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ALPHA, BETA AND GAMMA MOTONEURONS: FUNCTIONAL DIVERSITY IN THE MOTOR SYSTEM'S FINAL PATHWAY

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Since their discovery in the late 19th century our conception of motoneurons has steadily evolved. Motoneurons share the same general function: they drive the contraction of muscle fibers and are the final common pathway, i.e., the seat of convergence of all the central and peripheral pathways involved in motricity. However, motoneurons innervate different types of muscular targets. Ordinary muscle fibers are subdivided into three main subtypes according to their structural and mechanical properties. Intrafusal muscle fibers located within spindles can elicit either a dynamic, or a static, action on the spindle sensory endings. No less than seven categories of motoneurons have thereby been identified on the basis of their innervation pattern. This functional diversity has hinted at a similar diversity in the inputs each motoneuron receives, as well as in the electrical, or cellular, properties of the motoneurons that match the properties of their muscle targets. The notion of the diverse properties of motoneurons has been well established by the work of many prominent neuroscientists. But in today's scientific literature, it tends to fade and motoneurons are often thought of as a homogenous group, which develop from a given population of precursor cells, and which express a common set of molecules. We first present here the historical milestones that led to the recognition of the functional diversity of motoneurons. We then review how the intrinsic electrical properties of motoneurons are precisely tuned in each category of motoneurons in order to produce an output that is adapted to the contractile properties of their specific targets.

Keywords: Spinal cord; historical perspective; electrophysiological studies; physiological types of motor units; intrinsic properties of motoneurons; voltage-dependent currents.

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1. Introduction

Since the pioneering work of the great physiologist Sir Charles Scott Sherrington, it is widely recognized that a specific group of CNS neurons, called motoneurons, link the nervous system to the muscles. They are the final common pathway, i.e., the seat where all the peripheral and central neural pathways converge to elicit the motor output. A single motoneuron drives a subset of muscle fibers within a muscle, thereby defining the concept of motor unit [111]. Since Charles Sherrington's work we know the function of motoneurons: each of them transforms inputs arising from the numerous paths involved in motricity into an output that drives the contraction of the innervated muscle fibers. The Nobel Prize was awarded jointly to Sir Charles Scott Sherrington and Edgar Douglas Adrian, in 1932, "for their discoveries regarding the functions of neurons". The motoneurons are unique in the mammalian central nervous system, in the sense that they are the only neurons for which their function is so precisely known.

Moreover, the spinal motoneurons were the first central cells to be intracellularly recorded by one of Sherrington's pupils, John C. Eccles, who was awarded (alongside Hodgkin and Huxley) the Nobel Prize in 1963. Eccles designed elegant methods that allowed him and his numerous collaborators to decipher the many pathways that synapse onto motoneurons. This enterprise initiated by the Canberra group was pursued in Göteborg by Lundberg, Jankowska and many colleagues. A number of excellent reviews have already been written on this topic (see for instance [3, 84]). In the mean time Granit (who also was awarded the Nobel prize in 1967) initiated the study of the intrinsic properties of motoneurons [56]. His pupil, Kernell demonstrated that the discharge properties of motoneurons are well adapted to the mechanical properties of muscle fibers [92]. We had to wait the end of 1980s and the work of Hultborn and Hounsgaard in Copenhagen to discover that dendrites of motoneurons are not passive but endowed with active properties that play a critical role in the input–output transformation [69, 70].

Our conception of the motoneuron has considerably evolved since their discovery, when they were implicitly considered a uniform population. Nowadays, we know that they constitute indeed a very heterogeneous class of neurons. They differ by their function (that is, the muscle fibers they innervate), their intrinsic electrical properties, the pathways that control them, their molecular properties, and their susceptibility to degeneration. Provocatively, one may even argue that there is no such thing as a "canonical" motoneuron. The aim of this review is to explore the differences between classes of motoneurons. In the first part, we will review the historical milestones that led to the recognition of the enormous functional diversity of motoneurons. In the second part, we will review how the intrinsic electrical properties of motoneurons are precisely tuned in each category of motoneurons in order to produce an output that is adapted to the contractile properties of their specific targets. Our goal was not to make an exhaustive review of the literature, but instead to point out important physiological principles that we believe deserve attention in order to understand the motor system.

2. Motor Nuclei Comprise Many Functional Subclasses of Motoneurons: A Brief Historical Summary

2.1. *Two distinct populations of motoneurons: Alpha and gamma motoneurons*

Since the discovery of muscle spindles by Kölliker [96] in frog and by Kühne [99] in mammals, we know that muscle spindles contain a bundle of thin muscle fibers that look different from the ordinary muscle fibers. A first milestone, remarkably summarized in Matthews's monograph [120], was the recognition that, in a muscle nerve, only the largest motor axons innervate the ordinary muscle fibers whereas the smallest specifically innervate the intrafusal muscle fibers. Already, Eccles and Sherrington [45] clearly demonstrated, after degeneration of the afferent fibers, a bimodal distribution of the motor axon diameters. However, they believed that the smallest fibers branched less than the largest ones and that they innervated fewer ordinary muscle fibers. Surprisingly, they overlooked at that time the possibility that the two different sizes of motor axons might have different functions despite the fact that Langley [101] has proposed few years earlier that the smallest axons might specifically target the spindles.

The two populations were called "alpha" and "gamma" motor axons on the basis of electrophysiological experiments demonstrating that small motor axons with high electrical threshold conduct the action potentials more slowly than large motor axon with low electrical threshold. Indeed, Erlanger, Bishop and Gasser [52] showed, in frogs, that the large motor fibers contribute to the first peak of the compound action potential recorded on the ventral roots upon stimulation of the sciatic nerve. They called this peak the "alpha peak". Later on, Leksell [107], in Granit's laboratory, demonstrated in cat experiments that another wave, which he named the "efferent gamma wave", appeared when the stimulation was increased about four times the threshold for the most excitable fibers in the alpha peak. Leksell found that the motor axons in the gamma wave conduct the action potentials at much slower velocities than the fastest axons in the alpha peak. The fast and slow conducting fibers in the alpha and gamma peaks were then routinely called alpha and gamma motor axons in the Granit laboratory. By extension, the corresponding cell bodies in the ventral horn were called alpha and gamma motoneurons. This nomenclature has been rapidly adopted by the community of motor system physiologists.

2.2. *Alpha and gamma motoneurons have different functions*

Elegant electrophysiological works provided the definitive demonstration of the fusimotor function of small axons and the ordinary function of the large ones. Matthews [117] was the first to demonstrate an excitation of spindle afferents when increasing the stimulation of the nerve above that required to cause the maximal contraction of the muscle. By selectively blocking the large axons with a mechanical pressure applied on the nerve, Leksell [107] was able to demonstrate that the

stimulation of gamma fibers did not elicit any force at the tendon and that they, therefore, do not have the same function as the largest motor axons. This conclusion was later confirmed by Kuffler, Hunt and Quilliam [98] who, using their novel elegant technique of isolation of a single functional motor axon in the ventral root (and single functional afferent fiber in the dorsal root), demonstrated that the stimulation of a single gamma motor axon did not produce any force. Instead, stimulation of a gamma axon increased the discharge from single spindle afferent endings. Each spindle afferent was influenced by a number of gamma motoneurons with axonal conduction velocities ranging from 15 to 55 m/s [76, 97].

2.3. Two subtypes of gamma motoneurons

It was recognized early that spindles bear two types of sensory endings: the primary ending, innervated by the Ia afferent fiber, and secondary endings innervated by group II fibers [75]. Cooper [37] was the first to show that primaries and secondaries do not exhibit the same sensitivity to muscle stretches. She found that only primary endings are sensitive to dynamic stimuli, i.e., their rate of discharge increases with the stretch velocity, whereas the secondary are relatively insensitive, i.e., their firing rate does not depend on the velocity. These results were largely confirmed by Matthews *et al.* on de-efferented spindles (ventral roots cut) in which any fusimotor action was eliminated [118]. An elegant demonstration of differences between primary and secondary endings was further given by Bessou and Laporte [13] when they recorded from one afferent of each type belonging to the same spindle situated in the tenuissimus muscle of the cat. A consensus was soon reached; primary endings exhibit a strong dynamic sensitivity, whereas secondary endings have a better ability to encode muscle length, i.e., a higher static sensitivity.

During that time, the effects of stimulating single gamma motor axons, isolated in ventral root filaments, on the response of primary and secondary endings to stretching were also found to be of two types. In Bessou and Laporte's work, a gamma axon was found to increase the dynamical response during the stretch of the primary ending but had virtually no effect on a secondary ending of the same spindle [13]. This was a fusimotor axon with a "dynamic" action. Reciprocally, another gamma axon had no effect on the dynamic sensitivity of the primary ending but increased the response of the secondary endings. This axon had a "static" action only. At the same time, Matthews and his collaborators extensively investigated the actions of gamma axons on primary endings during servo-controlled stretches [20]. In their experiments, the dynamic sensitivity of primary endings was quantified using a "dynamic index". The gamma motor axons were classified in dynamic or static depending on their effects on responsiveness of primary endings during the ramp stretch. The dynamic gamma axons increased the dynamic index of primary endings whereas static gamma axons caused a decrease in the dynamic response even though they increased the overall excitability of the ending. A further argument in favor of two gamma axon types was given by experiments in which the action of a

single gamma axon was investigated in several spindles. Each gamma axon has the same fusimotor effect, either dynamic or static, in every spindle it innervates [14, 19]. This provides the best evidence that the classification between dynamic and static gamma axons genuinely reflects functional properties.

