MECHANISMS UNDERLYING FIRING IN HEALTHY AND SICK HUMAN MOTONEURONS

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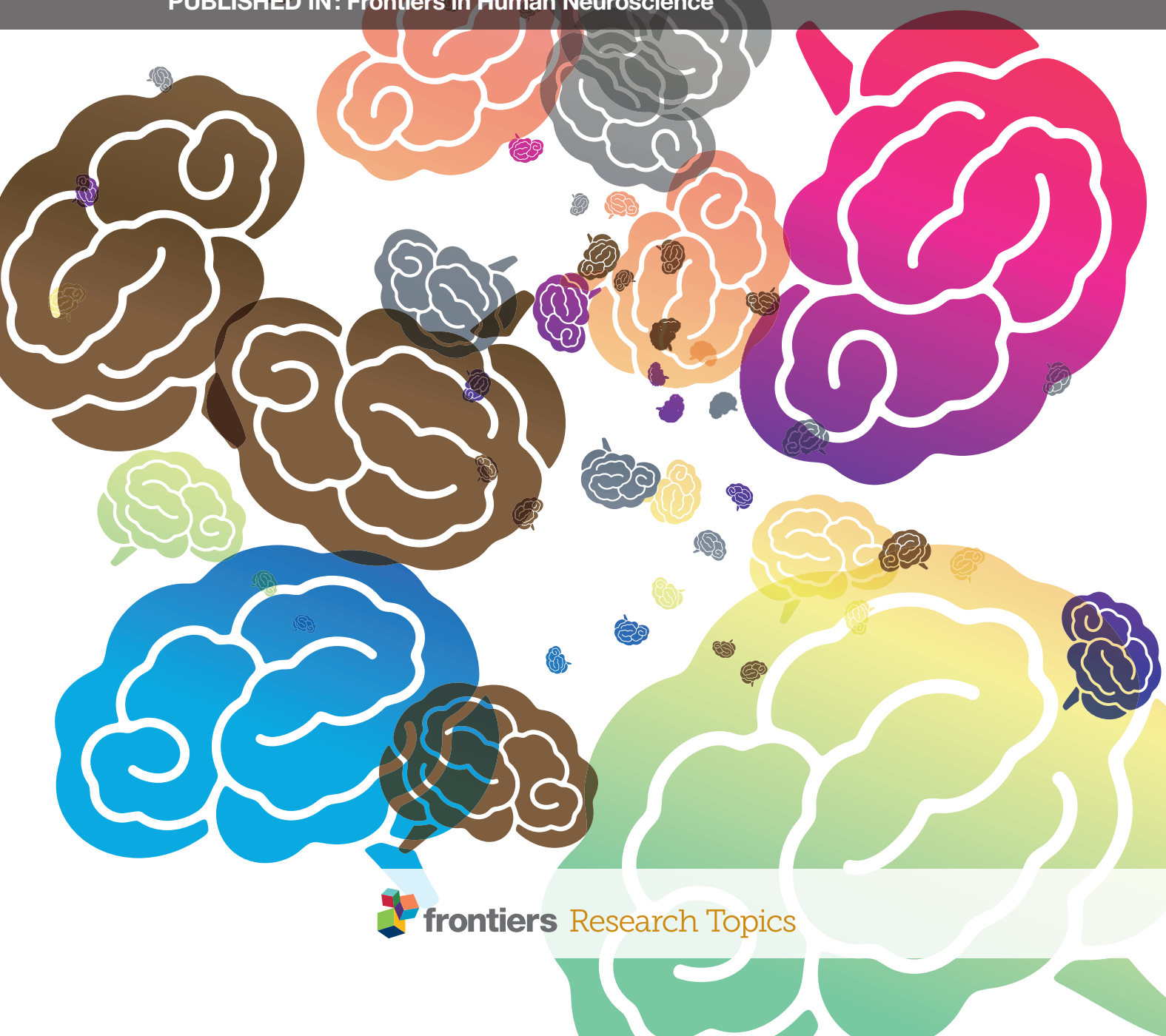

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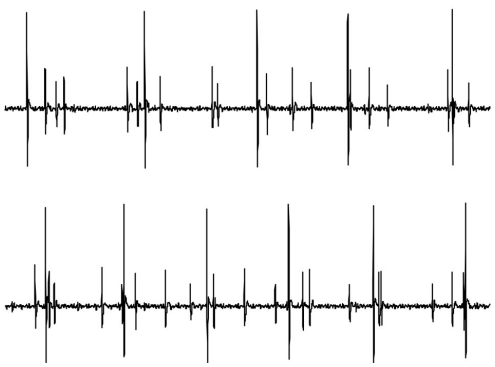
MECHANISMS UNDERLYING FIRING IN HEALTHY AND SICK HUMAN MOTONEURONS

Topic Editors:
Parveen N. S. Bawa, Simon Fraser University, Canada
Maria Piotrkiewicz, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Poland
Annie Schmied, National Center for Scientific Research (Centre National de la Recherche Scientifique), France



The information on human motoneuron firing characteristics can be obtained by studying single motor unit potential trains. The figure presents two fragments of needle EMG recording with potentials of 5 simultaneously active motor units from the soleus muscle (lower fragment is the continuation of the upper one). Data were provided by the courtesy of Kemal Türker.

Since the latter half of the twentieth century an enormous amount of knowledge about mammalian motoneuron pools has been collected. This progress was enabled mostly by the development of the precise techniques of intracellular recordings in acute animal experiments, many of which were conducted under deep anaesthesia. Recently obtained evidence indicates that anaesthetics used at that times changed certain properties of the cell membrane, which might affect firing of the neuron. Experiments on normal humans gets around this problem, which lets one compare motoneuron firing characteristics in humans and reduced preparations.

Firing pattern of human motoneurons is obtained indirectly by recording from a few muscle fibres of a motor unit. Since there is one-to-one relationship between motor unit and motoneuron firing, the statistical analysis of motor unit firing is equivalent to the analysis of motoneuron firing. This analysis, based on the essential knowledge about motoneuron physiology, gained from the direct measurements in animal experiments and verified by computer simulations, allows one to draw conclusions about the physiological properties of human motoneurons. For obvious reasons, the deductions made on properties of human motoneuron from these analyses should be accepted with caution. On the other hand, human experiments provide the unique opportunity to study intact motoneurons during normal physiological behavior. Thus, combining information obtained from animal and human experiments, and computer simulations, gives insight into underexplored problems of motor control.

This E-book contains a collection of articles with range of exciting findings on the physiology and pathology of human motoneurons. The collection covers such important issues concerning firing of healthy motoneurons as recruitment and rate coding as well as motoneuron excitability, discusses intrinsic motoneuron properties disclosed by studying double discharges, and provides information on broad spectrum of motoneuron pathology. It is our hope that this collection promotes further expansion of knowledge on human motoneurons.

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Motoneuron pathology

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EDITORIAL
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Mechanisms underlying firing in healthy and sick human motoneurons

Maria Piotrkiewicz[1]*, *Parveen N. S. Bawa*[2]* and *Annie Schmied*[3]*

[1] *Engineering of Nervous and Muscular System, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland,* [2] *Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada,* [3] *Centre National de la Recherche Scientifique, Plasticité et Pathophysiologie du Mouvement, Institut de Neuroscience de la Timone, University Aix Marseilles, Marseille, France*

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In an address to the British Association for the Advancement of Science in Cambridge, Professor Sherrington introduced the terms "*motor neurone*" and "*the final common path,*" the latter term implying that all motor commands converge onto the motoneuron which integrates the incoming information and passes the net information to the muscle for contraction (Sherrington, 1904). The relative ease of access of the spinal motoneuron made it feasible to set up techniques for investigating the physiological, biophysical and molecular properties of these neurons. It became the most investigated neuron of the CNS in the twentieth century and the information gained from studies on motoneurons formed the basis for examining the other neurons of the CNS. Since the compound action potential of a muscle unit is strictly related one-to-one to the action potential arriving from the innervating motoneuron, the statistical analysis of muscle unit action potentials provides an investigator with an elegant way to probe the properties of motoneurons in behaving humans. In the following review the terms *motoneuron* and *motor unit* might be used interchangeably. Different aspects of human motoneuron investigations in health and disease are presented in 16 articles of this topic which are summarized below.

An increase in the net excitatory synaptic input to the motoneuron pool results in an increase in the level of muscle contraction by recruitment of additional motor units (MUs) and an increase in firing rates of the already recruited units (Milner-Brown et al., 1973; Henneman et al., 1974). The principle of orderly recruitment of motoneurons by size was originally proposed by Henneman (1957) but was later questioned by other researchers presenting examples of selective, rather than orderly recruitment (e.g., Smith et al., 1980). These controversies are assessed by Bawa et al. (2014), and the opinion unifying the concept of orderly recruitment is presented. In humans, increases in firing rates of motor units have been shown to follow the "onion skin" pattern at lower levels of contraction, meaning that the lower-threshold motor units discharge with higher rates than higher-threshold ones. However, studies performed on the whole range of muscle forces indicated that for higher force levels the motor unit firing rate follows a "reverse onion skin" pattern. Hu et al. (2014) decided to approach this problem using small surface electrodes and step increases in force instead of the "ramp and hold" protocols used by previous authors. They showed that the "onion skin" pattern was preserved until 15% of maximal voluntary contraction, and from their results predict this pattern to be valid for the whole range of muscle forces, which is not supported by the previous published works. However, the reported rate saturation of the MUs discharging with higher rates implies that at the higher forces the "reverse onion skin" pattern may be expected. In another paper, Duchateau and Baudry (2014) show that during ballistic contractions the maximal discharge rates are higher than those observed in ramp contractions. It should be noted, however, that during ballistic contractions one deals with instantaneous rates, while during ramp and hold contractions one refers to tonic firing rates defined as the average

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Edited and reviewed by:
Hauke R. Heekeren,
Freie Universität Berlin, Germany

***Correspondence:**
Maria Piotrkiewicz,
masia@ibib.waw.pl;
Parveen N.S. Bawa,
bawa@sfu.ca;
Annie Schmied,
annieschmied@yahoo.fr

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over 1 s. One cannot compare maximal rates during the two patterns of contraction. The authors also suggest that the maximal rate of force development is determined by maximal instantaneous firing rate of the motoneurons confirming earlier work on reduced cat preparations using intracellular current injections (Baldissera et al., 1982). For information of firing rates in older adults, the maximal firing rates have been reported to decline (Duchateau and Baudry, 2014; Kallio et al., 2014).

