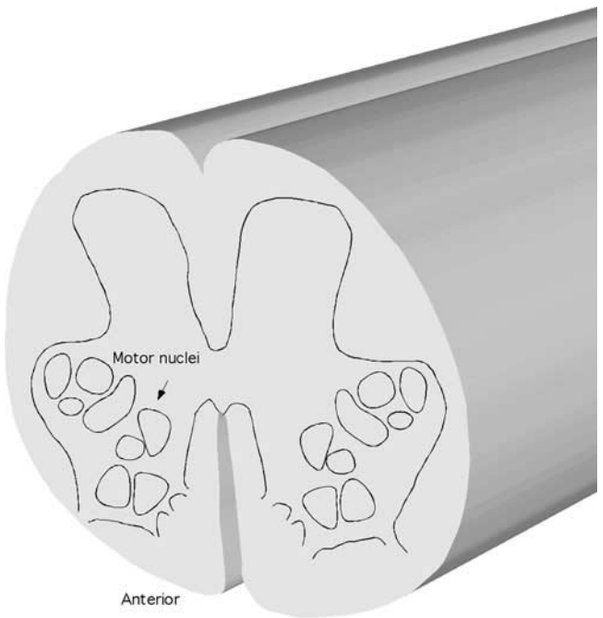

Figure 1.1. Cross-section of the lumbar spinal cord, showing the location of the motor neuron pools.

neurons are difficult to identify in physiological experiments, there is little direct evidence about their properties. What little is known indicates that the properties of beta motor neurons and their extrafusal muscle fibers are essentially the same as those of alpha motor neurons [16]. For this reason, alpha and beta motor neurons will not be distinguished in this chapter.

Alpha motor neurons have extensive dendritic trees that receive synaptic input over their entire extent [17, 18, 19]. Their myelinated axons have large diameters with correspondingly fast conducting velocities, ranging from 40 to 60 m/s in human motor nerves [20]. Faster conduction velocities, 50–120 m/s, have been reported in cats and smaller mammals [21]. The axons of motor neurons can be extremely long, up to a meter in length for those motor neurons innervating the distal foot muscles of a tall adult. The length and diameter of the motor axons mean that the volume of axoplasm may exceed the volume in the cell body and dendrites by tenfold or more (Figure 1.2). The large metabolic demands of maintaining the peripheral axon presumably account for the large size of the motor neuron cell body.

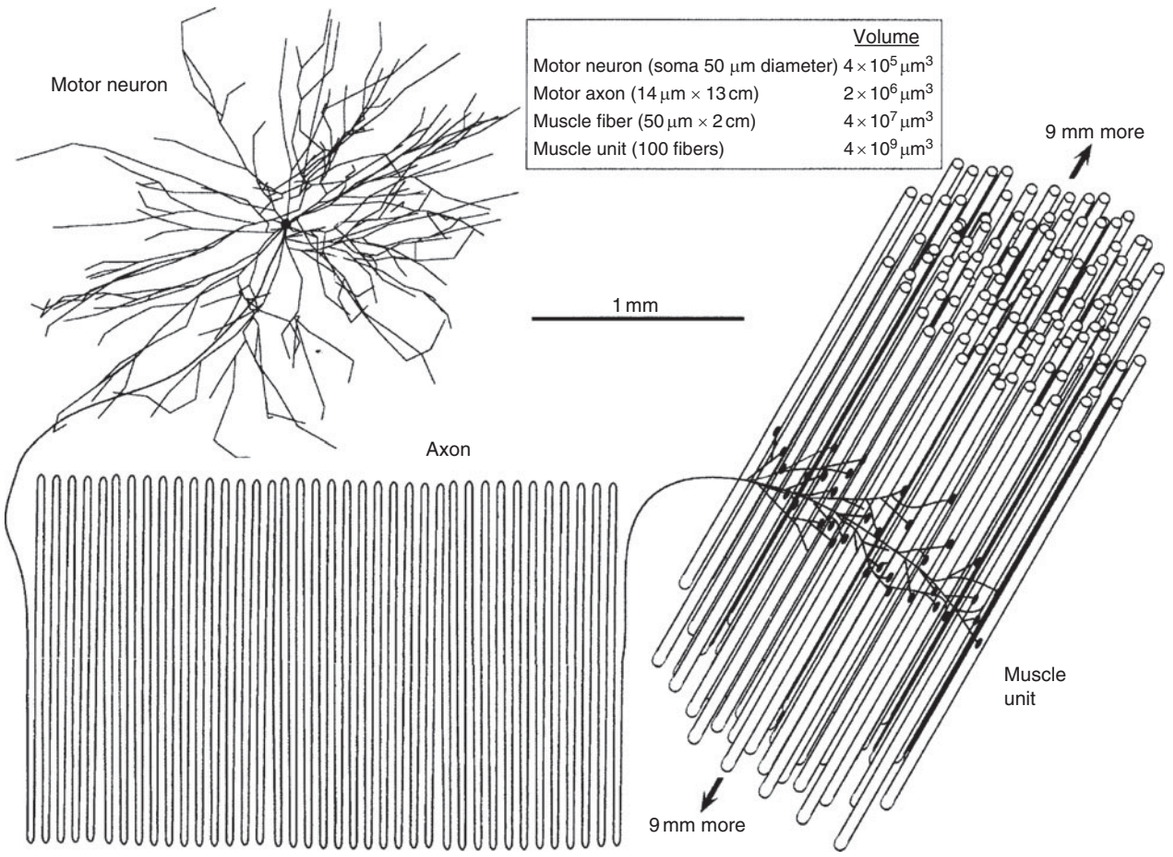

Figure 1.2. Diagram of a motor unit with its components drawn to scale. Note the smaller size of the motor neuron cell body compared with its extensive dendritic tree and very long motor axon. The volume of a single muscle fiber is more than tenfold greater than the volume of cytoplasm in the motor neuron plus its axon. Contributed by R. E. Burke.