It was further known that the spindles contain two main types of intrafusal fibers: the nuclear bag fibers with relatively large diameter and long length, and the nuclear chain fibers with small diameters and relatively short length [4]. This led Matthews [119] to hypothesize that the differential effects of the dynamic and the static gamma axons could be explained if they respectively innervated nuclear bag fibers and nuclear chain fibers with different viscoelastic properties. This view proved, however, to be incorrect, but not far off the mark. Indeed, degeneration experiments showed that gamma axons can innervate both nuclear bag fibers and nuclear chain fibers [6]. Moreover, remarkable experiments carried out by Bessou and Pagès [15] and by Boyd, Gladden, McWilliam and Ward [16], in which electrophysiological recordings (action of a single gamma motor axon on a primary ending) were coupled to kinematic analysis of the contraction of intrafusal fibers, demonstrated that: (1) the dynamic gamma axons innervate a single nuclear bag fiber (that was shown to contract slowly); and (2) the static gamma axons innervate the other nuclear bag fiber (that displays a fast contraction) and/or the nuclear chain fibers. Experiments using the glycogen depletion method later on confirmed the selective innervation of dynamic and static gamma axons [18].

To summarize, in addition to the classical alpha motoneurons, there is a specific class of motoneurons, the gamma motoneurons, which innervate the mammalian muscle spindles (Fig. 1). These motoneurons allow a control of the spindle sensitivity that is independent of the control of the motor units. Among the gamma motoneurons, some (the gamma dynamic motoneurons) innervate only the bag1 fiber and they act by enhancing the dynamic sensitivity of the primary ending. The others (gamma static motoneurons) innervate the bag2 fiber and the chain fibers and they mainly act by enhancing the overall stretch sensitivity of primary and secondary endings.

2.4. Three main subtypes of alpha motoneurons

The recognition that alpha motoneurons innervate different physiological types of muscle fibers (Fig. 1) arose in parallel with the distinction of the subtypes of gamma motoneurons. Indeed, we know since Ranzier's work [137] that the contraction is slower in red muscles than in pale ones. Histological studies have revealed that most mammalian skeletal muscles are made of a mosaic of muscle fibers with different histological characteristics (see [33] for a review). The technique of isolation of a single functional motor axon in the ventral root allowed investigating the force (isometric force recorded at the tendon) and the physiological properties of single motor units. This enterprise was initiated by Laporte's and Henneman's groups. It was shown that the fastest conducting axons supply large motor units (large force) that contract with a high speed whereas slow conducting axons supply small motor

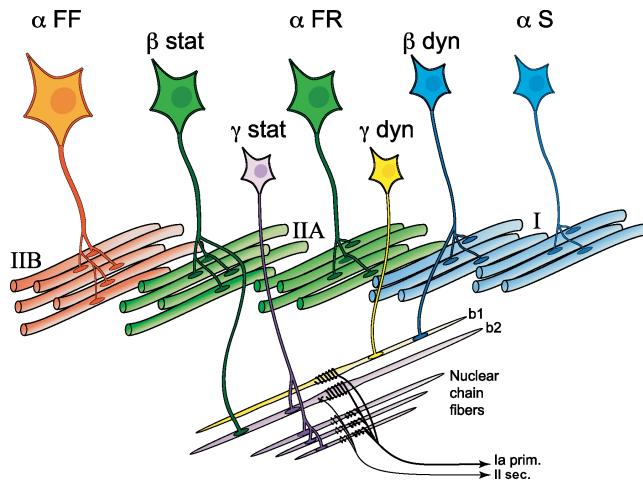


Fig. 1. Schematic representation of the different types of motoneurons. The figure represents seven motoneurons innervating either extrafusal or intrafusal muscle fibers. FF-type alpha motoneurons are the biggest motoneurons (in term of soma size and axon diameter), and innervate a large number of type IIB extrafusal muscle fibers. FR alpha motoneurons are slightly smaller and innervate type IIA extrafusal muscle fibers. S-type alpha motoneurons are the smallest of the alpha motoneurons, they innervate fewer type I muscle fibers. Beta motoneurons are skeleto-fusimotor: they innervate both extrafusal and intrafusal muscle fibers. Beta static motoneurons innervate either type IIA or IIB extrafusal fibers and the intrafusal bag2 fiber. Beta dynamic motoneurons innervate type I extrafusal muscle fibers and the intrafusal bag1 fiber. Gamma motoneurons innervate exclusively intrafusal muscle fibers and are the smallest of the motoneurons. Gamma static motoneurons innervate the intrafusal bag2 fiber and/or the nuclear chain fibers. Gamma dynamic motoneurons innervate the intrafusal bag1 fiber. Note that in a muscle, the various types of extrafusal muscle fibers are mingled together and organized in a mosaic, while the intrafusal muscle fibers are much smaller than the extrafusal fibers and are ensheathed in the spindle capsule. Primary and secondary endings of the spindle encode parameters of the muscle stretches that are sent to the central nervous system via afferent fibers Ia and II.

units that contract slowly [11, 150]. Each muscle was shown to contain motor units with a wide range of physiological properties and it was assumed that these properties must be related in some way to the molecular properties of their muscle fibers.

Progress of histochemistry in the 1960s revealed the ATPase activity (mitochondrial and myofibrillar), the content in mitochondrial oxidative enzymes (succinic dehydrogenase, NADH dehydrogenase) and the glycolytic activity of muscle fibers. Many classification systems of muscle fibers have then been proposed (for a review see [33]). Most of them distinguished three histochemical profiles of muscle fibers. In particular, Brooke and Kaiser [17] classified the muscle fibers into types I, IIA and IIB. This classification was based on the ATPase reactivity pattern of muscle fibers. Later on, it was shown that isoforms of myosin heavy chains are differentially expressed in the different types of muscle fibers, and nowadays classification of muscle fibers relies instead on immunohistochemistry of myosin heavy chains (for a review see [140]).

Edström and Kugelberg [47] were the first to use a method based on the depletion of glycogen in order to map the territory occupied by muscle fibers of a single motor

unit. Following a prolonged repetitive stimulation of a single motor axon, the glycogen is depleted in the innervated muscle fibers that can be revealed in cross muscle sections using the periodic acid-Schiff reaction. Succinic dehydrogenase and phosphorylase activities of the depleted muscle fibers were assessed in serial sections. This allowed them to investigate the “histochemical profile” of the muscle fibers and to correlate this profile with the physiological characteristics of the motor unit. They found a correlation between resistance to fatigue and activity of oxidative enzymes, but they did not find any relation between the twitch contraction time and histochemical profile. Moreover, the fibers of a given motor unit were not spatially grouped but scattered within the muscle.

A decisive progress was made by Burke *et al.* [26, 27] who combined intracellular stimulation of motoneurons innervating the gastrocnemius muscles with the glycogen depletion method. They found that two physiological parameters were best suited to separate the motor unit population into three physiological types. The first parameter was the presence or the absence of a sag on an unfused tetanus produced by a stimulus train in which the period was about 1.25 times the contraction time of the motor unit. The sag was present on the fast contracting motor units and absent on the slow contracting ones. The second parameter was the fatigue index. Burke *et al.* developed a stimulation paradigm (intermittent tetanization, i.e., short tetanus repeated every second during two minutes) that did not fatigue the neuromuscular transmission but induced some fatigue in the muscle fibers themselves. The fatigue index (ratio of the tetanus force at 2 minutes to the initial tetanus force) allowed them to distinguish fatigable motor units (fatigue index < 0.25) from resistant motor units (fatigue index > 0.75). It appeared that all motor units without sag were fatigue resistant, and they were thereafter called slow contracting motor units (S type). Most of the motor units that displayed a sag were either fatigable (fast contracting fatigable motor units, FF type) or fatigue resistant (FR type). A few fast contracting motor units had an intermediate fatigability (FI type). Thanks to the glycogen depletion method, Burke *et al.* [26, 27] further demonstrated that all the muscle fibers of a given motor unit exhibited the same histochemical profile. Furthermore, they found a correlation between the physiological type and the histochemical profile: type S motor units have type I muscle fibers, type FR motor units have type IIA muscle fibers, and type FF motor units have type IIB muscle fibers. The glycogen depletion technique also allowed counting the numbers of fibers in a single motor unit (i.e., the “innervation ratio”). The largest number was found in the FF motor units and the smallest in the S ones (intermediate number in the FR motor units) [28]. This fitted with the fact that FF motor units had the fastest axonal conduction velocity (presumably because the large number of axonal intra-muscular branches necessitate a large diameter axon) and developed the highest force whereas S motor units had the slowest axonal conduction velocity and developed the smallest force. Since this pioneering work, the three physiological types of motor units have been demonstrated to be present in many skeletal muscles, not only in cats, but also in many mammal species including humans [23].

The experiments done by Burke *et al.* were indeed very powerful. Since the motoneurons were stimulated with an intracellular microelectrode, the authors could also record the basic intrinsic properties of motoneurons. They were then able to correlate these properties with the physiological type. Their work contributed to show (along with many works from different groups including Henneman's group) that the electrical properties of the motoneurons are in keeping with the supposed function of the motor unit (S and FR motor units likely involved in postural activity, FF motor units likely involved in transient and powerful movements; see below part II, and also [23] for a review). The demonstration by Burke *et al.* that motor units can be classified within three physiological types that correlate well not only with the three histochemical profiles of muscle fibers but also with the electrical properties of the motoneurons proved to be conceptually most important.