The high instantaneous firing rates have also been observed at low, slower speeds of muscle contractions. They are generated by some motoneurons, which occasionally fire pairs of closely spaced spikes (doublets); each pair is followed by a prolonged post doublet interval suggested to result from the summation of successive afterhyperpolarizations (AHPs). The "true" doublets (Bawa and Calancie, 1983) should be distinguished from the short interspike intervals fired during ballistic contractions. The former are attributed to the delayed depolarization, an intrinsic property of the motoneuron (Kernell, 1964; Calvin, 1973), while the latter are due to the high rate of rise in synaptic inputs (Baldissera et al., 1982). Doublets have been reported by several authors contributing to the present topic. Piotrkiewicz et al. (2013) observed doublets in the soleus muscle where they have never been documented before. Repetitive doublets, where doublet/postdoublet intervals alternate, were first reported by Bawa and Calancie (1983). Kudina and Andreeva (2010) suggested earlier that repetitive doublets resulted from suprathreshold delayed depolarization supported by plateau potentials. In the present article, Kudina and Andreeva (2013) pose the question whether there are common attributes of motoneurons in the spinal cord which can fire repetitive doublets. They suggest a cranio-caudal gradient of the number of motoneurons that can discharge doublets and more frequent repetitive doublets observed in the cervical motoneurons compared to the motoneurons of the lumbar region. Piotrkiewicz and Kuraszkiewicz (2014) investigated the relationship between duration of motoneuron AHP and confirmed an earlier observation by Kernell (1964) that motoneurons with shorter AHPs discharge doublets more easily. Doublets were also mentioned in Søgaard et al. (2014). Triplet discharges have been reported by Piotrkiewicz and Kuraszkiewicz (2014) and the authors speculate on the possible mechanisms to explain this pattern.

Three papers deal with problems of motoneuron excitability. Various methods used to date to test excitability of human motoneuron pools are reviewed by McNeil et al. (2013). While synaptic inputs to a motoneuron pool recruit motoneurons from small to large, electrical stimulation of a muscle nerve recruits motoneurons in reverse order, from larger to smaller axons. It has been acknowledged for some time that the diameter is not the only factor determining axon excitability. In a mixed nerve, sensory and motor fibers have different biophysical properties making sensory afferents more excitable than motor axons of the same size. Human studies of axon excitability have obvious limitations, thus Lorenz and Jones (2014) explored this problem in a rat model. They have shown that several biophysical parameters differ between axons innervating slow soleus and fast tibialis anterior. These mechanisms may underlie the bimodal distribution of axon excitability observed in the tibialis anterior by Kudina and Andreeva (2014). Since the axons of larger motoneurons are more excitable, electrical stimulation used for rehabilitation purposes might only recruit and strengthen the larger motor units and let the small units succumb to atrophy. Dean et al. (2014) have proposed a method to recruit smaller low threshold units using specially tailored electrical stimulation of a mixed nerve. The stimulus consists of a high frequency pulse train with current strength subthreshold for eliciting M or H waves. In soleus, stimulation of the posterior tibial nerve with such parameters has been shown to recruit motor units in an orderly fashion from small to large, and at physiological firing rates thus leading to activation and strengthening of small motor units.

Six papers deal with motoneuron discharge related to pathology. Søgaard et al. (2014) recorded electromyographic activity bilaterally from upper trapezius muscles. They observed a tonic low level motor unit activity and suggested that such activity, whether it is built into the motor program to stabilize the limbs or results from stress of paying attention during computer work, may be the underlying cause of myalgia observed in computer workers. Garland et al. (2014) reviewed stroke-related changes in motor unit discharge characteristics and suggested that residual motor control strategies may remain after stroke. McNulty et al. (2014) investigated motor unit firing characteristics in different muscles of stroke survivors showing that the effects on peak firing rates and their dynamic range differ not only between joints of the upper and lower limbs but also between muscles of different joints of the same limbs. Furthermore, they have shown that motor units on both paretic and non-paretic sides changed after stroke. Confirming the common observation that firing rates on the affected side are lower than normal, they have also shown that motor units from the unaffected side discharge with firing rates higher than normal. The authors conclude that motor unit properties on both sides should be compared to data from age- and sex-matched healthy subjects.

Neuroscientists have used peripheral inputs to investigate motor output and various properties of motoneurons. Yet, when it comes to voluntary control of movement, the peripheral afferents are generally ignored. In their paper describing extensive single motor unit recordings from a deafferented subject, Schmied et al. (2014) demonstrate the importance of peripheral afferents in the control of motoneuron excitability, variability in firing, synchronization and coherence between different motor units. The viability of motoneurons and the dependence of their excitability on peripheral afferents are discussed by Zijdewind et al. (2014). In incomplete spinal cord injured subjects the mean firing rates decrease compared to those in normal subjects. The authors argue that the decrease in firing rates is not due to changes in motoneuron properties resulting from a decrease or complete elimination of descending inputs. These motoneurons are capable of reaching normal discharge rates during spasms. However, the covariation in firing rates among various motor units mentioned above by Hu et al. (2014) is lacking during spasms, which implies that the weight of different inputs onto different motoneurons of a pool might alter in spinal cord injury. This might also apply to persistent inward currents of the members of a pool.

Motoneurons form the final motor path for all the motor commands; an animal without motoneurons would die. Such is the case in amyotrophic lateral sclerosis (ALS), a deadly disease of unknown etiology, which causes selective degeneration of spinal motoneurons and corticomotoneuronal cells ("lower" and "upper" motoneurons, respectively). De Carvalho et al. (2014) review the properties of the upper and lower motoneurons in ALS, aiming to show how changes in the pattern of motor unit firing could help to delineate the underlying pathophysiological disturbance as the disease progresses. However, some of the few single motor unit studies performed in patients with ALS have not been covered in this review. The interested reader may find additional information in Schmied et al. (1999), Attarian et al. (2006, 2008).

The articles collected in this exciting Research Topic cover a broad spectrum of human motoneuron research, from their intrinsic properties such as afterhyperpolarization following delayed depolarization to pathological changes in neuromuscular disorders. We hope that this collection will be equally exciting for the potential readers. Our sincere thanks are expressed to all the authors and reviewers who contributed to this important topic of human neurophysiology.

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Assessment of size ordered recruitment

Parveen N. S. Bawa[1], Kelvin E. Jones[2]* and Richard B. Stein[3]

[1] *Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada*
[2] *Faculty of Physical Education and Recreation, University of Alberta, Edmonton, AB, Canada*
[3] *Department of Physiology, University of Alberta, Edmonton, AB, Canada*
**Correspondence: kejones@ualberta.ca*

Edited by:
Maria Piotrkiewicz, Polish Academy of Sciences, Poland

Reviewed by:
Piotr Krutki, University School of Physical Education in Poznan, Poland

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The spinal motoneurons innervating a limb muscle are heterogeneous: they vary in diameter of cell bodies, axons and surface area of the dendritic trees, electrophysiological differences (e.g., input resistance, afterhyperpolarization, spike threshold) and contractile properties of the associated muscle units. Considering the range of motoneuron and corresponding muscle unit properties, the obvious question arose about how and which motoneurons were selected by the central nervous system for various types of contractions and movements. Elwood Henneman and his coworkers proposed an unequivocal simple pattern in a series of papers starting in 1957 from experiments conducted on reduced cat preparations (Henneman, 1957; Henneman et al., 1974). The *Size Principle* of motoneuron recruitment stated that for any net excitatory input to the motoneuron pool, motoneurons were recruited in an orderly fashion, always from small to large. Stein and coworkers demonstrated that the size principle generalized to voluntary isometric contractions in humans, which they called *Orderly Recruitment* (Milner-Brown et al., 1973). The literature on this topic is well summarized in a recent review by Heckman and Enoka (2012).

Since the publication of Henneman's seminal paper in 1957, publications on this topic have steadily accumulated. The purpose of this short article is to clarify the basic essence of size ranked or orderly recruitment of motoneurons by addressing conclusions about the alternative: selective recruitment. This requires stretching the strict boundaries imposed by some authors in understanding the *Size Principle*. For example, the anatomically defined motoneuron pools may not always coincide with the group of motoneurons being excited for a particular task; the precision of rank-ordered recruitment suggested from reduced animal preparations (Henneman et al., 1974) may not be that precise in a noisy physiological system (Stein et al., 2005). Here we elaborate on some of the factors that may have lead to the conclusions of selective or random recruitment.

Though the size principle was stated for a motoneuron pool, the initial observations made by Henneman and coworkers were not restricted to one pool. They recorded from ventral roots while stretching all triceps surae muscles; the recorded activity in individual ventral roots could have its origin in any one of the synergistic pools. One recorded action potential could be from soleus and the second from gastrocnemius. Yet orderly recruitment was observed between the collection of all motoneurons that were excited by the input, specifically the total motoneuron population of soleus, medial and lateral gastrocnemius (LG) muscles. Likewise, an anatomically defined motoneuron pool may under certain conditions be compartmentalized into task groups (Riek and Bawa, 1992) and orderly recruitment is observed within each task group. The existence of task groups does not imply size independent selective recruitment of motor unit types. Wakeling (2009) has reported recruitment of different compartments of triceps muscles in goats and humans depending on the mechanics of the movement. Selective recruitment of different compartments is akin to recruitment of different task groups; again, this observation does not imply selective recruitment if excitatory input is restricted to a subset of motoneurons. This same line of reasoning applies to the condition of fast paw shake in the cat (Smith et al., 1980). In these experiments EMG activity was observed in the *fast* LG muscle but not in the *slow* soleus muscle during the paw shake. This observation is often cited as evidence for selective recruitment of motor unit types. However, there was no discussion as to whether the motor units within the LG muscle, which is composed of type I and II muscle fibers, were recruited according to size. A subsequent study that included EMG sampling from histochemically regionalized muscles showed high activation of all muscle regions during paw shaking, which is not consistent with preferential recruitment of muscle regions rich in type II fibers during this condition (Chanaud et al., 1991). We suggest that when discussing recruitment order, the motoneuron pool should be operationally defined as the group of motoneurons that receive excitatory synaptic input to drive the functional movement, not the pool of motoneurons defined by anatomy. The validity of the *Size Principle* should then be evaluated within this operationally defined motoneuron pool to determine if recruitment proceeds from small to large.