Neuromuscular junctions

As the myelinated motor axons near their target muscle, they begin to divide into tens or hundreds of terminal branches, which lose their myelin sheaths as they near the neuromuscular junctions (NMJs). The NMJ is a large, highly specialized synapse between the motor nerve terminal and the muscle fiber [22]. In somatic muscles there is only one NMJ per muscle fiber [23], but exceptions are found in some cranial muscles, such as the laryngeal [24] and extraocular muscles [25]. On a given muscle fiber, the NMJ is located approximately equidistant from its ends, allowing action potential depolarization to spread equally to both ends from the center of the muscle fiber. The NMJ is a complex structure that undergoes remodeling during development and aging and in response to denervation. At the NMJ, the motor nerve terminal is separated from the postsynaptic muscle membrane by a synaptic space containing basal lamina with synapse-specific glycoproteins. On the postsynaptic side, the muscle membrane is highly folded. Acetylcholine receptors are found on the crests of the junctional folds apposing the vesicle release sites on the presynaptic terminal, whereas the voltage-gated sodium channels responsible for action potential generation are densest in the depths of the folds [26]. NMJs exhibit structural specializations related to the size and type of muscle fiber [27]. The structure and function of NMJs will be covered more fully in Chapter 23.

Muscle fibers

The skeletal muscle fiber is a cylindrical, multinucleated cell that is formed by the fusion of myoblast cells during development. The muscle fiber has a highly organized structure, with several distinct spatial domains. Nuclei are positioned along the periphery of the fiber beneath the plasma membrane, or sarcolemma. The center of the muscle fiber is packed with the contractile apparatus, which consists of longitudinally oriented myofibrils and scaffolding proteins. The contractile apparatus is encircled by a network of sarcoplasmic reticulum (SR), a form of endoplasmic reticulum specialized for calcium release and reuptake. The sarcolemma has numerous narrow infoldings, called T-tubules, that penetrate deep into the muscle fiber, where they become closely apposed to regions of the SR at specialized junctions called triads or "calcium release junctions." The T-tubule membrane is continuous with the sarcolemma membrane, but it is specifically enriched in certain membrane proteins, such as voltage-gated calcium channels, chloride channels, and transporters (Figure 1.3) [28, 29]. The T-tubule "interior" is in continuity with the extracellular space, although diffusion occurs more slowly from this narrow space than at the surface membrane. The triads, where T-tubules meet the SR, are the sites where action potential depolarization is coupled to the mechanical contraction. Excitation–contraction coupling occurs through protein–protein interactions between the sarcoplasmic domains of the voltage-gated calcium channels on T-tubule membranes and the calcium release proteins, known as ryanodine receptors, on the SR membrane [30].

The contractile apparatus of the muscle is organized into a series of repeated units a few microns long called sarcomeres [31]. The sarcomere is the smallest unit of contraction. It consists of highly organized protein assemblies that give the muscle fiber a characteristic striated appearance (Figure 1.4b). The sarcomere contains the myofibrils, longitudinal arrays of thick and thin filaments that are maintained in a hexagonal lattice by a scaffolding network (Figure 1.4a). Proteins in the scaffolding network condense at the ends and middle of the sarcomere to form transverse bands called Z-disks and M-bands [32]. The thin filaments consist of filamentous actin entwined by tropomyosin and troponin, a calcium-binding protein. Thick filaments consist of myosin, a large molecule with heavy and light chains. The myosin heavy chains have a tail region and a globular head. Thick filaments are formed by the assembly of myosin monomers with their tails centrally and heads protruding outwards, with an antiparallel orientation on opposite ends of the filament. Z-disks, which mark the border between sarcomeres, serve to anchor the thin filaments. The Z-disks are formed by an ensemble of several proteins, including alpha-actinin. Titin, a large elastic protein spanning from the Z-disk to the M-band, binds to the myofibrils, keeping them centered in the sarcomere, and transmitting tension to the Z-disk during sarcomere shortening [33]. Titin and proteins that comprise the M-band essentially form an intrasarcomeric cytoskeleton that maintains the regular spacing of the thick and thin filaments [32, 34].

The myosin heads on the thick filament contain an ATPase activity and binding sites for actin. When contraction is initiated by a muscle fiber action potential, calcium released from the SR binds troponin, uncovering binding sites on actin. This leads to the formation of cross-bridges between actin and myosin. The ATPase activity of myosin is enhanced by formation of cross-bridges, and as ATP is hydrolyzed the cross-bridge is broken, freeing the myosin head to swivel to the next actin-binding site. The repeated formation and cleavage of actomyosin cross-bridges produces the sliding action of thin and thick filaments that causes shortening of the sarcomere and muscle contraction [35, 36]. The actomyosin cross-bridges serve as the mechanical linkage between thick and thin filaments for transmitting tension to the insertions of the muscle fiber. The amount of tension is proportional to the number of cross-bridges, reaching a maximum at sarcomere lengths when thick and thin filaments have the greatest overlap [37, 38].

The muscle fiber has a rich cytoskeletal network underlying the membrane and surrounding the myofibrils. In subsarcolemmal regions, protein complexes of dystrophin, syntrophins, and other molecules bind to F-actin and other cytoskeletal proteins. By binding as well to intracellular domains of membrane proteins such as sarcoglycans these effect a linkage between the muscle interior and the extracellular matrix. Beneath the subsarcolemmal cytoskeleton, networks of