2.5. *The beta motoneurons: A third distinct category of motoneurons*

The specific innervation of intrafusal muscle fibers by gamma motoneurons seems to be the result of the evolution since it appears only in mammals. In lower vertebrates, such as amphibian and reptiles, the intrafusal innervation arises from branches of the same axons as those that innervate the ordinary (extrafusal) muscle fibers [144]. These axons have been called skeleto-fusimotor axons, or beta axons. However, it should be noted that the name beta does not refer to the conduction velocities of these axons and was chosen only to differentiate them from the alpha and gamma axons.

It was long discussed whether or not mammalian spindles are also innervated by skeleto-fusimotor axons in addition to their specific gamma innervation. Definitive answer to this question was provided by elegant experiments carried out by Laporte's group. To be undoubtedly identified as skeleto-fusimotor, an axon must produce both an extrafusal contraction and an excitation on spindle ending. However, the difficulty of the experiments was to differentiate the *direct* activation of the spindle ending that is elicited by the contraction of intrafusal fibers themselves from the *indirect* activation that could be caused by a passive stretching of the ending due to the contraction of adjacent extrafusal fibers. The demonstration of the direct character of the spindle activation is required to identify with certainty the axon as skeleto-fusimotor. On a very small cat muscle (the first deep lumbrical muscle) that contains less than 10 motor units, Bessou *et al.* [10, 12] stimulated, in ventral root filaments, single motor axons innervating this muscle while they recorded in dorsal root filaments afferent fibers innervating primary spindle endings of the same muscle. They found that some slowly conducting axons (that innervate slow contracting motor units) elicit a direct activation of spindle primary endings. Their demonstration relies on two observations: (1) The firing frequency of the spindle ending still increased when the stimulation frequency of the axon was increased above the frequency that elicits the maximal contraction of the motor unit (tetanic

fusion frequency). (2) The discharge of the primary ending persists after a light curarization sufficient to block completely the neuromuscular transmission to extrafusal muscle fibers but not the neuromuscular transmission to intrafusal muscle fibers. Both observations indicate that the spindle activation was not correlated with the extrafusal contraction and point out to a skeleto-fusimotor innervation. Interestingly, Bessou *et al.* [12] also showed that the presence of the beta innervation does not preclude a concomitant gamma innervation. The same spindle might be innervated both by one beta axon and by gamma axons with dynamic and static actions. Neuroanatomical evidence of the beta innervation was soon provided. Adal and Barker [1] were able to trace under microscope the innervation of the first deep lumbrical muscle. They found that some axons innervate both extra- and intrafusal fibers confirming the presence of beta axons. In the steps of Laporte's group, physiological arguments in favor of beta innervation was also provided for rat tail muscles [93] and cat tibialis posterior muscle [19].

2.6. Two subtypes of beta motoneurons: Slow contracting motor units have a dynamic action on spindle endings; fast contracting motor units have a static action on spindle endings

In their pioneering experiments, Bessou *et al.* [10, 12] have also investigated the action of the beta axons on the responsiveness of the primary ending during a muscle stretching. They found that these axons increased the dynamic sensitivity of the ending. Barker's group and Laporte's group then joined their efforts to use the glycogen-depletion method in order to study the intra- and extrafusal fiber types involved in the beta-innervation pattern. The dynamic beta axons were found to innervate the bag1 intrafusal muscle fiber, i.e., the effector of dynamic action, and extrafusal muscle fibers of the I type, i.e., the slow contracting motor units (see Fig. 2 and [5]).

Interestingly, in rabbit lumbrical muscles, Emonet-Denand *et al.* [49] using the same methods as in Bessou *et al.* [10, 12], found that despite the fact that most beta axons have a dynamic action on spindle primary endings, a fraction of them instead elicit static actions. However, a systematic investigation in various hindlimb muscles of the cat carried out by the same experimentalists [50] have revealed only exceptionally the presence of beta axons with static action. Almost all of the beta axons were found to elicit a dynamic action in cat muscles. Moreover, most dynamic beta axons have relatively slow conduction velocities (between 40 and 85 m/s, [50]). It was then speculated that the discrepancy between rabbit and cat experiments might be due to the fact that, for some reason, the intrafusal neuromuscular junctions, and particularly those of the chain fibers, are more sensitive to curare in the cat spindles than in the rabbit spindles. In cats, the neuromuscular junctions of the chain fibers are blocked as easily as the neuromuscular junctions of the extrafusal fibers precluding the use of the differential curarization test to identify static beta axons.

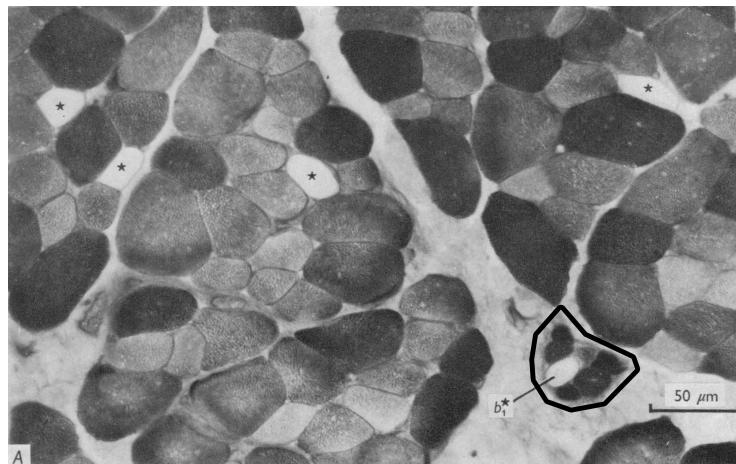


Fig. 2. Glycogen-depletion method revealing extrafusal and intrafusal muscle fibers innervated by a beta axon. An axon, with a faster conduction velocity (77 m/s) than gamma axons, was found to both increase the dynamic response to a muscle stretch in three spindles and to activate ordinary fibers in the cat peroneus brevis muscle. Glycogen depletion was obtained by the repetitive stimulation of the axon during which the blood flow was reduced by occluding the artery that supplied the muscle. After freezing, the muscle was cut in 10-mm thick sections. The present plate shows a section stained for glycogen using the periodic acid-Schiff (PAS) method. In the spindle visible on this plate (circled), the b1 fiber was depleted indicating that it was innervated by the axon (the b1 fiber of two other spindles were also depleted). In addition, extrafusal fibers (four on this plate, pointed out by asterisks) were depleted from their glycogen. Histochemistry on serial sections of myofibrillar ATPase activity and succinate dehydrogenase activity showed that these extrafusal fibers were of type I. Adapted from Barker *et al.* [5], with permission.

However, histophysiological studies using the glycogen-depletion method actually suggested the presence of static beta innervation in cat muscles [62, 81]. In these studies, the prolonged stimulation of groups of motor axons with fast conduction velocities (above 85 m/s) was found to induce a glycogen depletion essentially in nuclear chain fibers, i.e., the effectors of static actions, and more specifically in the longest of the chain fibers [62, 81]. In three experiments, in which a single fast-conducting motor axon was investigated, the depleted extrafusal muscle fibers were of the group IIA type, i.e., fast contracting and fatigue resistant motor unit [82].

Jami *et al.* [83] then designed new physiological tests that revealed the presence of static beta axons in the peroneus tertius, a small muscle of the cat. Beta static axons were identified using a combination of several protocols: (1) A differential fatigue of the neuromuscular junctions of extrafusal and intrafusal muscle fibers was elicited by prolonged periods of stimulation at 100–250 Hz. The fact that the activation of the spindle primary ending outlasts the complete block of the extrafusal contraction was taken as a sign of intrafusal action of the axon. (2) The excitation of spindle excitation should increase with stimulation frequencies above that eliciting maximal extrafusal contraction. (3) The discharge of the primary ending should still be modulated by these high stimulation frequencies. This indicated that the beta axon innervates intrafusal chain fibers that are known to exhibit tetanic fusion frequency

much higher than the extrafusal fibers. (4) Finally, the stimulation of the beta axon should exert a static action on the response of the spindle to ramp stretches.

Jami *et al.* [83] tested a large number of motor axons in the alpha range of conduction velocities. Among them, 21% proved to be static beta axons and 10% were dynamic beta axons (a total of 31% of the axons were thus beta axons). This figure was likely to be conservative since it was not possible in each experiment to test the action of every motor axon in all the spindles. Convergence on the same spindle of two (generally one static and one dynamic) or even three beta axons was frequently observed. Furthermore, the physiological type of the extrafusal muscle fibers was assessed using the same protocol as Burke *et al.* [26] (see above). Remarkably, all but one beta axons with static intrafusal action innervated FR or FF motor units whereas all but one beta axons with dynamic intrafusal action innervated S-type motor units (Fig. 1). The relative incidence of static versus dynamic beta axons depends on the proportion in FR/FF versus S motor units. The peroneus brevis (in which S motor units predominate) has more dynamic beta axons and less static beta axons than the peroneus tertius, in which FR/FF motor units predominate [51]. Dynamic beta effects occur when slow-contracting motor units are recruited. Since the dynamic sensitivity of primary endings is increased, one might speculate that dynamic beta motoneurons help to restore the balance and to maintain the posture. Static beta effects are related to the recruitment of fast-contracting motor units. One might speculate that they prevent the discharge rate of spindle endings from slowing or even from pausing during rapid muscle shortening. The fact that in mammalian muscles about one third of motor units are indeed “beta units” and that about three out of four spindles are beta innervated [51] indicate that beta motoneurons play a significant part in the regulation of spindle activity and consequently in the control of posture and movement.