What do orderly recruitment and selective recruitment really mean? The accepted narrative is that orderly recruitment of motor units from small to large twitch force, results in a more precise control of force and movement; this precision is

more important for small and mid range forces. By maintaining the same order of recruitment, the central nervous system minimizes the computational load across a wide range of desired outputs (Henneman et al., 1974). A range of quantitative theoretical studies support this qualitative description. For example, Senn et al. (1997) used an information theoretical approach to demonstrate that orderly recruitment maximizes information content of motoneuron output that in turn minimizes the error in muscle force generation. Selective recruitment, on the other hand, refers to the hypothesis that under certain conditions the central nervous system selects motor units to enhance the force and rate of force output irrespective of the rank order of the motor unit within a motoneuron pool. To achieve this goal, selective recruitment may use preferential inhibition of small motor units. The most commonly proposed examples of selective recruitment include ballistic contractions, lengthening contractions and the preferential recruitment of fast motor units during cutaneous stimulation. However, empirical evidence from a number of laboratories failed to support the hypothesis of selective recruitment in these conditions (reviewed by Heckman and Enoka, 2012). Electrical stimulation of some pathways could produce inhibition of small motor units and excitation of larger motor units. Yet none of the behavioral studies demonstrated selective recruitment of large units with inhibition of the small ones. A possible basis for this discrepancy comes from Kernell and Hultborn (1990). They proposed that the selective excitatory and inhibitory synaptic inputs change the gain of the input-output curve of the motoneuron pool. To increase the recruitment gain, the small motoneurons are biased with inhibitory currents. Alternatively, or concurrently, the large motoneurons can be biased with excitatory currents. The opposite synaptic bias scheme can be used to decrease the gain. All motoneurons receiving excitatory input to drive the final movement, despite underlying bias inputs, should be considered part of a functionally defined pool of motoneurons. In the high gain situation, there will be a higher likelihood of random departures from strict recruitment as a result of noise—however, the general principle of rank ordered recruitment will remain.

Another factor that has contributed to a misunderstanding of the orderly recruitment is the expectation for precise rank ordered recruitment (Henneman et al., 1974). The degree of precision suggested from reduced animal preparations is not expected to hold for a noisy physiological system (Stein et al., 2005). It is important to remember that rank-ordered recruitment was originally defined for movement under healthy conditions. However, in conditions such as ageing, reinnervation, pain and fatigue, when precision decreases, other processes may obscure rank-ordered recruitment. With ageing, some motoneurons die leaving behind orphaned muscle fibers. The surviving motoneurons will sprout new terminals to reinnervate some of the orphaned fibers, thus changing the size of the muscle units (Chan et al., 2001; Gordon et al., 2004). Under these conditions, the order of recruitment will be less orderly which will lead to a decline in precision of motor output. During prolonged contractions in healthy adults, it has been shown that motoneurons fatigue and rotation of activity occurs among motor units (Manning et al., 2010). If rotation occurs among almost similar sized motor units, precision will remain unaffected. However, rotation among motor units of quite different sizes would increase the noise in motor output. Another example of disrupted rank ordered recruitment is the activity of motor units during pain (Tucker et al., 2009). These examples are not evidence for selective recruitment, but rather a decrease in precision of rank-ordered recruitment. In our experience the situation in which common sense reasoning favors selective recruitment is movements with maximum velocity over a short period of time: a ballistic movement. The reasoning is that slow motor units impede ballistic movements while exclusive recruitment of fast units would be optimal. However a recent study demonstrated that the recruitment of slow motor units does not pose any such problem (Holt et al., 2014).

The ultimate meaning of recruitment order lies in the force output of the entire musculoskeletal system for the purpose of behaviorally relevant movements. Clearly in *laboratory* conditions with simple muscles and a limited set of contractions, recruitment is orderly in terms of force (Milner-Brown et al., 1973; Calancie and Bawa, 1985; Zajac and Faden, 1985; Riek and Bawa, 1992; Jones et al., 1993). In biomechanically complex musculotendon-skeletal systems, accurately measuring the size of a motor unit may be difficult (Clamann and Schelhorn, 1988; Bodine-Fowler et al., 1990; Roy et al., 1995). The challenge for the field is to measure size and recruitment order, over relevant ranges of force during ecologically valid behavior (Jones et al., 1994). Until this is done, the question remains whether the laboratory defined *size principle of orderly recruitment* will generalize to movements of everyday life.

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Control of motor unit firing during step-like increases in voluntary force

***Xiaogang Hu*[1]*, *William Z. Rymer*[1,2] *and Nina L. Suresh*[1]**

[1] *Single Motor Unit Lab, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL, USA*
[2] *Department of Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*

Edited by:
Maria Piotrkiewicz, Nalecz Institute of Biocybernetics and Biomedical Engineering – Polish Academy of Sciences, Poland

Reviewed by:
Kemal Sitki Turker, Koç University School of Medicine, Turkey
Richard Stein, University of Alberta, Canada

***Correspondence:**
Xiaogang Hu, Single Motor Unit Lab, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, 345 E Superior Street, Room 1406, Chicago, IL 60611, USA
e-mail: xiaogang.hu@northwestern.edu

In most skeletal muscles, force is generated by a combination of motor unit (MU) recruitment and increases in the firing rate of previously active MUs. Two contrasting patterns of firing rate organization have been reported. In the first pattern, the earliest recruited MUs reach the highest firing rates as force is increased, and later recruited MUs fire at lower rates. When firing rate of multiple MUs are superimposed, these rate trajectories form a concentric layered profile termed "onion skin." In the second pattern, called "reverse onion skin," later recruited MUs reach higher firing rates, and crossing of firing rate trajectories for recorded MUs is common (although such trajectories are assembled routinely from different trials). Our present study examined the firing rate organization of concurrently active MUs of the first dorsal interosseous muscle during serial, step-like increases in isometric abduction forces. We used a surface sensor array coupled with MU discrimination algorithms to characterize MU firing patterns. Our objective was to determine whether "onion skin" profiles are contingent upon the force trajectory of the motor task, examined here using step-like increases of force output, and also whether they are manifested at different force levels. Our results revealed that the overall "onion skin" firing rate profile was retained as the force level increased with each force step up to 15% MVC. However, the distribution of firing rates across MUs was compressed with increasing force, and overlapping firing rate of units were observed. This rate compression was largely due to rate saturation of the relatively high frequency discharging MUs. Our results reflect flexible firing patterns across MUs at different levels of excitation drive. It is also evident that many units did not follow all the step increases consistently. This failure to track firing rate increases at higher forces could be due to an intrinsically mediated saturation of firing rates for the low threshold MUs, or potentially to some form of inhibitory interactions between active MUs as the level of excitation of the MU pool is progressively increased.

Keywords: motor unit, firing rate, recruitment threshold, onion skin, rate saturation

INTRODUCTION

Changes of voluntary muscle force are realized by adjustments of both motor unit (MU) recruitment and MU firing rates for units belonging to a given muscle. MU recruitment has been shown to be organized in an orderly manner, in that smaller MUs are recruited earlier and larger MUs are recruited later with increasing excitation. This recruitment rank order is widely known as the "size principle" (Henneman, 1957). Although there are also systematic MU firing rate adjustments in relation to the threshold of recruitment, the specific patterns of firing rate change with increasing voluntary command remain controversial, largely because of conflicting experimental observations.

One potential firing pattern is that earlier recruited units tend to fire slowly, while later recruited MUs fire at higher rates. This form of firing rate organization (termed here the "reverse onion skin" property) shows intersecting rate trajectories with increasing force, and has been reported in both cat (Kernell, 1965; Burke, 1968) and human muscles (Gydikov and Kosarov, 1974; Grimby et al., 1979; Moritz et al., 2005; Oya et al., 2009). This organization is intrinsically appealing because of the hypothetical match of MU firing rate profiles with MU twitch properties. Specifically, earlier recruited MUs tend to have smaller-sized but more prolonged twitches (Milner-Brown et al., 1973), meaning that firing rates can be slower while still maintaining partial fusion of MU forces during repetitive activation.

Conversely, the twitch force profile for later recruited and larger MUs tends to have a shorter duration, with a shorter rise time and a faster decay, which would require a higher MU firing rate for effective fusion of force twitches. Thus in this reverse onion skin scheme, the firing rates would (in theory) be well-matched to the contractile properties of the muscle fibers innervated by the motoneuron, and force output would be maximized for a given set of activated MUs. This strategy would also minimize the fluctuations of muscle force especially at high force levels (Hu et al., 2014b).

The assumption here is that the reverse onion skin pattern is a design feature of the pool that maximizes efficiency and force production, based on the assumed recruitment order of MUs.

The firing rates of different MUs would then be expected to be a function solely of the absolute recruitment threshold of the MUs, regardless of the form of force trajectory. However, the firing rate data in those studies reporting this reverse onion-skin pattern were obtained using intramuscular recordings, which are highly selective, yielding few MUs in each trial. As a consequence, earlier studies had to pool results from multiple recording sessions collected at different force levels and even from multiple subjects. This is potentially problematic when making inferences about the MU pool properties.

In contrast, the other firing rate pattern that has been reported is that later recruited MUs tend to fire at lower rates than do earlier recruited MUs, generating a layering effect of the firing rate trajectories over time (termed the "onion skin" property). This scheme has also been reported in both cat (Hoffer et al., 1987) and human muscles (Tanji and Kato, 1973; Freund et al., 1975; De Luca et al., 1982; De Luca and Hostage, 2010) during voluntary contractions. The issue regarding this paradigm is that the later recruited units potentially discharge at an unfused frequency, potentially producing force in an inefficient and fluctuating fashion. One functional benefit regarding this firing organization is that later recruited MUs are more fatigable (Burke, 1967); thus a lower firing rate for these larger MUs could limit fatigue and help maintain a sustained muscle contraction, and could also help fine control of muscle force. Additionally, the lower firing rates in later recruited larger MUs could allow for greater force reserves when needed (De Luca and Hostage, 2010; De Luca and Contessa, 2012).