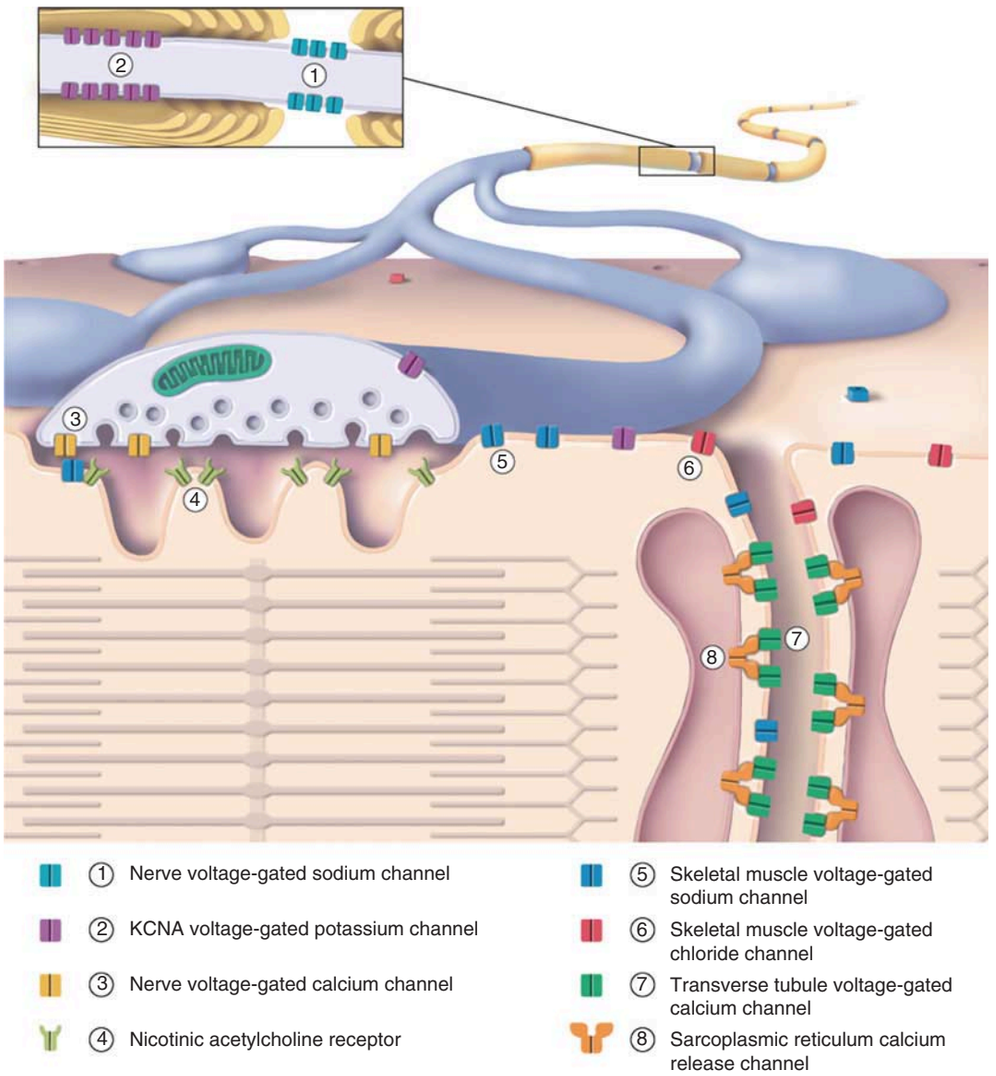

Figure 1.3. Spatial organization of ion channels of the motor nerve, neuromuscular junction (NMJ) and skeletal muscle. The drawing shows a myelinated axon branching to form synaptic contacts with a muscle fiber. The upper inset shows the location of the channels at the node of Ranvier and internodal regions of the motor axon. The lower portion of the drawing depicts the outer surface of a presynaptic terminal and muscle fiber in cut section. Note the location of acetylcholine receptors at the crests of the junctional folds at the NMJ, and the location of channels on the T-tubules and sarcoplasmic reticulum (SR). Used with permission from Cooper and Jan (1999) [175].

intermediate filaments, of which desmin is the most prominent, play a role in the positioning and morphology of organelles within the muscle (reviewed in [39, 40]). Desmin connects Z-disks, SR, myofibrils, and other organelles to the subsarcolemmal cytoskeleton. Mitochondria are usually found in two locations within the muscle fiber, beneath the sarcolemma and among the myofibrils, mostly near the Z-disks. Subsarcolemmal and interfibrillar mitochondria appear to be functionally distinct, with differing cytochrome content, capacity for ADP-stimulated respiration, and susceptibility to apoptotic stimuli [41, 42]. Deficiencies of desmin lead to subsarcolemmal accumulation of mitochondria in mice, supporting a key role for desmin in mitochondrial positioning [43]. Intermediate filaments also bind to proteins on the surface of lysosomes, which are relatively sparse in normal muscle, but become prominent in some myopathies. Glycogen particles, sometimes termed glycosomes, are found in myofibrillar and subsarcolemmal locations.

Extracellular matrix

The muscle fiber is surrounded by an extracellular matrix which consists of several distinct layers [44]. The innermost layer, the basal lamina, contains the carbohydrate-rich extracellular domains of membrane proteins, such as dystroglycan and integrins, that interact with the muscle cytoskeleton; secreted glycoproteins such as members of the laminin family;

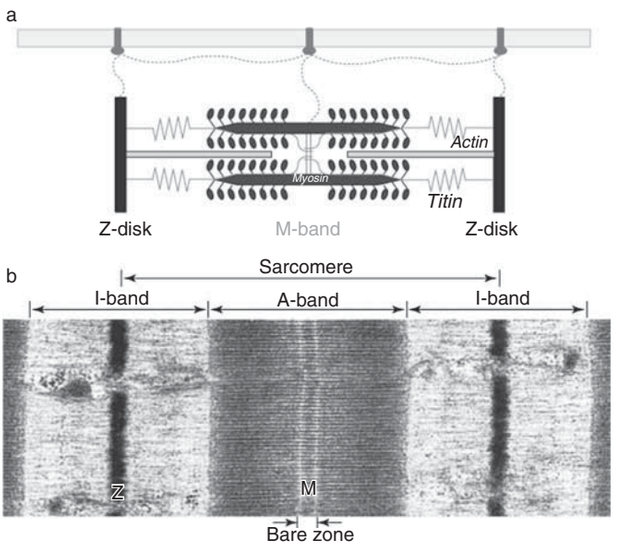

Figure 1.4. Sarcomere structure. The upper drawing shows the myofibrillar proteins, actin and myosin, in longitudinal orientation with titin in the sarcomere. The Z-disk and M-band are transversely oriented. Intermediate filaments (dotted lines) anchor to the cytoskeletal proteins. The lower figure shows the appearance of a complete sarcomere, bordered by two partial sarcomeres, in an electron microscope picture. The A-band is formed by the overlap of actin and myosin filaments. The I-band is formed by thin filaments anchored to the Z-disk, which forms the border between adjacent sarcomeres. (From Agarkova and Perriard (2005) [34], with permission).

and a variety of ligands and proteoglycans that bind to the extracellular matrix proteins. The outermost layer is rich in collagen fibers, forming a connective tissue layer, the endomysium. The extracellular matrix is specialized at the NMJ, containing synaptic laminins, ligands such as agrin, and the enzyme acetylcholinesterase. The basal lamina and the extracellular matrix molecules play a key role in supporting muscle fiber development and regeneration after injury. Lying beneath the basal lamina are satellite cells, myogenic precursors that are able to proliferate and differentiate into myoblasts [45].