To summarize, it is now clear that the mammalian spindles are innervated by both gamma and beta motoneurons. Similarly to gamma motoneurons, beta motoneurons exert both dynamic and static actions in the spindle endings. However, the intrafusal and extrafusal innervations of beta motoneurons is very precisely organized (Fig. 1). Dynamic beta motoneurons innervate the intrafusal bag1 fiber and the extrafusal slow contracting fibers (S-type motor unit). Static beta motoneurons innervate the longest of the intrafusal chain fibers and the extrafusal fast-contracting fibers (FR or FF motor units).

3. Differences in Electrical Properties Create Subtypes of Motoneurons That are Functionally Adapted to Their Targets

As we have discussed so far, even though motoneurons share a common function, to elicit muscle fiber contraction, there are many different muscle fibers: intrafusal fibers (among them the bag1 fiber whose mechanical properties are very different from those of bag2 and chain fibers) and extrafusal fibers which can be slow contracting, fast contracting, fatigable or fatigue resistant (each extrafusal fiber is

innervated by a single motoneuron). The contractile properties of the muscle fibers also depend on the function of the muscle. It is clear that flexing one's biceps is a vastly different task than protruding one's tongue or producing an eye saccade. Among different species, it might also be self evident to the reader that the properties of the muscles of a tiny animal like the mouse need to be different than those of a larger animal like an elephant, or even a man. This extraordinary functional diversity has hinted, especially since the works of Burke, at a diversity in the electrical, or cellular, properties of the motoneurons that innervate different types of muscle fibers. Even though the notion of the diverse properties of motoneurons has been extensively studied, it tends to fade away in today's scientific literature, where all the motoneurons become a single cell population, which develop from a given population of precursor cells, and which express a common set of molecules. The aim of this section is therefore to provide an overview of the differences that can nevertheless exist between cells that are remarkably similar to each other, but still need to exhibit different electrical responses to produce a force adapted to their function. The description of the various channels expressed by motoneurons was the subject of several excellent recent reviews [21, 65, 132], and we will therefore only focus on the properties that differ between motoneurons.

Unfortunately, very little is known about the properties of gamma motoneurons, mostly because of their smaller size, which makes them harder to record from intracellularly. Even less is known about betas because it is very difficult to identify a motoneuron as beta while making intracellular recordings. Therefore, most of this section will be concerned with the differences among alpha motoneurons, with some details about the others when available.

3.1. The size principle or the orderly recruitment of motoneurons

Since the muscle fibers that constitute most muscles have different contractile properties, the order in which each motor unit is recruited is important for muscle force gradation and metabolic efficiency.

The recruitment of motoneurons depends on numerous factors. First and foremost, like all cells, the membrane of the motoneurons acts like a parallel RC circuit with a resistance and a capacitance. The input resistance of a motoneuron depends on its geometry and its specific membrane resistance, that is the resistance of the membrane per unit area, which is related to the amount of passive channels inserted in the membrane per unit area [136]. Current injected into the neuron through the recording electrode in experimental conditions, or through the opening of synaptic receptors in more physiological conditions, translates into a change of membrane potential proportional to the input resistance of the neuron.

Morphological analyses have shown that all motoneurons do not have the same size. There is approximately a threefold range in soma area [92] among motoneurons and those with the smallest somas have fewer primary dendritic branches and a smaller overall dendritic tree (Fig. 3). Furthermore, there is also a threefold range in

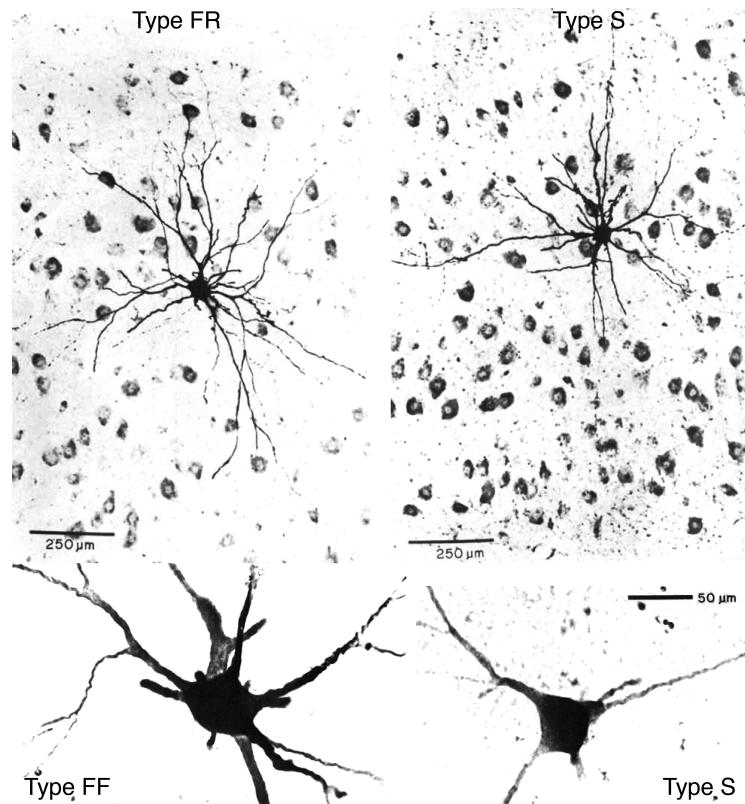


Fig. 3. Size differences among type identified motoneurons. The top two panels illustrate the difference in dendritic arborization between a FR motoneuron from the gastrocnemius pool (left) and an S motoneuron from the soleus pool (right). Adapted from Burke *et al.* [24], with permission. The bottom two panels illustrate the difference in soma size and the number of primary dendritic branches between a FF motoneuron (left) and an S motoneuron (right). Adapted from Burke [23], used with permission from the Am Phys Soc.

specific membrane resistance, such that the smallest motoneurons have the highest resistance per unit area of membrane, while the largest have the smallest specific resistance [58, 59]. The combination of the geometrical and electrical properties yields a 10-fold range in input resistance among motoneurons (although other intrinsic properties must also be considered, see below and [61]). This range seems to be critical for the orderly recruitment motoneurons, as it has been found in cats [151], rats [2] and mice [113].

Henneman *et al.* were the first to argue that an orderly recruitment of motoneurons according to their size (the “size principle”, see Ref. [67]) would allow a smooth gradation of the force produced by a motor pool as the “common drive” to the pool increased. This idea was supported by the fact that synaptic inputs are broadly distributed on all the motoneurons of a motor pool. For example, a single Ia afferent was shown to make contact with more than 90% of the motoneurons of the pool [124], which suggests that motoneurons receive a common input. However, the synaptic inputs are not uniformly distributed on motoneurons, and extensive studies by the

Binder laboratory have shown that different pathways can be biased toward smaller or larger motoneurons [65, 87]. Nevertheless, a multitude of studies in humans have shown the size principle to apply in multiple tasks, muscles and movement speeds [41, 53, 85, 143], which fully validate it as a genuine physiological principle.

The functional significance of the size principle was made especially clear when Burke was able to match the electrical properties of motoneurons with the contractile properties of their muscle fibers (see Part I above) [23]. He showed that there are very good correlations between the electrical properties of motoneurons and their physiological type (S, FR and FF, see Part I). As such, the smallest, most excitable motoneurons belong to the S-type, i.e., they innervate fibers that contract slowly and develop little force, but are highly resistant to fatigue. The FR motoneurons have a slightly lower resistance (are less excitable) and innervate fast-contracting, fatigue-resistant fibers. Finally, FF motoneurons are the biggest and the last to be recruited, they innervate fast and powerful muscle fibers that fatigue rapidly. From a metabolic point of view, the size principle allows optimizing the energy consumption of the motor system by first recruiting units that are metabolically efficient, however developing small forces and recruiting units that develop large amounts of force, but with poor efficiency, only when the task requires it [46].

What about gamma motoneurons? Gamma motoneurons are smaller than alphas but their input resistances are in the same range as those of S-type motoneurons suggesting a lower specific membrane resistance [149]. One might think that they would be recruited in the same time as the S-type motoneurons, which would imply that any motor tasks are always accompanied by static and dynamic gamma activation. However, the physiology seems more complex. In some motor tasks, gamma and alpha motoneurons are co-activated but in others they are activated independently (see [74] for a review). Indeed, gamma motoneurons do not share the same common inputs that alphas receive. In particular, gamma motoneurons do not receive monosynaptic Ia inputs [43, 89] (see also one example in [153]). Furthermore, it was shown that gamma dynamic and static motoneurons are differentially driven by descending supra-spinal inputs [74]. Consequently, one can assume that there are dedicated pathways on gamma motoneurons that can activate them specifically depending on the task to be performed.