A majority of the studies that have shown the "onion skin" firing pattern used a ramp-hold task, in which voluntary force is increased slowly followed by a steady hold of the force. However, it is possible that the observed lower firing rates of the higher threshold units recruited close to the end of the ramp force were due to a smaller effective excitation drive, since motor commands necessarily should diminish before the required force transition can take place. However, with further increases of excitatory drive, the initially plateaued firing rate of the later recruited MUs might well increase to a higher rate and potentially surpass the firing rates of the earlier recruited units. In this case, the onion skin pattern is potentially a manifestation of the drive to the motoneuron pool during the single ramp-hold task, and not necessarily a predetermined firing paradigm based on the properties of the motoneuron pool, although the size principle would still determine the order and thus the relative drive to each motoneuron in the pool.

To test this hypothesis, we examined the firing rate organization of concurrently active MUs of the first dorsal interosseous (FDI) muscle during serial, step-like increases of isometric forces. With sequential increases of force levels (excitation drive), we were able to follow the firing rate patterns of the same MUs and quantify the consistency of specific firing patterns at different force levels.

To discriminate MUs, we used a surface electromyogram (sEMG) sensor array coupled with a high-yield MU decomposition algorithm to characterize MU firing patterns. We then relate these firing patterns to recruitment threshold for each unit. The accuracy of the decomposition results for this approach has been assessed previously, and is described in more detail in the Materials and Methods section.

The results reveal that the firing rates of earlier recruited MUs indeed increased further with force level or excitatory drive. However, the overall "onion skin" profile was retained as the force level increased in sequential steps. We also found that the layering pattern was less distinct as muscle force increased, due to a saturation of firing rate of the earlier recruited MUs. Our findings indicate that the "onion skin" profile was retained during different types of isometric contractions including incremental step-like force increases addressed in the current study, as well as for trapezoidal force trajectories examined in earlier studies. The different patterns of firing rate modulation across MUs reflect a flexible firing organization with an increase of the excitation drive to the pool.

MATERIALS AND METHODS

PARTICIPANTS

Six right-dominant neurologically intact individuals (three male, three female) volunteered to participate in this study. All participants gave informed consent via protocols approved by the Institutional Review Board under the Office for the Protection of Human Subjects at Northwestern University.

EXPERIMENTAL SETUP

Participants were seated upright in a Biodex chair with their upper arm comfortably resting on a support. To standardize hand position and to minimize contributions of unrecorded muscles, the forearm was immobilized with a cast and placed in a ring mount interface attached to a forearm rest. The forearm was placed in full pronation and the wrist was held neutral with respect to flexion/extension. The little, ring, and middle fingers were extended away from the index finger and strapped to the support surface. The thumb was secured at an approximately 60 degree angle to the index finger. The index finger was placed in line with the second metacarpal and the long axis of the forearm creating a 0 degree or neutral metacarpophalangial joint angle (**Figure 1A**). The proximal phalanx of the index finger was fixed to a ring–mount interface attached to a six degrees-of-freedom load cell (ATI, Inc.). The recorded forces from the abduction-adduction direction were low pass filtered (cutoff = 200 Hz) and digitized at a sampling frequency of 2 kHz. The subjects were instructed to produce required abduction forces while minimizing the off-axis forces.

EMG recordings

The subject's skin was sterilized with alcohol pads to ensure proper electric contact and low baseline noise. sEMG was recorded from the FDI using a surface sensor array (Delsys, Inc.) as shown in **Figure 1B** that consists of five cylindrical probes (0.5 mm diameter). The probes are located at the corners and at the center of a 5×5 mm square. Pairwise differentiation of the five electrodes yields four channels of sEMG signals (**Figure 1C**). The sEMG sensor and a reference electrode were connected to four channels of a Delsys Bagnoli sEMG system. The signals were sampled at 20 kHz and were amplified and filtered (Butterworth) with a bandwidth of 20 Hz to 2 kHz.

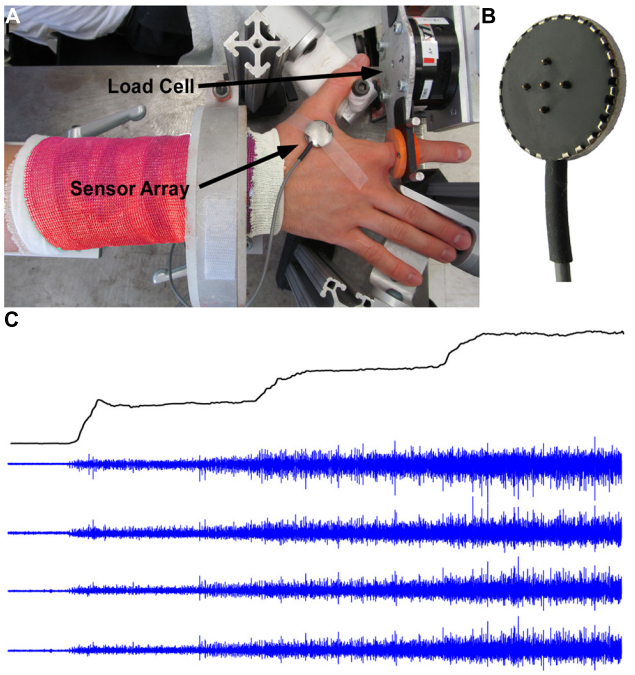

FIGURE 1 | Experimental setup, force, and EMG signals. **(A)** Experimental setup with surface EMG and force signal recordings. **(B)** The five-pin surface EMG sensor array. **(C)** Force trajectory with three force steps, and four channels of surface EMG signals.

PROCEDURES

Prior to the main testing session, subjects were asked to perform maximal voluntary contractions (MVCs) for 3 s. This maximum contraction was repeated three times in total, with 60 s rest between trials. The largest value of the three trials was designated as the MVC. The rest of the session consisted of a series of isometric voluntary contractions during which the subject was asked to follow step-like force trajectories displayed on a computer screen. The force output in one exemplar trial is shown in **Figure 1C**. The force trajectory contains four segments: a 3-s quiescent period for baseline noise calculation, a 5% MVC force step (a 0.5-s up-ramp increased at 10% MVC/s, a 9.5-s constant force at 5% MVC), a 10% MVC step (a 0.5-s up-ramp increased at a rate of 10% MVC/s, a 9.5-s constant force at 10% MVC), and a 15% MVC step (a 0.5-s up-ramp increased at 10% MVC/s, a 9.5-s constant force at 15% MVC). Given that the decomposition algorithm is template based and the algorithm works the best in a trapezoid force profile where the action potential template shape is relatively stable. To comply with the algorithm and ensure reliable decomposition results, we limited the force at low levels, such that the force increment at each step is relatively small (i.e., 5% MVC increase per step) and the template shape change is minimal, and meanwhile, the force increment is still large enough to induce measurable changes in firing rate and recruitment of MUs. To ensure that the subjects could follow the force target trajectory closely, they practiced a minimum of five trials of the force steps before the main experiment. For the main part of the experiment, the subjects performed 30 trials with a 60-s rest period between repetitions in order to minimize fatigue.

DATA ANALYSIS

Data processing

The sEMG and force trials were selected for further analysis based on the following criteria:

(a) there was no sudden change (i.e., larger than 20% MVC/s) in the up-ramp force,
(b) the force variability during each steady step was low (within $\pm$ 2 standard deviation of background force level), and
(c) the signal to noise ratio >5. The signal to noise ratio was calculated based on the peak–peak amplitude of the baseline noise and peak–peak amplitude of the EMG signal at steady state contractions.

These criteria were based on the suggestions for robust MU discrimination using the dEMG decomposition system (De Luca et al., 2006; Nawab et al., 2010). For each subject, based on the preceding criteria, approximately 10–15 trials were selected for further analysis. The dEMG decomposition algorithm was used to extract single MUs from the EMG data.

For each identified MU, the output from this algorithm consisted of the firing times and four normalized action potential templates from each of the four recorded sEMG channels. Our confidence in this approach is based on prior observations affirming the decomposition accuracy, which has been validated using simulation approaches (Hu et al., 2013a) and a two-source cross-validation method (Hu et al., 2014a). Specifically, in the simulation, we injected random errors to the decomposed spike timing and randomly shuffled the decomposed spike trains as well as action potential templates through a surrogate analysis. We found that the perturbed decomposition did not resemble either the action potential templates or the original EMG, suggesting that the original decomposition results were reliable. In the two-source validation, simultaneous intramuscular and surface EMG signals were recorded, and both signals were decomposed independently using separate decomposition algorithms. We found that the decomposition accuracy was 95% on average, based on approximately 120 commonly identified MU pairs from the two types of recordings.

The timing accuracy of the identified MU action potential train was assessed using a spike triggered averaging technique (Hu et al., 2013b), and the validity of the spike triggered averaging has been previously assessed using simulated sEMG signals (Hu et al., 2013c). Specifically, the spike triggered averaging was performed on each of the four channels of the sEMG signals, resulting in four action potential estimates for each MU. The identified firing times for each MU were then used as triggering events for the spike triggered averaging calculation. To ensure reliable estimate of firing rate, we then performed two separate tests to determine which MUs would be retained for further analysis. These tests were designed to assess the stability of the waveform over the trial duration and the degree of match with the decomposition estimated templates.