Muscles

Most mammalian muscle fibers are only a few centimeters long, much shorter than the length of most muscles. The length of a muscle fiber is thought to be limited by the need for sarcomeres to be activated nearly simultaneously to produce an effective contraction, which in turn is limited by the time needed for an action potential to travel the length of the muscle fiber. The conduction velocity of muscle fibers is relatively slow, in the range of 2–10 m/s [46, 47]. To achieve an effective mechanical action over a larger length, groups of muscle fibers, called fascicles, are bound together by perimysial connective tissue to form a muscle. Muscle fascicles are arranged in various ways that allow a common direction of force to be delivered to the muscle's points of origin and insertion [48]. There are two general schemes [49]: pinnate, in which the muscle fibers are oriented at an angle to the muscle's primary direction of force; and parallel, in which

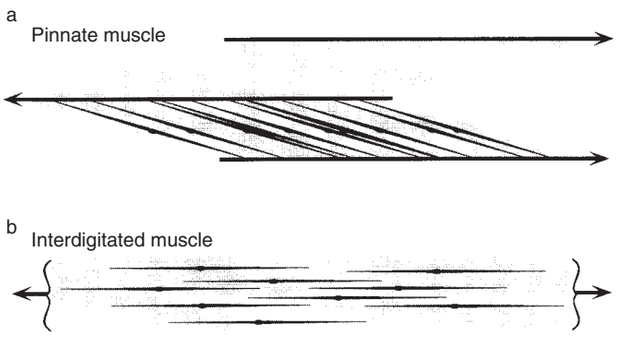

Figure 1.5. The two basic designs of muscle architecture. (a) Pinnate arrangements of muscle fibers in parallel arrays that run at an angle between the aponeuroses of origin and insertion. The fibers of an individual muscle are depicted in the lower half, with central neuromuscular junctions aligned along the axis of the muscle belly. All of the muscle unit fibers contribute to the effective cross-sectional areas of the muscle unit in force generation. (b) An interdigitated muscle, showing tapered muscle unit fibers and their neuromuscular junctions scattered along the length of the muscle belly in irregular arrays. Forces produced by individual fibers are transmitted to the tendons of origin and insertion by internal connective tissue stroma. The effective cross-sectional area of the muscle unit is less than its total cross-sectional area. Contributed by R. E. Burke.

the orientation of muscle fibers is the same as the force vector. In pinnate muscles, the fascicles are arranged in parallel bundles, often in a feather-like pattern along one or more tendinous aponeuroses (Figure 1.5a). Muscles with pinnate architecture have relatively limited distensibility, but can deliver large output forces. Pinnation is commonly seen in muscles with relatively short lever arms that operate over a limited range of physiological lengths, for example the gastrocnemius muscles of the leg. At the other extreme are muscles with parallel arrangements of interdigitated muscle fascicles, staggered at different longitudinal locations along a web-like intramuscular stroma (Figure 1.5b; [47, 50]). This arrangement allows a small amount of slippage of fascicles past each other, and is commonly seen in muscles that span multiple joints or undergo large changes in length during movement. As might be expected, some muscles exhibit mixtures of these designs (e.g., tibialis anterior in the cat; [51]). A few long, strap-like muscles, such as the biceps femoris, have two or more bellies arranged in series separated by tendinous inscriptions that create distinct anatomical compartments [52].

Most muscles have an optimal range of working lengths. When muscles are stretched during natural movements, they offer some resistance. Most of the tension is related to the number of cross-bridges between overlapping thick and thin filaments [37, 38]. Additional contributions from tendons and internal connective tissue enter into consideration primarily when a muscle is stretched beyond its optimal working range. Because connective tissue is less elastic than muscle fibers, tension rises quickly at these lengths. Contributions from connective tissue to muscle length–tension curves are referred to as passive, in contrast to the active contributions from the myofibrillar cross-bridges. Passive contributions to muscle tension differ between healthy and diseased muscle.

Degenerative muscle diseases, or even the prolonged disuse of muscles, such as after a stroke, may result in markedly increased connective tissue within the muscle with stiffness and increased resistance to stretch [53].

Functional organization of motor units

Distribution of motor unit fibers

The spatial distribution of muscle fibers belonging to an individual motor unit has been studied experimentally with the glycogen depletion technique [54]. In this method, prolonged stimulation of a motor axon is used to deplete muscle fibers of endogenous glycogen stores, enabling the depleted fibers to be identified histochemically. The glycogen depletion method showed that muscle fibers belonging to the same motor unit were arranged in a mosaic fashion among muscle fibers belonging to other motor units [54, 55]. Relatively few muscle fibers from the same unit occurred immediately adjacent to one another [56, 57]. Statistical studies suggest that the distribution of fibers in single units is basically random [58]. Nevertheless, the arrangement of the muscle unit's fibers must accommodate to the internal architecture of the parent muscle to produce a meaningful pattern of force. In pinnate muscles, fibers from one motor unit were found to be scattered more or less evenly through territories that were relatively large, but smaller than the total cross-section of the muscle (Figure 1.6). In multicompartment muscles, motor unit fibers were usually distributed only within one compartment [59]. However, there are examples, such as the extensor digitorum muscle of the monkey forelimb, in which fibers of one motor unit are distributed among several compartments to exert a common force on multiple tendons [60].