Burke and Tsairis [29], while examining the muscle fibers that were depleted by the prolonged stimulation of a soleus motoneuron, fortuitously found in their material, one intrafusal bag1 fiber that was depleted in addition to extrafusal fibers. This motoneuron was therefore a beta motoneuron. Interestingly, this motoneuron was receiving monosynaptic Ia EPSPs suggesting that, unlike gamma motoneurons, beta motoneurons share the same synaptic drive as alpha motoneurons [29].

However, motoneurons, like other neurons, in particular are not biophysically passive because, even in the resting state, voltage-dependent channels are open and can influence their responsiveness. In cat spinal motoneurons for example, when one injects a small hyperpolarizing current step through the recording microelectrode, the membrane potential first reaches a peak value in 15–20 ms, then settles at a

smaller (more depolarized) value about 100 ms later [80]. This “sag” in the response is due to the presence of a mixed cationic current activated by hyperpolarization, which is known as the *h*-current (I_h) [121]. The HCN channels mediating this current are partly open at rest and contribute therefore to the input resistance of the motoneuron. When the membrane is hyperpolarized, more channels open, which increases their inward current, and thus depolarizes the membrane in return. Conversely, the HCN channels close when the membrane is depolarized, which lets less inward current in, and thus hyperpolarizes the membrane. As such, it is believed that the function of I_h is to stabilize the resting membrane potential [80].

Yet, the presence of I_h does not invalidate the size principle. Indeed, it was shown very early that the amplitude of the sag, which is roughly proportional to the conductance of the *h*-current, depends on the size of the motoneurons; small motoneurons have little or no sag, while larger motoneurons have a much stronger sag [59, 114]. Therefore, since the open HCN channels decrease the resistance of large motoneurons, the presence of I_h expands the range of input resistance between small and large motoneurons, and thus contributes to the mechanisms underlying the size principle.

The time constant of I_h is slow, however [121], which means that it can only follow slow changes in membrane potentials but not fast changes. As a consequence, the effective input resistance (which is then called “impedance”) depends on the frequency of the input. I_h acts as a high pass filter (Fig. 4(b)). Moreover, all cells, because of their parallel RC membrane property, have an impedance that declines at high frequency (Fig. 4(b)). The combination of the low pass filtering by the passive membrane properties and the high-pass filter by I_h creates a band pass filter, i.e., a peak in the impedance curve also known as “membrane resonance” (Fig. 4(b)) [77, 134]. We have shown that a membrane resonance due to I_h exists in cat motoneurons (Fig. 4(a)) [116], as well as in mouse motoneurons (Fig. 4(c)) [113]. Since I_h is stronger in large motoneurons than in small ones, the resonance is also stronger in large motoneurons.

3.2. Persistent inward currents and the amplification of synaptic inputs

In addition to I_h , motoneurons possess other currents that can alter their response to synaptic inputs. Indeed, Schwindt and Crill, in the late 1970s described the presence of a negative slope region in the current–voltage relationship of certain motoneurons [141, 142] and hypothesized that this current could amplify and change the time course of synaptic inputs. This current was later called “persistent inward current” (PIC), because it inactivates slowly after it has been opened. This property of the motoneurons was previously unknown because the PIC is highly dependent on the level of neuromodulation [34, 40, 70, 104, 128, 129, 131], but the neuromodulatory pathways are strongly depressed in the commonly used barbiturate anesthetized cat preparations, and the PIC can be directly blocked by the barbiturate

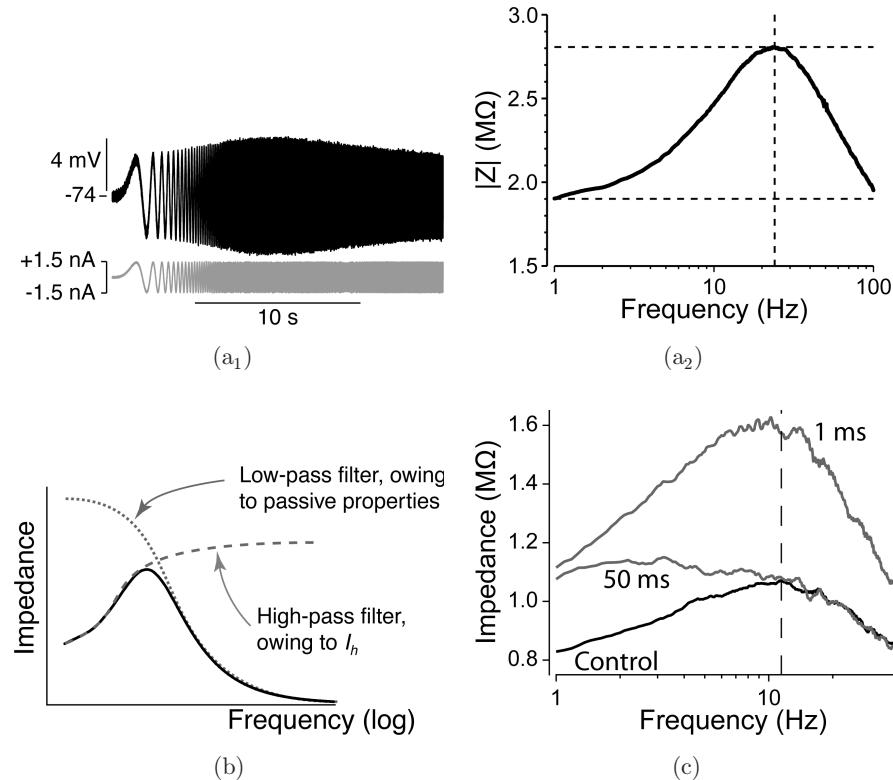


Fig. 4. Resonance properties of motoneurons. (a₁) Response of a mouse motoneuron (top trace) to the injection of a sinusoidal current of increasing frequency (bottom trace). Notice how the response is smaller in response to low frequencies, reaches a peak in the middle of the injected current, and then decreases again when the frequency of the sinusoidal current gets too high. (a₂) Frequency response curve (FRC) of the same motoneuron as in (a₁). The FRC is obtained by plotting the modulus of the complex impedance $|Z|$ versus the frequency. Notice that the curve shows a peak at 24 Hz in this motoneuron. This peak is the signature of the “resonance”. Adapted from Manuel *et al.* [113]. (b) Cartoon illustrating how the combination of a low pass filter, due to the passive filtering properties of the membrane, and a high pass filter created by the slow kinetics of I_h , creates a band pass filter, also called “resonance”. Adapted from [78]. (c) Effect of the PICs on the resonance. In this experiment, an artificial PIC, either activating quickly (time constant 1 ms) or slowly (time constant 50 ms) was added to a cat motoneuron using dynamic clamp. Notice than in control condition (without added PIC, black trace), this motoneuron showed a resonance at 12 Hz (in cat motoneurons, the resonance frequencies are lower than in mouse motoneurons). Adding a slow activating PIC canceled the resonance by amplifying the low frequencies (grey trace, 50 ms). Adding a fast activating PIC amplified the resonance but amplified preferentially the frequencies around the resonance frequency (top grey trace, 1 ms). Adapted from Manuel *et al.* [116].

anesthetics [57]. The presence of the PIC is readily apparent, however, in decerebrate preparations [7, 8, 40, 70], or by using pharmacological agents reproducing the action of neuromodulators [34, 70, 71, 104, 126].

The presence of the PICs has been found in virtually all types of motoneurons, cat lumbar motoneurons [7, 8, 34, 40, 70, 104], rat lumbar motoneurons [30], rat hypoglossal motoneurons [133, 146], rat sacral motoneurons [9], mouse lumbar

motoneurons [32, 123] and turtle spinal motoneurons [73]. However, the precise molecular substrate of this current is not necessarily the same in all motoneurons. In spinal motoneurons, it was established that a large part of the PIC is mediated by calcium ions, entering the cell through dihydropyridine-sensitive L-type channels [71, 146], most likely Cav1.3 because of their low activation voltage. A significant body of evidence has been accumulated that showed that the location of these channels is dendritic: it was shown that synaptic activity (either excitatory or inhibitory) can change the apparent activation voltage of this current as measured from the soma [8], and it can be activated with a field potential that selectively depolarizes the dendrites [72]. Recently, immuno-labeling, however, demonstrated the presence of these channels on the soma as well as the dendritic tree of motoneurons [65]. Since the dendritic tree is covered with synaptic boutons [25], these channels are in a perfect location to amplify the synaptic inputs to motoneurons. But other channels can also participate in the PIC. For example, in turtle motoneurons, part of the PIC is mediated by a nonselective calcium-activated cationic current (I_{CAN}) [130], while in rat hypoglossal motoneurons, it was argued that the calcium current was carried predominately by Cav2.1 and 2.2 channels [133]. The same group then found that a prolonged PIC can be observed on nucleated patches of membrane, and that this current is blocked by specific agonists of Cav1 channels. They concluded that the PIC in rat hypoglossal motoneurons is mediated by both Cav2 (in the dendrites) and Cav1 (in the soma) channels [127]. Regardless of their exact origin, the calcium PIC (CaPIC) is a slow-activating current that does not (or little) inactivate [32, 109, 141]. It has the potential of producing long tail currents in voltage clamp mode, and “plateau potentials” in current clamp mode.