MU recruitment and firing rate estimation

To estimate the recruitment threshold, the threshold force of the selected MU was calculated from the averaged isometric force data

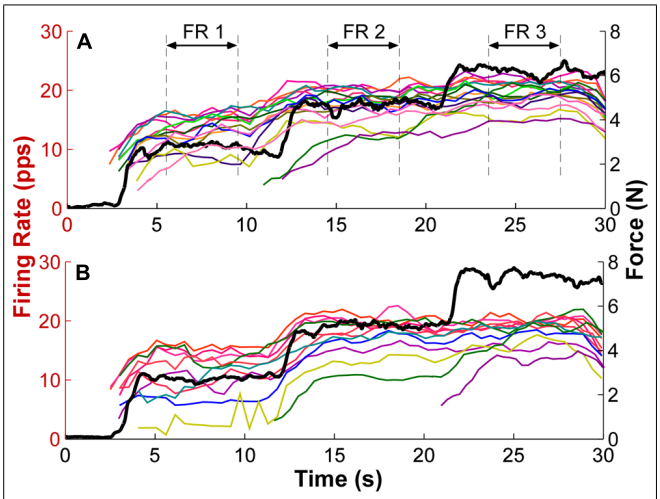

FIGURE 2 | Exemplar firing rate and force output profiles. (A) Each thin trace represents the firing rate over time of one exemplar trial. Different colors represent different motor units (MU). The mean firing rate (FR) of a 4-s window as marked by the dashed lines at each force step was calculated as the mean firing rate for each step. The thick black trace represents the force output. PPS represents pulses per second. **(B)** Firing rate and force traces of a second exemplar trial. Additional MUs were also recruited with step increase of force.

over an interval (−50 to 150 ms relative to the onset of the first firing event with an inter-spike interval smaller than 300 ms, which was the minimal discharge rate at recruitment). An averaged force was calculated to reduce the influence of force fluctuations registered at the load cell. The window was asymmetric relative to the firing time because of the electromechanical delay (ranging from 30 to 100 ms) between the occurrence of an action potential and a registered force increment (Cavanagh and Komi, 1979; Ce et al., 2013a,b). The mean firing rate was calculated using a 2 s moving window with a step of 0.5 s. The firing rate profiles of individual MUs with overlaid force output from two exemplar trials are shown in **Figure 2**. The mean firing rate (FR1, FR2, and FR3) for each force step was then calculated from a 4-s window as marked by the dashed lines. The middle 4-s window at each step was used because the force was relatively constant at the steady state muscle contraction and the firing rate was relatively stable. In order to track the change of firing rate organization, only the MUs recruited during the first step was used for analysis.

STATISTICAL ANALYSIS

The organization of MU firing properties as a function of the recruitment threshold was examined at each step increase of force output. A least-squared linear regression between the mean firing rate and the threshold force was performed on the concurrently active MUs at each step. The goodness of fit and the regression slope were compared between the three steps. Given that the regression slope varied considerably between subjects due to different MVC values across subjects, the change of regression slope was compared; specifically, the slope at the first step was used as a reference, and the relative difference between the step 1 and step 2 as well as between step 1 and step 3 were calculated:

$$Change\ of\ slope = \frac{Slope_i - Slope_1}{Slope_1} \times 100\% \quad (1)$$

where $Slope_i$ represents the regression slope at step 2 or step 3, and $Slope_1$ represents the regression slope at step 1. A negative *change of slope* value means that the regression at step 2 or 3 was shallower than the reference step 1, given that $Slope_1$ was negative as shown in the Results section.

The mean firing rate and the coefficient of variation (CV; standard deviation normalized by the mean) of firing rate across the concurrently active MUs were also compared cross the three force steps. A one-way repeated measures analysis of variance (ANOVA) was used to test whether the goodness of fit, the change of regression slope, the mean firing rate, and the CV of firing rate differs between force steps. When necessary, *post hoc* pairwise multiple comparisons with Bonferroni's correction method were used. $P < 0.05$ was considered as statistical significance.

RESULTS

We recorded surface EMG from the FDI using the array sensor in six intact right-handed subjects. Each trial consisted of a series of step-like increases in voluntary isometric abduction force. Each step sequence provided a substantial body of data, generating typically 10–20 MU recordings that were followed successfully over the step sequence. In total we were able to track several hundreds of units over six subjects.

The firing rate profiles of individual MUs from two exemplar trials are shown in **Figure 2**. Different MUs are represented in different colors, and the force trajectory is also plotted in thick lines. At force step 1, the earlier recruited MU discharge faster and the later recruited discharged slower, forming an "onion skin" pattern. As force increased to higher levels, the initially plateau in firing rate was interrupted, and firing rates increased further. Furthermore, this rate increment was more evident in the later recruited units (e.g., the yellow traces in both panels). As a result, the range of firing rate across units was compressed. Meanwhile, additional MUs were also recruited, and the firing rate of these newly recruited MUs also followed the force steps more closely than the earlier recruited units at step 1. Across the three force steps, the overall "onion skin" layering pattern was retained, although occasional firing rate crossovers were observed.

FIRING RATE IN RELATION TO THRESHOLD FORCE

The firing rate profiles in relation to threshold force at each force step are shown in **Figure 3** for one representative subject. Each symbol represents one MU and the symbols with the same color represent concurrently active MUs from a single trial. When the force was increased voluntarily in sequential steps, the overall firing rate of the recorded MUs increased accordingly. This increment of firing rate was especially evident in later recruited MUs with initially low firing rate. An inverse relation between firing rate and threshold force (i.e., an "onion skin" pattern) was observed consistently across the three force steps. However, as force increased, the rate increments narrowed, and as a result, the regression slope

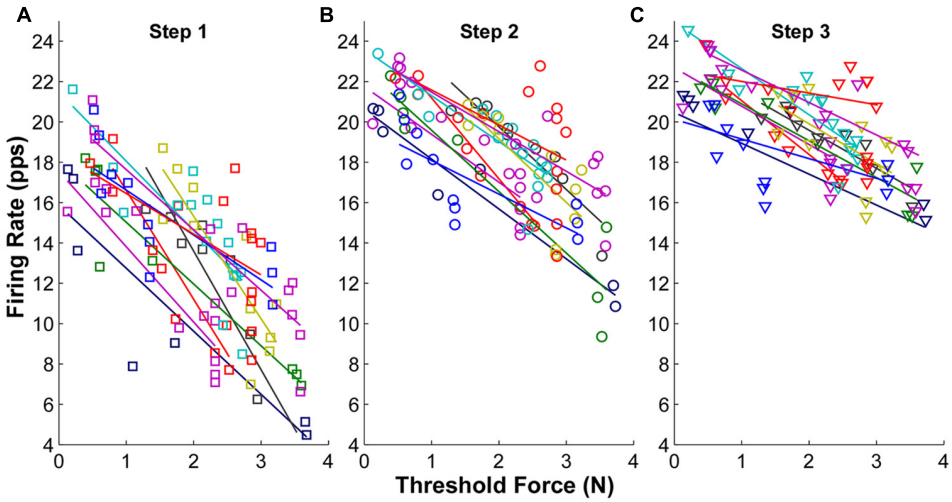

FIGURE 3 | Mean firing rate as a function of threshold force at each step from one exemplar subject. (A) Mean firing rate at step 1. Each symbol represents one motor unit, and different colors represent different trials. The linear regression line was also plotted for each trial. **(B)** Mean firing rate at step 2. **(C)** Mean firing rate at step 3.

(between firing rate and threshold force) became shallower, largely due to increased firing rates of the later recruited MUs.

The goodness of fit (R^2) plots across the three force steps are summarized in **Figure 4A**. The ANOVA results revealed a significant reduction of R^2 with an increment of force steps ($p < 0.05$) across the whole data set. The R^2 was 0.68 ± 0.03 in step 1, and reduce to 0.55 ± 0.06 in step 2 and 0.41 ± 0.05 in step 3. The reduction of R^2 was significant from step 1 to step 2 and 3 ($p < 0.05$) and was also significant from step 2 to step 3 ($p < 0.05$). Regarding the change of slope (**Figure 4B**), a negative value represents a shallower slope than the reference step 1 [calculated from Equation (1)]. The value at step 1 was strictly zero. The results showed

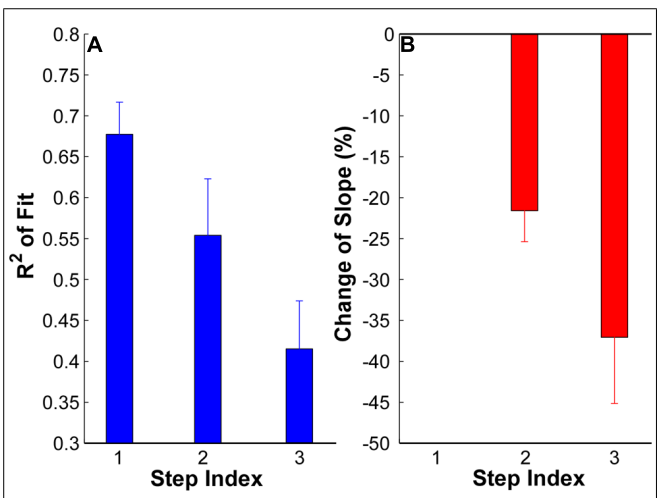

FIGURE 4 | (A) Goodness of fit (R^2) at each step of all the subjects. Error bars represent standard errors across subjects. **(B)** Change of regression slope in reference to the first step. The values at step 1 are strictly zero. A negative value here means a shallower slope in step 2 and 3 than in step 1.

that the regression slope was significantly shallower in step 2 and 3 compared with step 1 ($p < 0.05$). The regression slope in step 3 was also significantly shallower than in step 2 ($p < 0.05$).

MEAN FIRING RATE AND CV OF FIRING RATE ACROSS MUs

The mean firing rate of the MUs at the three force steps from one exemplar contraction is shown in **Figure 5A**. Each dot represents one MU and the same MU across the three steps is connected by solid lines. The red lines represent the group average from one representative trial. The firing rate increased from step 1 to step 2 consistently across the identified MUs; however, such a rate increment was not evident in most of the MUs with initially high firing rate in the step 1 and 2, and the degree of rate increment in the initially low firing rate MUs was also reduced.

To quantify the compressed range of firing rate with increasing force during a contraction, the CV of mean firing rate across the identified MUs in a single contraction was calculated at each force step (**Figure 5B**). One dot represents the CV from one trial, and the CV from the same trial is connected by solid lines. The red lines represent the group average of one particular subject. As shown in **Figure 5B**, the CV reduced substantially with an increment of force level, especially from step 1 to step 2. However, the CV reduction was not evident from step 2 to step 3 in certain trials.

The averaged firing rate and CV across subjects are summarized in **Figure 6**. The ANOVA results revealed that there was significant increase of firing rate in step 2 (15.19 ± 1.24 pps) and step 3 (16.05 ± 1.23 pps) compared with step 1 (12.69 ± 1.22 pps; $p < 0.05$); and the firing rates in step 2 and 3 were not significantly different ($p > 0.05$). Regarding the CV of firing rate, the CV reduced from 0.26 ± 0.02 in step 1 to 0.18 ± 0.01 in step 2 and 0.14 ± 0.01 in step 3. The reduction of CV was significant from step 1 to step 2 and 3 ($p < 0.05$) as well as from step 2 to step 3 ($p < 0.05$).