Electromyographic (EMG) studies of single motor units in humans suggest a similar spatial organization of muscle unit fibers. Using a technique called scanning EMG, in which a motor unit action potential is recorded as an electrode is advanced in successive steps of 50 µm through the muscle, Stalberg and colleagues [61, 62] recorded territories with cross-sectional areas of 2–10 mm for single motor units in the biceps and tibialis anterior muscles. Within the same region of muscle, they found that several dozen motor units had overlapping territories. For an individual motor unit, at some places the muscle fiber action potentials were grouped, and separated from other regions, suggestive of fractions of the muscle unit innervated by different branches of the motor axon (arrows, Figure 1.7).

One way to describe the size of a motor unit is according to its innervation ratio: the number of muscle fibers innervated by a given motor neuron. The number of muscle fibers

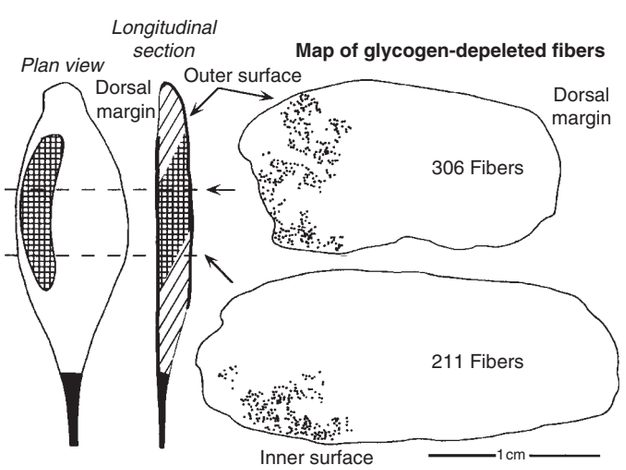

Figure 1.6. The distribution of glycogen-depleted fibers in a Type FR motor unit (fast twitch, fatigue resistant) in the medial gastrocnemius muscle of the cat. The cross-hatched areas in the whole muscle diagrams on the left indicate the extent of the motor unit territory, which occupies only a fraction of the muscle volume. The diagonal hatching on the longitudinal section denotes the angulation of the fibers in this unipinnate muscle. Maps of the spatial distribution of depleted fibers at two levels along the muscle belly are shown on the right. Note the irregular boundaries of the unit territory but relatively even distribution of fibers within it. Adapted from Burke and Tsairis (1973) [56], (with permission from Wiley-Blackwell Publishing Ltd and the authors).

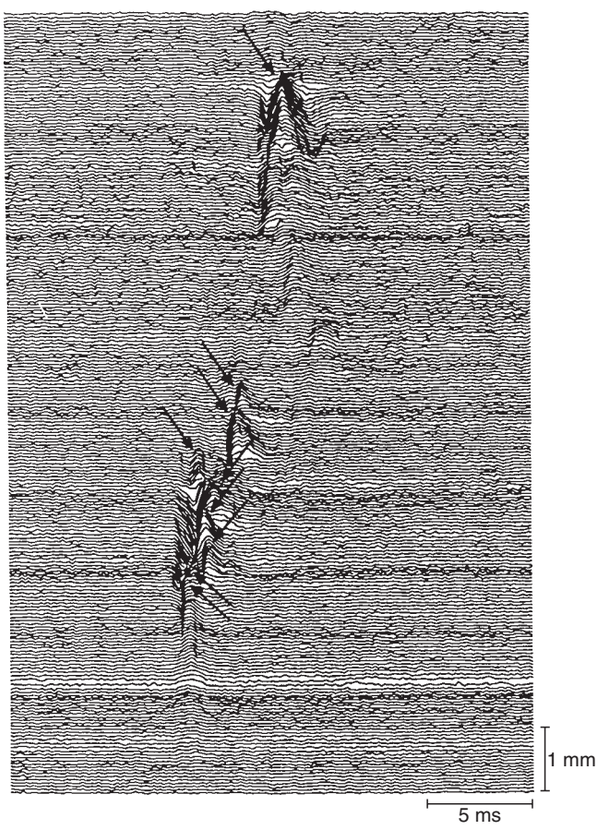

Figure 1.7. Topographical territory of a motor unit from human biceps, as measured by scanning EMG. Each line represents successive steps of 50 µm through the muscle and the motor unit action potential is recorded at each step. For the biceps, the mean cross-sectional length of a motor unit territory was approximately 5 mm. In patients with nerve injury and reinnervation, the territories were of similar size. From Stalberg and Trontelji (1994) [62] with permission.

Table 1.1. Estimates of innervation ratios of motor units in human muscles

Muscle	Number of motor axons	Number of muscle fibers	Innervation ratio	Reference
Biceps	774	580 000	750	Buchthal, 1961 [64]
Brachioradialis	315	129 000	410	Feinstein *et al.*, 1955 [63]
First dorsal interosseous	119	40 500	340	Feinstein *et al.*, 1955 [63]
Medial gastrocnemius	579	1 120 000	1934	Feinstein *et al.*, 1955 [63]
Tibialis anterior	445	250 200	562	Feinstein *et al.*, 1955 [63]

innervated by one motor neuron varies widely between different muscles. In humans, innervation ratios have been estimated by dividing an estimate of the total number of muscle fibers in a muscle by counts of the number of large axons in cross-sections of the muscle nerve. Such calculations have produced estimates of innervation ratios ranging from less than a dozen for the extraocular muscles to over a thousand for motor units of large limb muscles (Table 1.1) [63, 64]. Physiological methods have also been used to estimate the number of motor units innervating certain muscles, and these studies have also shown similar ranges [6]. However, using the glycogen depletion method to identify the fibers of individual motor units in animals, Burke and Tsairis [56] found considerable variation in the innervation ratios for different units within a given muscle. The innervation ratio of the motor unit is a major factor governing its force output. Variation in innervation ratios is likely to provide much of the variability in force output produced by different motor units within a muscle [65, 66].