In addition to the calcium component of the PIC, a substantial portion (about 40–50%) of the PIC is mediated by a persistent sodium current [64, 109, 110, 133]. The molecular origin of this persistent sodium current (I_{NaP}) is less clear. The axon initial segment of motoneurons is very rich in channels Nav1.1 and Nav1.6 [42], but it is unlikely that I_{NaP} is mediated by a specific isoform of the channels. It more likely arises from an alternate activation state of the same channels that generate the spikes [39, 68]. Contrary to CaPIC, this current activates very quickly (with a time constant in the order of the millisecond) [39], and despite not being fully “persistent”, it inactivates slowly.

Regardless of their origin, the PICs augment the effective synaptic current that reaches the spike initiation zone of motoneurons [65, 132] amplifying, for example, synaptic inputs elicited by muscle stretches or tendon vibration [8, 86, 105]. Moreover, the fact that motoneurons possess two PICs with very different kinetics allows them to amplify synaptic inputs relevant to their physiological function. We have shown (Fig. 4(b)), through a combination of experiments using dynamic clamp *in vivo* and the study of theoretical models, that I_{NaP} is able to amplify the subthreshold resonance present in motoneurons (see above). In other words, I_{NaP} amplifies preferentially the dynamic components of the inputs (with frequencies close to the resonant frequency) in large — most likely F type — motoneurons,

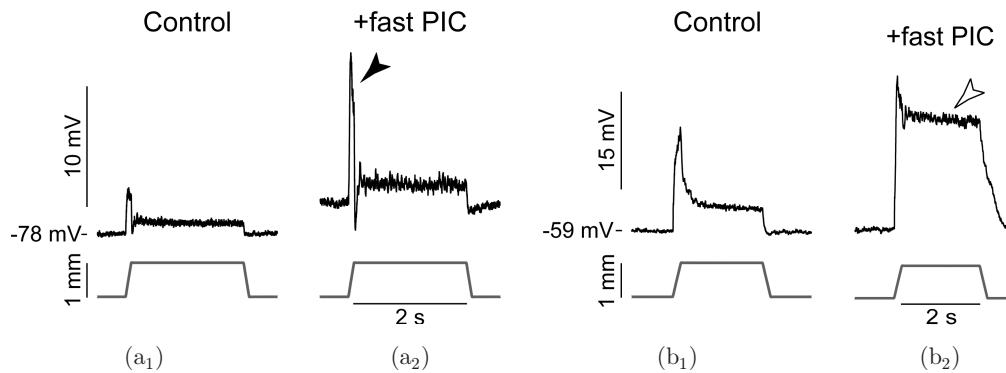


Fig. 5. Differential amplification in resonant and non-resonant motoneurons. (a₁) Response of a *resonant* cat Triceps Surae (TS) motoneuron (top trace) to a ramp-and-hold stretch of the TS (bottom trace). (a₂) Adding, using the dynamic clamp technique, an artificial fast activating PIC (time constant 1 ms) amplifies greatly the dynamic component (filled arrowhead) of the response (top trace) to the same stretch (bottom trace). (b₁) Response of a *non-resonant* (most likely S-type) cat TS motoneuron. (b₂) In this motoneuron, adding a fast activating artificial PIC amplifies all the components of the response, but mostly the static component, by eliciting a plateau potential (empty arrowhead). Adapted from Manuel *et al.* [116].

since they are the one with the strongest resonance (Fig. 5(a)) [116]. On the other hand, because of its slow kinetics, CaPIC amplifies only the low frequency inputs and thereby counteracts the effect of I_h and tend to suppress the resonance (Fig. 4(b)). If CaPIC is strong enough to cancel the resonance, then I_{NaP} amplifies the static inputs. The properties of CaPIC can be modulated by neuromodulatory inputs, in particular by serotonin (5HT), via 5HT2 receptors, and by norepinephrine (NE), via alpha1 receptors [104, 108, 131]. Likewise, I_{NaP} is also under monoaminergic neuromodulation [63]. Provided that the modulation of CaPIC and I_{NaP} is done through different subtypes, or subpopulations, of receptors, it is interesting to imagine that the motor system might be able to modulate independently the relative strength of CaPIC and I_{NaP} . This would allow to adjust the amplification of dynamic and static synaptic inputs depending on the task [116]. In non-resonant motoneurons however (i.e., S-type motoneurons), we have shown that both PICs amplify the static component of the inputs (Fig. 5(b)). This effect is further accentuated by the fact that the properties of the PIC (especially CaPIC) are different between S and F motoneurons. Lee and Heckman [102] have indeed shown that, in putative S motoneurons, the PIC activates at a lower voltage, and tend to persist longer, showing a marked hysteresis between the upward and downward portion of a voltage ramp, than in putative F motoneurons. These differences translate into distinct responses in the two populations of motoneurons. When activated in F motoneurons, the PIC induces a steady depolarization ("plateau potential") but cannot sustain it for more than 1–2 s. By contrast, in S motoneurons, the plateau potential always last longer than 3 s [103]. These long-lasting plateau potentials might be necessary for the function of these small motoneurons that are heavily implicated in postural task where a steady firing is required, as

opposed to larger motoneurons that would be recruited more transiently. There are indeed evidences showing that extensor motoneurons, which play a critical role in postural tasks, and during the stance phase of locomotion, have a greater capacity for self-sustained firing thank to a plateau potential caused by the activation of the PICs than flexor motoneurons (see Sec. 3.3.3) [38, 70].

3.3. *The firing properties of motoneurons*

With the discovery of PICs and its continued study in more and more species, our understanding of the firing properties of motoneurons has dramatically evolved during the past two decades. We will first review the firing properties that were originally described in motoneurons of cats deeply anesthetized with barbiturates, and then how this view was challenged by recent studies.

3.3.1. “Speed matching” in cat motor units

Once synaptic input has sufficiently depolarized the motoneuron, like in any other excitable cell, an action potential is generated. This action potential is followed by a phase of hyperpolarization, called the “after hyperpolarization” (AHP) [35], which has been extensively studied since the very first intracellular recordings of motoneurons. It was shown to be mediated by channels permeable to potassium [36]. These channels, contrary to those discussed so far, are not voltage dependent but are opened by intracellular calcium [121, 147] which enters the cell via high threshold, voltage-sensitive channels [146, 147]. The channels mediating the AHP were identified as “SK” channels by their sensitivity to the bee venom apamin [147, 152]. Along with the difference in input resistance (or size), the AHP characteristics were the first to be shown to be different in the different types of motoneurons. Eccles *et al.* [44] already showed that the motoneurons innervating slow contracting muscles have generally a longer AHP than the motoneurons supplying fast muscles, which has subsequently been confirmed by many groups [58, 151]. It was suggested that the strong sag in large motoneurons could be responsible for this difference [60], but the difference in duration persists when one takes care to select motoneurons with an *h*-current too slow to affect the kinetics of the AHP [114]. Today, it is agreed that the time course of the AHP is due to the speed of buffering of internal calcium [139], which might therefore be different between S and F motoneurons, either because of their size difference, or because of a difference in the expression of calcium buffers. Similarly, the amplitude of the AHP depends on the physiological type of the motoneuron: FF motoneurons have a shallower AHP than S motoneurons [151], but we have shown that this difference is due to the difference in input conductance because the AHP conductance recruited by a spike is not different in large versus small motoneurons [114].

The differences in the AHP duration in different types of motoneurons play an important functional role, a long lasting AHP limits the firing to low frequencies [92]. The extensive studies by Kernell’s group have shown that, at the minimal

amount of current that elicits repetitive firing in spinal motoneurons of deeply anesthetized cats, the period between two spikes (the minimum firing frequency) is, in fact, equal to the duration of their AHP [90]: S motoneurons have therefore a lower minimal firing rate than FF motoneurons. As the intensity of the injected current is increased, the frequency increases in a linear fashion (“primary range”), up to a limit that is also dependent on the duration of the AHP [90]. The slope of the linear relationship between the current and the discharge frequency in the primary range is essentially controlled by the AHP, as we have shown both theoretically and experimentally (Fig. 6(a)) [114, 115]. In each cat motoneuron, the AHP duration is precisely adapted to the twitch duration of the muscle fibers that the motoneuron innervates (“speed matching”) [92]. As such the AHP allows the precise adaptation of the discharge to the contractile properties of the muscle fibers. At recruitment, the firing frequency is lower in S motor units that have a longer lasting contraction, and faster in FF motor units that contract quickly. The minimal firing frequency thus corresponds to the frequency at which twitches just start to sum, and the force produced by the motor unit is small [92]. As the amount of excitation increases, the firing frequency increases linearly, which in turn allows the force to be finely graded (Fig. 6(b)). The maximal frequency at the end of the primary range is also controlled by the AHP in such a way that it corresponds to the frequency for which the twitches are fully fused and the force reaches its maximum (“tetanic fusion frequency”) [92]. The AHP therefore controls the rate of firing of motoneurons