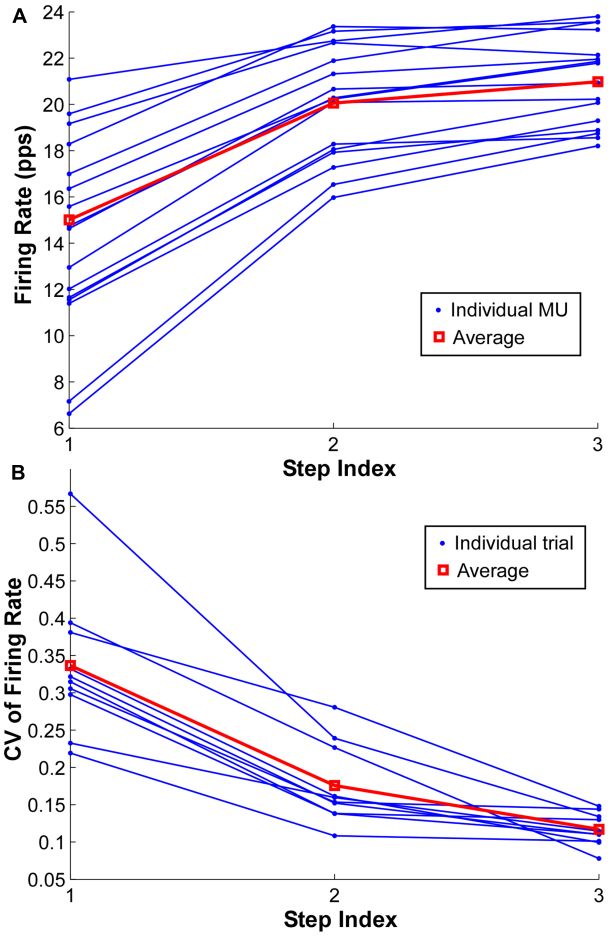

FIGURE 5 | Firing rate and coefficient of variation (CV) of firing rate at each step. (A) Mean firing rate at each step of individual motor units in one exemplar trial. The group average is shown as red square. **(B)** CV of firing rate at each step of individual trials from one exemplar subject. One dot represents one trial and the red square represents the group average.

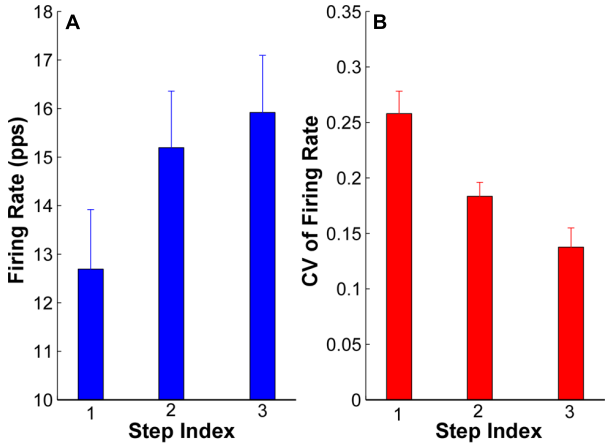

FIGURE 6 | Mean firing rate and CV of firing rate at each step from all the subjects. (A) Mean firing rate at each step of all the subjects. Error bars represent standard errors across subjects. **(B)** CV of firing rate of all the subjects.

DISCUSSION

This study examined the firing rate organization in relation to recruitment threshold of concurrently active MUs of the FDI muscle during step-like increases of isometric force. We used a sEMG sensor array together with a high-yield MU decomposition algorithm to characterize MU firing patterns in relation to recruitment threshold. The results showed that the "onion skin" firing pattern was retained as the force level was increased in sequential steps and no systematic firing rate crossovers were evident. We did find that the initial plateau in firing rate can be exceeded with these serial steps, and that firing rates of the units can be driven higher with increase of excitatory drive. The further increase of the initial firing rate plateau suggests that the observed onion skin is, at least in part, a consequence of the variation in effective drive to the MUs as a function of motoneuron recruitment threshold. Namely, the earlier recruited MUs received higher effective drive than the later recruited units leading to a higher firing rate observed in the lower threshold units.

We also found the "onion skin" layering pattern was less prominent (i.e., a worse goodness of fit and shallower regression slope) as muscle force increased. The weaker "onion skin" profiles at higher forces was largely due to a compressed range of firing rate in concurrently active MUs (i.e., a reduced CV of firing rate across MUs, due to an increase of firing rate of the later recruited MUs and a minimal increase of firing rate of the earlier recruited MUs). The sequential force steps allowed us to track the firing organization in the same group of active units at different force levels. With an increase of the excitatory drive, the initial plateau in firing rate indeed increased to higher levels. Although the rate increment of the later recruited units was larger, the increment was not large enough to cause systematic crossing of rate trajectories on our rate-time plots.

Given that the force levels tested were up to 15% MVC in the current study (due to the recommended force profiles as described in the Materials and Methods section), more rate crossovers might well be expected at higher force levels, if the excitatory drive is increased to even higher levels. However, we believe this outcome is relatively unlikely, because earlier studies have tested force levels close to maximum effort (De Luca and Hostage, 2010), and a strong "onion skin" firing pattern was still evident.

Two different mechanisms might lead to such a lack of systematic crossovers. First, additional MUs are being recruited during the force ramp, and these newly recruited MUs typically discharge at a low rate, therefore, maintaining the overall layering pattern. Second, as the force ramp is further increased, the later recruited units might eventually plateau at their peak firing rates. In order to confirm the second mechanism, it will be necessary to use a different MU recording technique that is capable of tracking firings from a MU pool over a larger force range than the one currently used in our study.

"ONION SKIN" vs. "REVERSE ONION SKIN"

Our findings are consistent with earlier reports that have shown concentric "onion skin" firing patterns in either concurrently

active MUs (De Luca et al., 1996; McGill et al., 2005; De Luca and Hostage, 2010; De Luca and Contessa, 2012) or in pooled MUs from multiple contractions (Tanji and Kato, 1973). Previous studies have also reported a weaker layering pattern with increasing force. For example, Monster and Chan (1977) showed that there is a consistent concentric layering pattern in steady firing rates from multiple intramuscular recordings, and that the firing rates of later recruited MUs rises at a steeper rate with increasing force, and do eventually catch up and discharge at a rate comparable to the earlier recruited MUs. However, it should be noted that the weaker concentric layering patterns observed in these earlier studies were assessed between different contractions, and thus presumably with different MUs. Our current study was able to extend these findings by tracking the firings of the same active MUs at different force levels during a single contraction. Similarly, with increasing force, a shallower regression slope between firing rate and recruitment threshold has also been reported (De Luca and Hostage, 2010), although again, the degree of change of slope does decline at high force levels (∼50% MVC), which is outside the force range tested in the current study.

Conversely, our current results did not reveal any recordings conforming with the general "reverse onion skin" profile at any force level, although occasional firing rate profile crossings (a key marker of "reverse onion skin" rate profiles) were observed as the force step increased (**Figures 2** and **5**). This "reverse onion skin" firing pattern has been reported in decerebrate animal models, in which motoneurons were activated by tonic muscle stretch, and firing rate profile crossings between MUs were observed (Eccles et al., 1958; Burke, 1968). Similar firing patterns have also been reported during voluntary contractions in different human muscles at different age groups (Moritz et al., 2005; Barry et al., 2007; Oya et al., 2009; Jesunathadas et al., 2012). One common feature of these firing patterns, regardless of the experimental conditions, is that the later recruited MUs tend to show a steeper rise of firing rate as excitation level increases and the rate eventually bypasses the firing rate of the earlier recruited MUs, as the firing rate of the earlier units saturates at rates lower than rates achieved by later units. Whereas in the "onion skin" firing pattern, the firing rate of the later recruited MUs tend to increase in the same or even slower rate compared with the earlier recruited ones.

It is also possible that the two different firing patterns arise from the differences in the MU composition (slow vs. fast) of the muscle; however, both firing patterns have been observed in a large range of muscles with different range of MU types. Specifically, the "onion skin" firing pattern has been observed in the FDI, biceps brachialis, deltoid, tibialis anterior, and vastus lateralis muscles, and the "reverse onion skin" pattern has been observed in FDI, soleus, and tibialis anterior muscles. Therefore, it is unlikely that the range of MU types in a muscle is responsible for to the two different firing patterns.

MECHANISMS OF LESS DISTINCT "ONION SKIN" LAYERING EFFECT

When the force was increased in sequential steps, the "onion skin" profile became less evident; namely, a poorer goodness of fit and a shallower slope of the linear regression were found at higher forces. The weaker layering effect is largely due to a narrowing of the distribution (i.e., a reduced CV) of the firing rate across MUs. Such a compression of firing rate can arise from a rate saturation of earlier recruited MUs and a relatively large rate increment of later recruited MUs. With increasing force output, presumably an increasing excitatory current input, the later recruited MUs with initially low firing rate have firing profiles followed the force trajectory. This rate increment primarily reduces the range of firing rate across MUs. This large rate increment also leads occasionally to firing rate profile crossovers as shown in **Figures 2** and **5**, which adversely affect the goodness of fit in the linear regression.

Unlike high threshold MUs, the firing rate of many low threshold MUs did not follow all the step increases consistently, especially from step 2 to step 3. This reduced rate modulation could be due to an intrinsically mediated saturation of discharge rates for the low threshold units (e.g., perhaps via persistent inward current (PIC) mechanisms). The PIC is a persistent depolarizing current that amplifies synaptic input. It can trigger an initial steep increase of firing rate, but can also limit the subsequent rate increase due to PIC saturation (Heckman et al., 2005). The reduced rate modulation could also be due to inhibitory interconnections between MUs. Typically, an isolated motoneuron will discharge faster with increasing excitatory current input. However, the recurrent inhibition circuits formed between Renshaw cells, motoneurons, and interneurons generate inhibitory current and can modulate the effectiveness of excitatory current increment (Burke et al., 1971; Hultborn et al., 1979).

In this case, with an increase of excitatory input, the inhibitory input also potentially increases disproportionally, leading to a reduced or unchanged net increase, and therefore rate saturation. Additionally, the high threshold motoneurons are likely to contribute more to the activation of Renshaw cells (Hultborn et al., 1988b) and the amount of inhibitory current received is higher in slower twitch units (Hultborn et al., 1988a). These scaled differences between slow and fast units can contribute to early saturation of firing rate, primarily in the low threshold units.