Muscle fiber types

For more than a century, it has been recognized that mammalian muscles fall into two general groups: dark "red" muscles with slow contraction times and lighter "white" muscles with fast contraction times. Histological and physiological studies have shown that most muscles contain a mixture of muscle fibers with differing contraction speeds and force outputs; muscles composed of purely fast or slow muscle fibers are exceptional (for reviews see [67, 68]). The isoform of the myosin heavy chain (MHC) expressed in the muscle fiber is one of the most important factors influencing the speed of contraction, because the rate of ATP hydrolysis determines the speed of cross-bridge cycling and sarcomere shortening [69, 70]. Other factors affecting the contractile speed of muscle fibers include the isoforms of the calcium reuptake and release proteins expressed and the density of the SR [71, 72, 73, 74]. There are three major isoforms of MHC expressed in adult human limb muscles: MHC I, also called slow myosin; and the two fast isoforms, MHC IIA and MHC IIX (also called MHC IID). Subtypes of these isoforms, as well as embryonic and neonatal forms of MHCs, generate further diversity. The fast and slow isoforms of myosin were first able to be distinguished histochemically because of their differing amounts of ATPase activity at acid and alkaline pH [75]. This histochemical difference allowed fast and slow muscle fibers to be classified into two types. Fast and slow muscle fiber types are further subdivided by their dependence on aerobic or anaerobic metabolic pathways. Muscle fibers that utilize oxidative metabolism for energy needs have abundant mitochondria and lipid droplets. In contrast, muscle fibers using anaerobic pathways for energy tend to be richer in glycolytic enzymes with more abundant glycogen stores. Histochemical methods for demonstrating mitochondrial enzymes combined with myosin ATPase activity have traditionally been used to define three major types of muscle fiber in adult human limb muscles, described below. The histochemical properties of different fiber types correspond fairly well to their contractile properties, allowing muscle fibers to be grouped into a small number of types by either histochemical or physiological measures. It should be recognized, however, that qualitative and quantitative differences in expression of fiber-type-specific proteins generate a continuous range of physiological properties.

Type 1 muscle fibers have a slow twitch and use oxidative metabolism. Type 1 fibers express MHC I, the slow isoform of myosin, and contain many mitochondria. These muscle fibers can be visualized histochemically by strong myosin ATPase activity at low pH and by dense staining for mitochondrial enzymes such as NADH dehydrogenase (i.e., nicotinamide adenine dinucleotide, reduced) and SDH (i.e., succinate dehydrogenase) (Table 1.2). Compared to Type 2 fibers, their SR is less abundant, and it contains a slower isoform of the SR calcium ATPase. Type 1 fibers contain myoglobin, a protein that binds oxygen and confers a red color, and have a rich capillary blood supply [76]. The metabolic profile and vascularization render Type 1 muscle fibers highly resistant to fatigue, and thus suitable for sustained contraction under aerobic conditions. The acronym "SO," slow oxidative, is used by some to denote these fibers.

Type 2 muscle fibers are fast-twitch fibers, expressing fast isoforms of myosin which exhibit strong ATPase activity at alkaline pH. There are several subtypes of Type 2 fibers, but two major subtypes occur in human limb muscles. Type 2A fibers express the MHC IIA isoform of myosin. Compared to Type 1 fibers, their SR is denser, and expresses isoforms of calcium handling proteins that allow a more rapid cycling of calcium ions from SR [71, 72, 73]. Mitochondria are relatively abundant in Type 2A fibers. In addition Type 2A fibers

Table 1.2. Features of muscle fiber and motor unit types. Cox, Cyclo-oxygenase; EPSPs, excitatory postsynaptic potentials; FF, fast twitch, fatigable; FR, fatigue resistant; IPSPs, inhibitory postsynaptic potentials; NADH dehydrogenase, nicotinamide adenine dinucleotide, reduced; PAS, periodic acid Schiff; S, slow twitch, fatigue resistant; SDH, succinate dehydrogenase

Histochemical properties	**Muscle fiber types**		
	1	**2A**	**2B**
Myosin ATPase (pH 9.4)	Low	High	High
Myosin ATPase (pH 4.6)	High	Low	Medium
Oxidative enzymes (SDH, NADH dehydrogenase, Cox)	High	Medium	Low
Phosphorylase	Very low	High	High
Glycogen (PAS)	Low	High	Medium
	Motor unit types		
Mechanical properties	**S**	**FR**	**FF**
Twitch contraction time	Slow	Fast	Fast
Maximum tetanic force	Small	Moderate	High
Fatigue resistance	Very high	Moderate/ high	Low
"Sag"	No	Yes	Yes
Motor neuron properties			
Axon conduction velocity	Slower	Fast	Fast
Soma diameter, membrane area	Smallest	Large	Largest
Input resistance	Highest	Low	Lower
Rheobase (excitability)	Low	Higher	Highest
AHP duration	Longer	Short	Short
Properties of synaptic organization			
Monosynaptic Ia EPSPs	Largest	Large	Small
Disynaptic Ia IPSPs	Largest	Large	Small
Recurrent (Renshaw) IPSPs	Largest	Large	Small
Cutaneous inputs from distal limbs	Mainly IPSPs	Mainly EPSPs	Mainly EPSPs

Notes: Adapted from Burke, R. E., The structure and function of motor units. In *Disorders of Voluntary Muscle*, 7th edn., ed. G. Karpati, D. Hilton-Jones, R. C. Griggs. (Cambridge: Cambridge University Press, 2001), pp. 3–25.

contain glycolytic enzymes, such as phosphorylase, and have abundant glycogen stores. These metabolic properties allow Type 2A to function under aerobic and anaerobic conditions, and provide them with a fairly high resistance to fatigue. Type 2A fibers have been denoted by the acronym "FOG" because they are fast twitch with oxidative and glycolytic metabolic capabilities. The third major muscle fiber type that occurs in human limb muscles is the Type 2B fiber. Type 2B fibers express the fastest isoform of myosin, MHC IIX (also known as IID). Their SR is dense and contains a fast isoform of SR calcium ATPase. Type 2B fibers have relatively sparse mitochondria, but contain glycolytic enzymes and stores of glycogen. Type 2B muscle fibers fatigue easily, but are suitable for short bursts of anaerobic exercise. The acronym "FG," fast, glycolytic, is sometimes used.