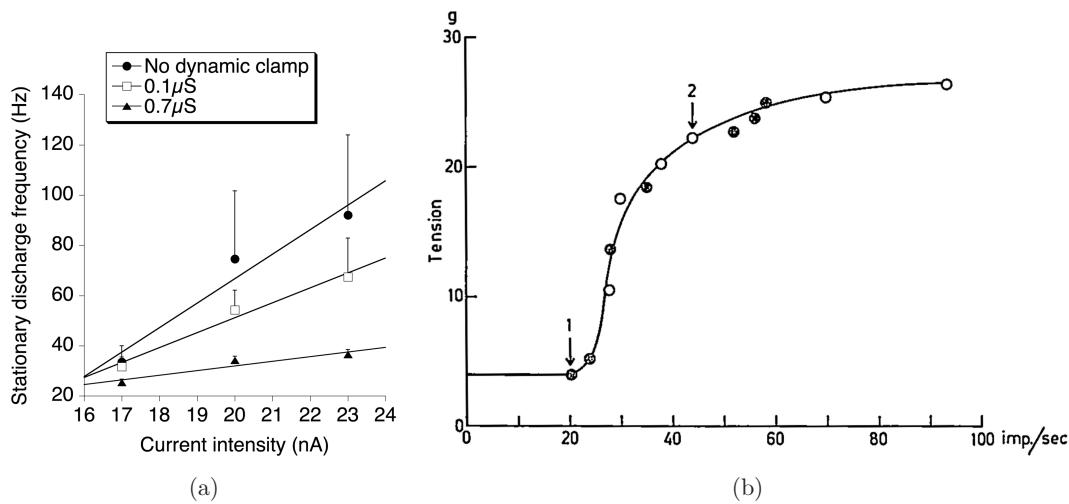


Fig. 6. Force gradation in the primary range, and control of the gain by the AHP. (a) In a motoneuron on which the AHP was dramatically reduced by the injection of the calcium chelator BAPTA, the gain was initially very high (about ten times the normal gain). Adding an artificial AHP with the dynamic clamp technique reduced the gain of the motoneuron. From Manuel *et al.* [115]. (b) Plot of the isometric force of a gastrocnemius motor unit vs. the discharge frequency of its motoneuron. Arrow 1 points to the minimal firing frequency of the motoneuron, while arrow 2 points to the maximal firing rate reached at the end of the primary range. Note that more than 80% of the force of this motor unit is recruited during the primary range. Reproduced, with permission, from Kernell *et al.* [91].

(i.e., the “gain” of the motoneuron) and by extension the gradation of motor unit force. The AHP is clearly a critical element of a motoneuron physiology. The AHP current is under tight neuromodulatory control, by 5HT and NE [63, 104, 108], but mostly by cholinergic C terminals that colocalize closely with SK channels [125]. Neuromodulation of the AHP has deep consequences. For instance, it was shown that during locomotion and the scratch reflex, the AHP is strongly reduced and the firing gain of the motoneuron is strongly increased [22]. The AHP plays the double role of adapting the discharge characteristics in the basal state so as to ensure a smooth gradation of the force, but also being a control variable that allows a dramatic increase of the gain and rate of force recruitment in any conditions where the movement to be performed requires it.

Very few gamma motoneurons have been intracellularly recorded for technical reasons [43, 89, 148]. Recordings of gamma motoneurons revealed that they are able to discharge at very high frequency (>200 Hz) and with a very high gain (20–60 Hz/nA), most likely because of a very shallow and short-duration AHP [74, 89, 148]. This fits to the properties of nuclear chain fibers, which display a very short contraction time and a high tetanic fusion frequency (see Sec. 2), suggesting that the discharge properties of gamma motoneurons are, in the same way as in alpha motoneurons, adapted to the contractile properties of their muscle fibers. However, despite the fact that bag1 fibers are slower than the nuclear chain fibers [16], gamma motoneurons with low gain and low firing frequencies have not been recorded. This might well be because of the small number of gamma motoneurons studied so far.

3.3.2. Firing properties of mouse and rat motoneurons

At least in alpha motoneurons, the AHP is not, however, the sole current that affects the repetitive discharge of motoneurons. The sodium persistent inward current, in particular, was shown to be critical for the initiation of each spike during a repetitive discharge, as it activates a few millivolts below the spiking threshold and provides an initial acceleration of the voltage trajectory, which allows the transient sodium channels to escape their inactivated state [64, 100, 106]. The voltage threshold of motoneurons does not depend on their physiological type [58] and no obvious differences have been observed across species. However, we have shown that, in mouse motoneurons, the fast-activating sodium current responsible for the spike generation is likely endowed with a very slow inactivation process, which creates a state of relative hypo-excitability [79], delays spike initiation, and induces subthreshold oscillations [113]. The presence of these oscillations creates a new regime of firing before the classical primary range, that we dubbed the “subprimary range”. In this range, contrary to the situation in cat motoneurons, inter-spike intervals can be longer than the duration of the AHP, and the number of oscillations at the end of the AHP essentially controls the period. Surprisingly, we have shown that, in this small animal, most of the motor unit force is recruited during the subprimary range and not in the primary range as in cats [112]. This new mode of

recruitment of force might be functionally important for small animals like rodents, as a subprimary range has also been recently described in rat lumbar motoneurons [145]. Note that, however, these results do not invalidate the “speed matching” of motoneurons and muscle fibers. In mouse as well as in rat motoneurons, the AHP duration displays systematic variations with the input resistance and the conduction velocities of the motoneurons [2, 112]. It seems instead that the “match” between the AHP duration and the twitch duration is done in such a way that it allows a substantial proportion of the force to be recruited during the subprimary range in rodents [112]. The AHP is likely to play an active role in controlling the subthreshold oscillations and thereby the subprimary firing range. The larger and longer AHP of cat motoneurons is more efficient at deactivating the sodium channels, and therefore allows a larger proportion of channels to be activated when the membrane reaches threshold. Indeed, we have shown that the subthreshold oscillations disappear in mouse motoneurons when one artificially increases the amplitude of the AHP using the dynamic clamp technique, or by adding some extra artificial persistent sodium current [79].

3.3.3. Impact of PICs on the discharge

Finally, it was shown, almost since their initial discovery, that PICs, and in particular CaPIC, can have a strong impact on the discharge properties of motoneurons. In their strongest manifestation, PICs are able to produce long lasting plateau potentials that can produce a “self-sustained firing” [34, 40, 70, 72, 73, 103]. This property is also called “membrane bistability” (Fig. 7(a)), as motoneurons can exist in two stable states: quiescent (not discharging), or firing continuously without the need to receive a sustained synaptic activation. However, as noted earlier, this property is not found in any type of motoneurons. S-type motoneurons, especially those innervating extensor muscles [38], seem more prone to exhibit a full bistability, while FF-type motoneurons only display a partial bistability; even though they exhibit self-sustained firing, it tends to stop on its own after a few seconds [103]. Even when they are not strong enough to turn the membrane bistable, the PICs can cause an acceleration of the discharge, and, in response to a triangular ramp of current for example, a counterclockwise hysteresis on the current–frequency relationship (Figs. 7(b) and 7(c)) [8, 9, 34]. It is not clear if and how these phenomena (bistability and counterclockwise hysteresis) are involved in the physiological control of motoneurons in normal humans (see next section), but a substantial body of evidence exists that shows that “abnormal” PICs can be implicated in pathologies like amyotrophic lateral sclerosis (ALS) [48, 122, 135] and spasticity after spinal lesion [9, 109, 110]. In the latter case, for example, Bennett’s group has elegantly demonstrated that, two months after complete spinal transection, the loss of serotonergic innervation from the brainstem on motoneurons causes a transformation of the 5HT2 receptors, which become constitutively active [54] and thus cause a pathological overexpression of the PICs.