ACCURACY OF THE DECOMPOSITION RESULTS

Given that the sEMG decomposition approach is developed recently, it is important to ensure that the decomposed MU results are reliable and that they do reflect physiological properties of the MU pool. Previous studies have evaluated the decomposition accuracy using both simulation approaches (Hu et al., 2013a) and a two-source validation method. Specifically, in the simulation, we introduced random timing noise/errors to the decomposed spike timing and randomly shuffled the decomposed spike trains as well as action potential templates through a surrogate analysis. We found that the perturbed decomposition does not resemble the action potential templates or the original EMG signal, when the waveform of action potentials and EMG signals were reconstructed using the perturbed decomposition results, suggesting that the original decomposition results were reliable, at least in the tested force levels up to 50% MVC. We also acknowledge that the simulation approach cannot detest missed firings (false negatives), and the goal of the simulation was to assess the general validity of the dEMG algorithm, rather than assessing the

explicit accuracy of particular spike timings. In the two-source validation, concurrent intramuscular and surface EMG signals were recorded, and both signals were decomposed independently using separate decomposition algorithms. We found that the decomposition accuracy was 95% on average in the 119 (10.4%) common MUs out of 1143 identified MUs from the sEMG signals. The two-source method provided critical assessment of the spike timing accuracy detecting both spurious and missed firings; however, the force levels were tested up to 15% MVC. The maximal force level in our current study was also limited at 15% MVC; therefore, the decomposed MU firings are reliable at these force levels.

In addition to the accuracy assessment described above, we performed a spike triggered averaging technique (Hu et al., 2013a) to filter potentially unreliable MU spike trains in the current study. Specifically, we evaluated the stability of the action potential waveform over the trial duration and the degree of match with the decomposition estimated templates, to ensure that decomposed firing train was accurate. However, the dEMG algorithm was developed originally based on a single trapezoid force profile, and a steady state contraction was required to perform the template matching process. It is possible that the series of force steps may induce decomposition errors due to action potential shape changes between force steps, which can provide erroneous firing spike trains. However, this possibility is unlikely because the template tracking algorithm allows a certain degree of progressive change of the action potential shape as in the case of a ramp-up state from 0% up to 90% MVC, and the current study only induced a 5% MVC force change. In fact, our current study shows that the algorithm can detect the increase of MU firing rate with step-increase of force output, indicating that the algorithm does not provide artificially pre-conditioned firing patterns.

CONCLUSION

In this study, we examined the firing rate patterns of a large number of concurrently active MUs at different force levels during a series of force steps. We found that the initially plateau in firing rate of lower threshold units increased further as excitatory drive is increased; however, the rate increment of discharge in later-recruited units was not strong enough to induce systematic crossings between firing rate profiles. Most importantly, we observed "onion skin" firing profiles across different force levels up to 15% MVC (although the layering pattern was compressed with increasing force). Further study is necessary to examine whether systematic cross-overs between MU firings can occur when higher force levels are tested.

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Maximal discharge rate of motor units determines the maximal rate of force development during ballistic contractions in human

Jacques Duchateau* and Stéphane Baudry

Laboratory of Applied Biology, ULB Neurosciences Institute, Université Libre de Bruxelles, Brussels, Belgium
**Correspondence: jduchat@ulb.ac.be*

Edited by:
Annie Schmied, Centre National de la Recherche Scientifique, France

Reviewed by:
Andrew Joseph Fuglevand, University of Arizona, USA
Dario Farina, Georg-August University, Germany

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INTRODUCTION

The magnitude of the neural activation, and hence the force produced by a muscle, depend on the number of motor units activated (recruitment) and the rates at which motor neurons discharge action potentials (rate coding). Although the recruitment order of motor units (size principle) is similar for contractions during which the force is gradually increased (ramp contraction) and those during which the force is produced as fast as possible (see Duchateau and Enoka, 2011), rate coding differs between the two types of contractions (Desmedt and Godaux, 1977a,b; Bawa and Calancie, 1983). Motor unit discharge rate increases progressively during slow ramp contractions (Milner-Brown et al., 1973) whereas fast contractions involve high instantaneous discharge rate that decreases thereafter (Desmedt and Godaux, 1977a; Van Cutsem et al., 1998). Maximal discharge rate during slow isometric ramp contractions usually reaches values of 20–50 Hz whereas it can attain much higher values (>100 Hz), albeit briefly, during fast contractions (for reviews, see Enoka and Fuglevand, 2001; Duchateau and Enoka, 2011)

Fast isometric contractions can be performed in different ways. A first possibility is to increase force as quickly as possible up to a certain level and to maintain this force for a few seconds (step and hold contraction). An alternative way is to produce force as fast as possible but to relax the muscle immediately after the target force is reached. Such impulse-like contractions have been termed *ballistic contractions* (Desmedt and Godaux, 1977a). Although both contractions involved reaching a target force as fast as possible, results from our laboratory indicate that the maximal rate of torque development is ∼16% greater for ballistic than step and hold contractions (465.2 ± 17.4 vs. 400.5 ± 20 Nm/s; mean ± SD) performed with the ankle dorsiflexor muscles. Considering the difference in motor unit discharge rate between slow and fast contractions, these data suggest that ballistic contractions could be used to assess the maximal discharge rate of motor neurons in humans.

MOTOR UNIT DISCHARGE RATE DURING BALLISTIC CONTRACTION

Desmedt and Godaux (1977a) were the first to provide a detailed description of motor unit discharge in the tibialis anterior muscle during ballistic contractions. They reported that during ballistic contractions, motor units usually began to discharge at high instantaneous rates (60–120 Hz) that thereafter declined progressively during their successive discharges, presumably reflecting the initial phase of discharge rate adaptation observed during repetitive activation of motor neurons (Sawczuk et al., 1995; Miles et al., 2005). Such discharge pattern has been also reported for the first dorsal interosseus (Desmedt and Godaux, 1977b) and the masseter (Desmedt and Godaux, 1979), with very brief interspike interval (<10 ms) mainly observed for the initial discharges (Desmedt and Godaux, 1977a; Van Cutsem et al., 1998; Van Cutsem and Duchateau, 2005). Similar brief interspike intervals have also been recorded in the flexor carpi radialis during fast and hold contractions (Bawa and Calancie, 1983). Such high motor unit discharge rates are similar to those reported for motor neurons in animal studies in response to fast current injection (Kernell, 1965; Baldissera et al., 1987; Sawczuk et al., 1995), and should mainly reflect the effect of the strong excitatory inputs required to produce ballistic contractions. However, these very high discharge rates could also be influenced by the trajectory of motor neuron membrane after de repolarization phase (delayed depolarization phase and/or after-hyperpolarization period—AHP) at the time of the activation (see Garland and Griffin, 1999; Kudina and Andreeva, 2013).

TASK-RELATED CHANGES IN DISCHARGE RATE

The discharge characteristics of single motor units during ballistic contractions can be modulated by the conditions under which the action is performed. For example, it has been observed that when a ballistic contraction with the ankle dorsiflexors was superimposed on a submaximal isometric contraction (20–25% of maximal force), the average discharge rate for the first three interspike intervals was significantly reduced by 22% (89.8 ± 14.6 vs. 115 ± 20.9 Hz; mean ± SD) compared with ballistic contractions performed from a resting state (Van Cutsem and Duchateau, 2005). The percentage of

motor units that exhibited discharges rate above 200 Hz at the onset of the activation was also diminished (6.2 vs. 15.5%). Interestingly, the instantaneous discharge for the first interspike interval was much reduced (−37%) than the second (−18%) and third (−8%) intervals. This lower motor unit discharge rate during superimposed ballistic contractions was accompanied by a decrease in the maximal rate of force development (∼16%). The slower rate of force development and reduced motor unit discharge rate during the superimposed ballistic contractions are, however, abolished when a brief silent period (usually called "premotor silent period") was observed at the transition between the pre-activation (sustained contraction) and ballistic actions (Van Cutsem and Duchateau, 2005). A similar observation has been reported when a brief voluntary agonist relaxation (deactivation) was inserted between the sustained and the ballistic action (Duchateau and Baudry, 2012). These silent periods (unintentional and voluntary) are thought to enable motor neurons to achieve a non-refractory state leading to a more synchronous recruitment and a greater discharge rate of motor units during the subsequent ballistic action (Tsukahara et al., 1995; Van Cutsem and Duchateau, 2005). The changes in maximal discharge rate achieved during ballistic contractions with initial conditions likely reflect the history-dependent changes of motor neuron excitability (Heckman and Enoka, 2012), and on a functional point of view supports the association between the maximal motor unit discharge rate and the rate of force development.

LONG-TERM CHANGES IN DISCHARGE RATE

A way to further investigate this association consists of studying long-term changes in the maximal discharge rate of human motor units, such as those occurring in response to training and ageing. For example, Van Cutsem et al. (1998) reported that 3 months of ballistic contractions of the ankle dorsiflexor muscles against a moderate load (30–40% MVC) enhanced the maximal rate of force development by 82% during ballistic contractions. Although no change was observed in the recruitment order of motor units, the average discharge rate of the first four action potentials increased by 38% after training (96.3 ± 39.5 vs. 69.9 ± 30.8 Hz; mean ± SD). The increase in discharge rate was significantly less for the first (+86%) and second (+70%) than the third (+124%) interspike intervals. In addition, training increased the number of motor units (5–33%) exhibiting discharges above 200 Hz at the onset of activation. Because the average time to peak force of motor unit mechanical responses was not significantly modified, the increase in the rate of force development during the ballistic contractions was mainly due to adaptation in motor unit discharge rate. Potential mechanisms that may explain the changes in motor unit discharge rate should involve different loci along the corticospinal pathway. Although some of these changes can occur at supraspinal level (Schubert et al., 2008), part of the adaptations presumably involve changes in the intrinsic properties of motor neurons, as observed after endurance training in rats (Gardiner et al., 2006).