Other isoforms of myosin are found in specialized muscle or at different developmental stages. In a number of animal species, Type 2B fibers express a very fast form of myosin, the MHC IIB isoform, particularly in muscles with very fast speeds of contraction [77, 78, 79, 80]. In humans, MHC IIB expression has been reported in some cranial muscles [81] but it is not expressed to a significant extent in limb muscles. Immature forms of myosin are expressed by muscle fibers prior to completing their differentiation during development [82, 83]. Fibers expressing immature forms of myosin that stain for ATPase activity at acid and alkaline pH, Type 2C fibers, are found in small numbers in normal adult limb muscles. The Type 2C profile occurs in regenerating fibers, which can be common in several muscular dystrophies. Muscle spindles also express a mixture of immature and slow isoforms of myosin [84].

The classification of the major muscle fiber types by their pattern of MHC expression agrees relatively well with the histochemical classification of fiber Types 1, 2A, and 2B that is based on myosin ATPase activity at differing pH. However, histochemical methods are relatively insensitive to hybrid muscle fibers expressing more than one MHC isoform. Hybrid muscle fibers can be demonstrated with immunocytochemical methods or *in-situ* hybridization for different isoforms of MHCs [80, 85]. Combinations of MHC IIA with IIx expression are relatively common in Type 2 fibers, for example [85, 86]. In some muscles hybrid fibers make up a sizeable fraction of the muscle fibers [78, 79, 85]. Hybrid fibers may play a role in the ability of muscle fibers to undergo rapid adaptations in response to training and use [87, 88, 89, 90].

Association of motor unit types with muscle fiber types

All muscle fibers belonging to the same motor unit have the same type, as judged from their staining for ATPase activity [54, 91, 92] and MHC isoforms [93, 94, 95]. Within a muscle unit the fibers also appear to have similar metabolic enzyme capacities [94, 96]. It is, therefore, assumed that muscle fibers within the motor unit also have essentially identical mechanical properties. Edström and Kugelberg [54] were the first to use the glycogen depletion method to examine the association between the mechanical properties and histochemical characteristics of the muscle fibers of individual motor units for two types of fast-twitch motor unit in rats. Burke and coworkers [55, 56] later used the same approach to examine the histochemistry of muscle fibers within the full range of physiologically identified motor units in the cat gastrocnemius muscle. In these studies, motor neurons were characterized physiologically with intracellular recordings, including stimulation with short stimulus trains while measuring force output and

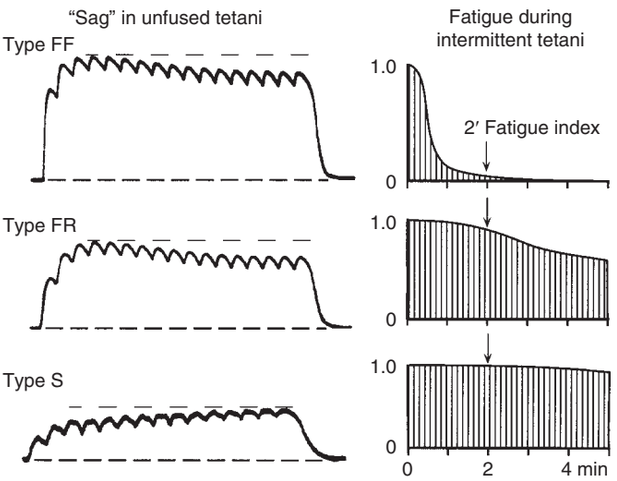

Figure 1.8. Mechanical responses from three muscle units to illustrate the properties used to identify motor unit types physiologically: FF, fast twitch, fatigable; FR, fast twitch, fatigue resistant; and S, slow twitch, fatigue resistant. The records in the left column are unfused tetani produced by repetitive stimulation at intervals near 1.25 times the respective twitch contraction times. The FF and FR unit responses show an early maximum force and subsequent "sag." The graphs on the right show the peak force produced by a sequence of short, unfused tetani produced by 13 stimulus pulses at 40 Hz, delivered every second for 5 min (duty cycle 0.33). The fatigue index is calculated as the ratio of the peak tetanic force after 2 min of repetitive stimulation (arrows) divided by the force produced by the first tetanus. The fatigue index of Type FF units was less than 0.25 while values for the FR and S units were greater than 0.75. The two properties taken together serve to distinguish three groups, with a fourth group, F(int), having a fatigue index between 0.25 and 0.75 and "sag" in unfused tetani. Contributed by R. E. Burke.

prolonged stimulation to deplete glycogen stores in active muscle fibers. Burke and coworkers found that motor units differed in several mechanical properties, not just the speed of contraction. These properties included the magnitudes of force produced by individual twitches (twitch force) and the maximal force produced by repetitive stimulation (tetanic force), resistance to fatigue during sustained activation, and the ratio of the twitch to the tetanic force [67]. These properties each exhibited continuous distributions that initially made it problematic to define distinct groups of motor units. However, two criteria were found that permitted relatively clear clustering of motor units into fast and slow groups in the cat: a "fatigue index" based on the decline in force output during a defined sequence of intermittent tetanization and a "sag property" based on the shape of unfused isometric tetanic contractions (Figure 1.8) [55, 91, 92, 97, 98]. Using these criteria, Burke and colleagues were able to define three main types of motor units: Type FF (fast twitch, fatigable), Type FR (fast twitch, fatigue resistant) and Type S (slow twitch, fatigue resistant). Some fast-twitch units exhibited fatigue resistance intermediate between those of FF and FR units and were, therefore, referred to as F(int) or FI [56, 92, 99]. Physiologically, there was a perfect match between S, FR and FF motor units with the histochemically defined muscle fiber Types 1, 2A, and 2B, respectively (Table 1.2; see also [97, 98, 100]). They also found some evidence that fibers in the minority F(int) unit type were histochemically distinct from the three main types [56, 98]. These same physiological criteria have been used with somewhat more variable success in classifying motor units in rat muscles (e.g., [101, 102]). It is possible that some of the variation in properties such as contraction time within a given motor unit type are associated with hybrid combinations of myosin isoforms, but this remains to be investigated systematically.