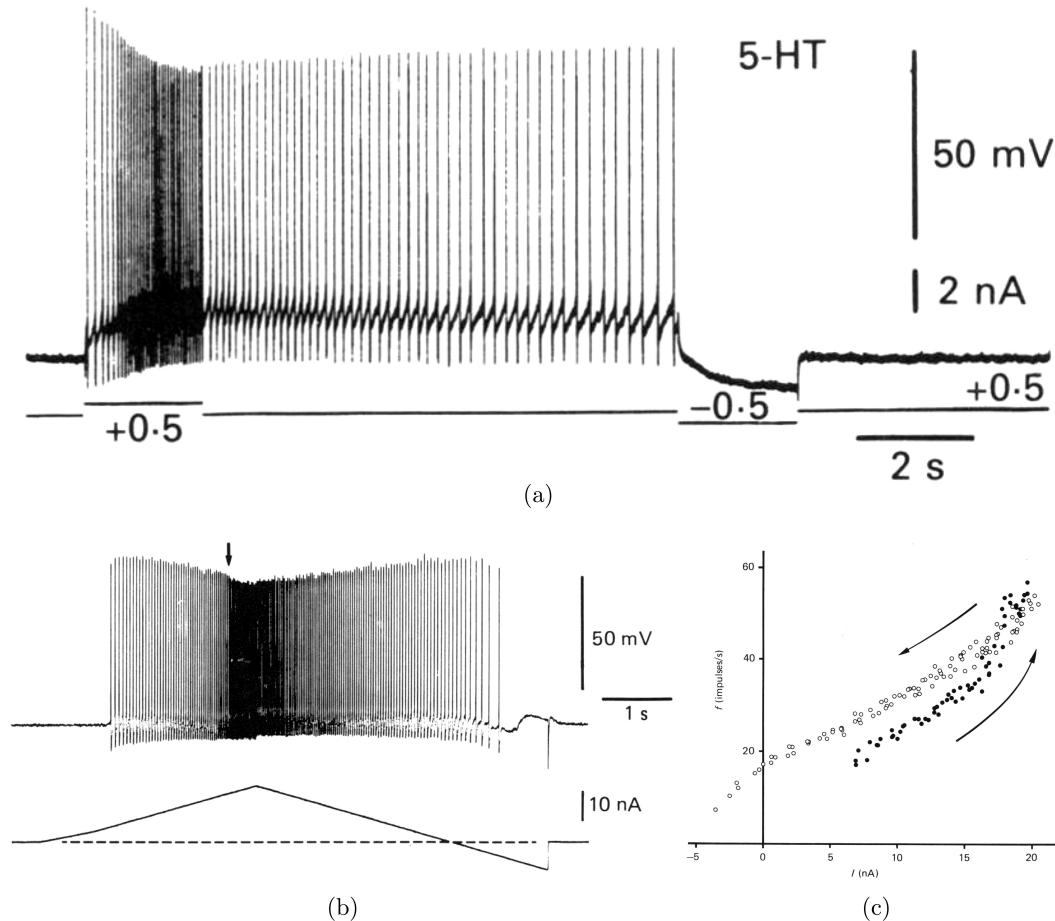


Fig. 7. Impact of the PICs on the discharge of motoneurons. (a) Membrane bistability in a turtle motoneuron. In this experiment, the PICs of a turtle motoneuron recorded *in vitro* were revealed by the addition of serotonin (5-HT) to the recording chamber. In these conditions, a depolarizing pulse of current (bottom trace) initiated the discharge (top trace), which accelerated during the pulse. Once the pulse was turned off, a self-sustained discharge is apparent, and it required a hyperpolarizing pulse of current to turn it off. From Hounsgaard and Kiehn [71], with permission. (b) Recording from a cat lumbar motoneuron (top trace) injected with 5-HT intravenously, in response to a ramp of current (bottom trace). The arrow points to an acceleration of the discharge on the ascending ramp. Note that the discharge last much longer on the descending ramp than on the ascending ramp, and that a negative amount of current is required to stop the discharge. (c) Frequency–current curve from the motoneuron in (b). The curve shows a clear counterclockwise hysteresis between the upward ramp (black dots) and the downward ramp (white dots). From Hounsgaard *et al.* [70], with permission.

3.4. PICs in human motoneurons and some consequences for human neurophysiological studies

As discussed above, in animals, PICs proved to play three important roles in spinal motoneurons: (1) they contribute to maintain a repetitive discharge during prolonged inputs (I_{NaP}), (2) they amplify synaptic inputs (I_{NaP} , CaPIC) and (3) they alter the shape of the current–frequency relationship (CaPIC). These effects largely depend on

the amount of 5-HT neuromodulation. It is likely that PICs induce similar actions in human spinal motoneurons. However, it is quite difficult to obtain and interpret the evidence of any such actions in human motoneurons (see [66] for a review).

The first evidence was given by the observation in some motor units of a prolonged EMG activity that continues after a short period of tendon vibration [94]. The prolongation of the motoneuron discharge was interpreted as resulting from a recruitment of a PIC by the tonic Ia EPSPs elicited by the spindle vibrations [94]. The PIC induces a self-sustained discharge that outlasts the vibration period [55, 94]. Paired motor-unit recordings provided a further argument in favor of PIC activation [55, 94]. The firing activity of a low threshold motor unit is used as a reflection of the synaptic drive. As the synaptic drive is largely common over the population of alpha motoneurons, any prolongation of the firing activity in the higher threshold motor unit suggests that this prolongation might be due to the activation of a persistent inward current in the motoneuron. Gorassini *et al.* [55] have used this technique to determine the relative strength of the PIC current during slow triangular movements. As a result of the PIC activation, the discharge of the motor units displays a counterclockwise hysteresis during triangular movements. Recently, Fuglevand has showed at the Paris Motoneuron Meeting (<http://motoneuron2010.parisdescartes.fr/>) that the hysteretic pattern of discharge during triangular forces tends to become linear when a cutaneous stimulation is applied during the contraction. The most likely explanation is that PIC was disengaged by the strong inhibition elicited by the cutaneous afferents. This result suggests again that PICs may shape the activity of human motoneurons.

Since PICs are present in human motoneurons, one might thereby wonder whether they influence the motor output during the tests of motoneuron excitability (H-reflexes and transcranial magnetic stimulation (TMS)) that are classically used in human neurophysiological studies [31]. Lessons from the animal experiments reviewed here prompted us to make the following suggestions. The excitatory potentials evoked in motoneurons during these tests might be amplified by the PICs, depending on their time course, thereby increasing the probability of reaching the threshold for discharge. Since the H-reflex is achieved by applying a single electrical shock on the nerve, it is very likely that the stimulation-induced Ia EPSPs are much too brief (a few milliseconds) to engage CaPIC. On the other hand, however, I_{NaP} might be able to amplify brief EPSPs [86, 116]. Furthermore, it is likely that the EPSPs amplification by I_{NaP} would be more important in the motoneurons innervating fast-contracting motor units that display a marked resonance than in those innervating the slow contracting motor units that hardly display any resonance (see above) [116]. Motoneurons innervating the fast-contracting motor units will then reach their firing threshold with a higher probability when the neuromodulatory state is such that I_{NaP} is strongly expressed. In conditions of strong neuromodulation, and assuming that the size principle is respected during the H-reflex, Ia input larger than the one necessary to recruit S-type motoneurons would recruit more F-type motoneurons. However, as the H-reflex is often tested in the soleus

muscle, composed nearly entirely of S-type motor units, such a consideration may not apply in this instance. In the same line, the impact of PICs in motoneurons during TMS depends on the shape of post-synaptic potentials induced by the descending inputs. Excitatory potentials on motoneurons evoked by TMS may be longer than those evoked by the H-reflex method but they do not exceed 10 ms [138]. This is due to the fact that, even in response to a single TMS shock, the cortical output is more complex than a single volley. Furthermore, differences in the conduction velocities in descending axons of cortical cells might result in further desynchronization of the command to motoneurons. However, the duration of excitatory inputs on motoneurons in response to TMS stimulation is still too short to substantially engage CaPIC [116]. I_{NaP} is likely to be the only one PIC active in motoneurons during TMS. Similarly as with the H-reflex, TMS might tend to recruit more F motoneurons in circumstances where the neuromodulation allows a significant I_{NaP} expression.

In conclusion, PICs have been inferred to exist in human motoneurons. Some caution must therefore be taken in interpreting the results from classical neurophysiological studies that, in part, depend on motoneuron excitability. It would be very useful to find methods to determine the “neuromodulatory state” of the subject in different motor tasks since any changes on this state can modify I_{NaP} [63], CaPIC [105], and the resonance acuity of motoneurons by changing I_h [95].

4. Conclusion: A Wide Functional Diversity of Spinal Motoneurons

Since Sherrington, our conception of the final common pathway has considerably evolved. Meticulous studies have shown that motoneurons innervate a multitude of muscle targets in a well organized plan, resulting in a heterogeneous functional population of motoneurons. Ordinary (extrafusal) muscle fibers are differentiated in three main types with contrasting physiological properties. Furthermore, each alpha motoneuron innervates muscle fibers that are all of the same type. It is then legitimate to consider that there exist three corresponding functional types of alpha motoneurons. Intrafusal muscle fibers differentiate in fibers that supply the dynamic (bag1 fiber) and the static (bag2 fiber and chain fibers) sensitivity of spindle endings. A gamma motoneuron elicits a dynamic or a static action depending on which group of intrafusal fibers it targets. Importantly, a substantial fraction of motoneurons (beta motoneurons) innervate both extrafusal and intrafusal fibers. Remarkably, there is a link between their intrafusal and extrafusal innervation that depends on the action, dynamic or static, they elicit on spindle endings.

What is more, the electrical properties of each motoneuron are precisely adapted to the contractile properties of their targets. Each type of muscle fiber (slow contracting or type I, fast contracting fatigue resistant or type IIA, fast fatigable or type IIB, intrafusal bag fibers and chain fibers) has indeed very different duration of contraction. Yet, despite the fact that all motoneurons share more or less the same set of voltage-dependent currents, the precise characteristics of these currents are

regulated so that the motoneurons can activate its target in the most efficacious way possible. The size difference among alpha motoneuron guarantees an energy efficient way to generate force. Furthermore, interactions between I_h , a fast I_{NaP} and a slow CaPIC (all of which can be regulated by neuromodulatory inputs) allow the differential amplification of the inputs that are the most relevant to the physiology of the motoneurons (static inputs in S motoneurons, dynamic inputs in F motoneurons). Finally, the AHP regulates the firing frequency range so as to guarantee that the firing will not be too rapid or too slow to generate the required muscle force.

It is obvious that both the innervation pattern of the different types of motoneurons and the membrane receptors and ionic channels that determine their electrical properties require a very precise control during development by sophisticated molecular signals. Only a few of these signals are known so far (for a review see [88]) and the search for the signals that guide a given axon towards its specific target remains an important issue. It is very likely that the fate of each motoneuron within a pool is to some extent determined quite early during development and recent works have uncovered some transcriptional factors or molecular signatures specific of a given motoneuron type [88]. However, it is still unclear whether the properties of motoneurons and muscle fibers are predetermined and each motoneuron seeks out compatible muscle fibers, or if, on the contrary, the properties of motoneurons and muscle fibers co-mature during development to obtain a properly adapted functional motor unit. The elucidation of these mechanisms would considerably advance on our understanding of the physiology of the motor system. It would also have promising therapeutic application for the treatment of diseases like ALS, which affect specific populations of motoneurons and not the others.

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