Motor units in human muscles

There is a wealth of information available from EMG studies in humans about the behavior of motor units in normal and diseased muscle, and it has been known for some time that fast- and slow-twitch muscle fibers coexist in human muscle [103]. However, for obvious technical reasons, it is difficult to examine the mechanical responses of individual motor units under the controlled conditions possible in animal experiments. Denny-Brown and Pennybacker [104] were the first to record individual twitches from the fasciculations of motor units in patients with motor neuron disease, using an indirect pneumatic transducer. Buchthal and Schmalbruch [105] used a mechanical transducer attached to a needle inserted into tendons, plus intramuscular stimulation of small nerve branches, to demonstrate that small groups of human motor units in normal muscles generate a wide range of twitch speeds, which varied in relation to the predominant local fiber type (see also [106]). The introduction of spike-triggered computer averaging into clinical neurophysiology made it possible to record the responses of individual motor units with greater assurance [107]. In this technique, discharges of single motor units during steady voluntary contractions are used to trigger an averaging computer while measuring the force produced by an appendage (e.g., a finger) attached to a force transducer. There are two limitations of this technique. First, the recorded twitch responses are not isolated twitches but rather components of unfused tetani, leading to errors in estimating the twitch forces and contraction times [108, 109]. Intra-neural stimulation of single motor axons to produce twitches has been used in an attempt to overcome this problem [110, 111, 112]. Secondly, the mechanical responses measured can be significantly degraded by the compliance of components between the active muscle fibers and the force transducer, including tendons of various lengths. Despite these technical limitations, most of the contractile properties measured from human motor units are generally similar to those from animals [113, 114]. There is disagreement about whether fatigability and "sag" can be used to classify human motor unit types in the same manner as in animals, and whether force measurements relate to the fatigability in the same way [110, 112, 115]. However, when motor units have been identified by glycogen depletion in muscle biopsy samples, these properties were consistent with histochemical identification [116]. Overall, the available physiological evidence and correspondence with

the histochemical classification strongly suggest that the basic characteristics of Types S, FF, and FR human motor units are similar to those described for the cat and rat.

Functional correlates of fiber properties and motor unit types

It is clear that many factors contribute to mechanical properties of the different motor unit types: in addition to the expression of MHC isoforms, there are fiber-type-specific differences in myosin light chains, troponin and tropomyosin proteins, proteins involved in calcium release and reuptake, and sarcotubular structures [72, 73, 74, 117]. It seems likely that the "sag" property, which differs sharply in fast and slow units, is produced by interactions among these factors [67, 68, 118]. Resistance to fatigue is directly related to the oxidative capacity of the different fiber types (Table 1.2; [91, 119]), as well as to their mitochondrial content [72] and local capillary supply [120]. These correlations are certainly causally related. The forces produced by individual motor units can vary by over two orders of magnitude during tetanization, and this variation is correlated with motor unit type (Figure 1.8 and Table 1.2). The force produced by a motor unit is a function of the effective cross-sectional area of its muscle fibers and the specific force output of that fiber type per unit area. Estimation of the effective cross-sectional area must take into account the effective innervation ratio [121], which may approximate the actual innervation ratio in pinnate muscles [91] but would be less in interdigitated muscles which have unit fibers in serial arrays (Figure 1.5). In general, Type 1 and 2A fibers have smaller diameters than Type 2B, making fiber area an important component of the equation. In humans, Type 2 fibers exhibit the greatest variability in diameter; in general fiber diameters tend to be larger in men than women [122]. There is some controversy about whether specific force output, which cannot be measured directly, differs between units with Type 1 and 2 muscle fibers [65, 69, 91, 92].

Motor neurons and synaptic specializations

In view of the differences between muscle fiber types, it is not surprising that the motor neurons that innervate them exhibit corresponding physiological differences (Table 1.3; reviewed by [67]). In general, motor neurons of Type S motor units have slower axonal conduction velocities, longer durations of post-spike hyperpolarized after-potentials (AHPs), and higher whole-cell input resistance values than the cells that innervate either FR or FF motor units. The AHP duration is particularly important because it is a key factor that controls the rate of motor neuron firing; motor neurons of Type S units have the longest AHPs and generally fire more slowly than those of FR or FF units. When examined with intracellular labeling methods, the motor neurons of Type S units tend to be smaller in membrane area than Type FF cells; Type FR motor neurons are intermediate in size [9, 10]. There is no systematic

Table 1.3. Functional specialization of motor unit types

Functions	Motor unit type		
	S	FR	FF
Recruitment threshold	Low	Intermediate	High
Duty cycle	Long/ continuous	Intermediate	Short/ intermittent
Fatigue resistance	High	Medium/ high	Low
Metabolic cost at rest	High	Medium/ high	Low
Metabolic optimum action	Isometric	Shortening	Shortening
Force gradation with recruitment	Fine	Intermediate	Coarse

difference between axonal conduction velocities of FF and FR unit groups [123]. Although the distributions of motor neuron properties are continuous and exhibit large overlaps when sorted according to muscle unit type, the relative excitability of the motor neurons to depolarizing currents injected directly, measured as the rheobase (the amount of current required to produce action potentials reliably), is more closely related to unit type than other measures [124, 125]. The rheobase data imply that intrinsic motor neuron excitability varies according to the sequence S > FR > FF, which has important implications for the recruitment order of motor units (Figure 1.9).

The strength of several synaptic inputs to motor neurons shows type-related differences that are undoubtedly related to the way in which the various types of motor units are used during activity. For example, the average amplitudes of monosynaptic excitatory postsynaptic potentials (EPSPs) produced in motor neurons by group Ia muscle spindle afferents, which are largely responsible for the stretch reflex, are ordered as S > FR > FF (Table 1.2) [126, 127]. The same ordering is evident with the disynaptic inhibition produced by stimulation of group Ia afferents from antagonist muscles [126] and with disynaptic recurrent inhibition produced by Renshaw interneurons activated from motor axon collaterals [128]. The organization of synaptic efficacy is a key factor that controls the function of motor unit populations [129], and for most inputs to motor neurons, the ordering of synaptic efficacy follows the size principle. However, there is evidence that certain cutaneous inputs and supraspinal systems, notably the rubrospinal tract, tend to excite relatively high-threshold motor neurons while inhibiting low-threshold cells [130, 131, 132], a pattern opposite to that found in group Ia excitation. Although there would be potential advantages to competing control systems that could bypass low-threshold, slow-twitch motor units that are slow to relax, the idea that large,