In contrast to training, the ageing process induces a decline in the speed-related capacity of individuals. For example, the maximal rate of force development during ballistic contractions performed with the ankle dorsiflexor muscles was significantly lower by 48% in elderly (71–84 year) than in young adults (∼20 year) (Klass et al., 2008). This age-related change was accompanied by a clear decline in the average motor unit discharge rate. As the decrease was less pronounced for the first (−19%) than for the second (−28%) and third (−34%) interspike intervals, this means that the aged motor units cannot sustain a high discharge rate during successive discharges. In addition, the percentage of motor units that exhibited initial discharges above 200 Hz was reduced (−45%) in elderly compared with young adults. As the rate of force development during electrically evoked contractions, that by-pass motor neurons activation, is less reduced than those during ballistic voluntary contractions, the decline in maximal motor unit discharge rate should significantly contribute to limit the performance of fast voluntary contractions with ageing. The age-related prolongation in the duration of motor neuron after hyperpolarization, as observed in the human biceps brachii by Piotrkiewicz et al. (2007), could be a relevant candidate to explain, at least in part, the reduced maximal rate of motor unit discharge during ballistic contractions in elderly adults.

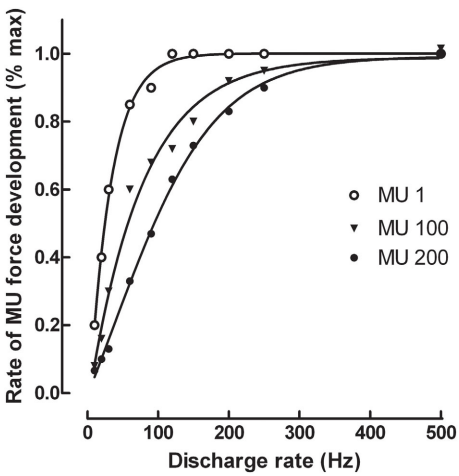

FIGURE 1 | Simulation of the relation between motor unit discharge rate and maximal rate of force development for the 1st, 100th, and 200th motor unit (MU) of a pool of 200 units in the tibialis anterior muscle. The simulation was based on a model developed by Fuglevand et al. (1993) with the inclusion of the spike-triggered average forces for motor units published by Van Cutsem et al. (1998). The force generated by each motor unit was simulated for 4 successive discharges generated at constant frequencies ranging from 10 to 500 Hz before the first derivative was computed to obtain the maximal rate of force development.

MODELING THE RELATION BETWEEN MOTOR UNIT DISCHARGE RATE AND RATE OF FORCE DEVELOPMENT

To further analyse the effect of a change in discharge rate on the maximal rate of force development, isometric force produced by single motor units was simulated from a model that contains a pool of 200 units (Fuglevand et al., 1993; Duchateau and Enoka, 2002). To that purpose, mechanical properties (peak force and time to peak force) of motor units obtained from the spike-triggered averaging method in the tibialis anterior (Van Cutsem et al., 1998) were inserted into the model. Data indicated that an increase in discharge rate up to 100–200 Hz augmented substantially the rate of force development for all units of the pool (**Figure 1**). Nonetheless, further increase in discharge rate has less influence excepted for the faster units (MU 100 and MU 200) of the pool, reflecting difference in speed-related properties between low- and high threshold motor units. These simulated data underscore the critical role of maximal motor unit discharge rate on the ability to rapidly develop force.

CONCLUDING REMARKS

Together, experimental and simulated data indicate that a high initial motor unit discharge rate at the onset of a fast contraction plays a critical role to reach a high rate of force development. Furthermore, and because the instantaneous discharge rates of motor units at the onset of ballistic contractions are much greater than those recorded during slow contractions and not yet influenced by history-dependent effects, ballistic contractions from a resting state can be used to assess the maximal motor neuron discharge rate in human. Nonetheless, as the acquisition of a simple motor task such as index finger abduction requires up to ∼300 repetitions to reach maximal acceleration capability (Lee et al., 2010), subjects must be familiarized beforehand with ballistic contractions of the muscle under study.

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Motor unit discharge rate in dynamic movements of the aging soleus

Jouni Kallio[1]*, Karen Søgaard[2], Janne Avela[1], Paavo V. Komi[1], Harri Selänne[3] and Vesa Linnamo[4]

[1] *Department of Biology of Physical Activity, Neuromuscular Research Center, University of Jyväskylä, Jyväskylä, Finland*
[2] *Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark*
[3] *LIKES, Foundation for Sport and Health Sciences, Jyväskylä, Finland*
[4] *Vuokatti Snowpolis, Department of Biology of Physical Activity, Neuromuscular Research Center, University of Jyväskylä, Jyväskylä, Finland*

Edited by:
Maria Piotrkiewicz, Polish Academy of Sciences, Poland

Reviewed by:
Piotr Krutki, University School of Physical Education in Poznan, Poland
Wojciech Zmyslowski, Institute of Biocybernetics PAS, Poland

***Correspondence:**
Jouni Kallio, Department of Biology of Physical Activity, Neuromuscular Research Center, University of Jyväskylä, Rautpohjankatu 8, P. O. Box 35, FIN-40351 Jyväskylä, Finland
e-mail: jouni.kallio@likes.fi

Aging is related to a variety of changes at the muscular level. It seems that the age-related changes in motor unit activation are muscle- and intensity dependent. The purpose of this study was to examine the motor unit discharge rate (MUDR) in both isometric and dynamic contractions of the aging soleus muscle. Eight elderly males participated in the study. The subjects performed isometric and dynamic plantar flexions while seated in an ankle dynamometer. The force levels studied were 10, 20, 40, 60, 80 and 100% of the isometric (ISO) maximal voluntary contractions (MVC) in ISO and 10, 20 and 40% in concentric (CON) and eccentric (ECC) contractions. Soleus intramuscular EMG was recorded with bipolar fine-wire electrodes and decomposed to individual trains of motor unit discharges. In ISO the MUDR increased with each force level from 40 to 100% MVC. In dynamic contractions the descriptive analysis showed a higher MUDR in CON compared to ISO or ECC. The difficulties of recording single motor units in dynamic contractions, especially in the elderly is discussed.

Keywords: aging, dynamic contraction, soleus, intramuscular electromyography, motor unit

INTRODUCTION

It is well known that ageing is related to changes at the muscular level, leading to a decline in motor performance. However, it seems that such changes in motor unit activation are muscle- and intensity dependent. Several studies have found the elderly to have a lower discharge rate during high intensity contractions in muscles, like the first dorsal interosseous, tibialis anterior (Kamen et al., 1995; Klass et al., 2008) and vastus lateralis (Kamen and Knight, 2004). In contrast, the soleus motor unit discharge rate (MUDR) was found to be lower in the elderly only at low force-levels, and for other muscles no age related decrease in MUDR was found in studies by Howard et al. (1988) and Galganski et al. (1993).

So far age-related differences in motor unit discharge behavior have mainly been investigated in isometric contractions. A large number of studies in young subjects have shown that neuromuscular control of isometric and dynamic contractions differ in many ways, including MUDR (Tax et al., 1989; Howell et al., 1995; Søgaard et al., 1996, 1998; Kossev and Christova, 1998; Del Valle and Thomas, 2005; Pasquet et al., 2006; Altenburg et al., 2009; Kallio et al., 2013) and discharge patterns (Søgaard, 1995; Søgaard et al., 1998) and double discharges (Søgaard et al., 2001).

Considering how essential dynamic contractions are in daily life activities, it is important to investigate the MUDR patterns also in dynamic contractions. This paper extends the scope of our previous study from the effects of contraction type on MUDR in young males (Kallio et al., 2013) to the effects of aging.

MATERIALS AND METHODS

Eight physically active old males (age: 69.1 ± 5.1 year height: 1.69 ± 0.4 m body mass: 75.3 ± 6.2 kg), volunteered as subjects for the study. Physical activity of the subjects was determined with a questionnaire, where the subjects wrote the type and frequency of their regular exercise routine. All men performed moderate to strenuous physical exercise three times a week or more. Before the measurements all of the subjects underwent a medical examination. Only subjects without any history of neuromuscular or vascular disease were approved for the study.

The measurement protocol and analysis has been described in more detail in Kallio et al. (2013). Shortly, the subjects performed isometric and dynamic plantar flexions while seated in an ankle dynamometer. The test battery included (1) maximal voluntary contractions (MVC); (2) isometric contractions 10, 20, 40, 60, 80 and 100% of the isometric MVC; and (3) dynamic contractions 10, 20 and 40% of the isometric MVC: In the dynamic trials the subjects lifted concentric (CON) or lowered (ECC) a weight stack that was attached to the foot pedal via a cable pulley system at a voluntarily controlled velocity of 10°/s (**Figure 1**).

Global EMG activity of the soleus (SOL) and gastrocnemius medialis (GM) muscles was recorded using surface electrodes (Beckman 650437, USA), calculated as root mean square (RMS) of the signal and normalized relative to the maximal EMG at isometric MVC. For the intramuscular EMG recordings, four separate bipolar fine-wire electrodes were inserted into the soleus muscle. Signal decomposition,

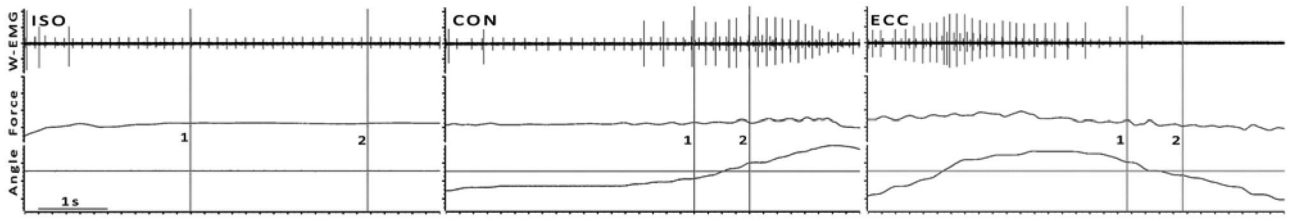

FIGURE 1 | Examples of ISO, CON and ECC trials from one subject at 10% MVC. Cursors 1 and 2 mark the beginning and end of analysis, respectively. In dynamic contractions Cursor 1 is 200 ms prior and Cursor 2 200 ms after the angle crosses the angle of the ISO trial, as indicated by the horizontal cursor. This figure is a typical wire electrode recording (W-EMG), where the recruitment of an additional motor unit in CON and a derecruitment of active units in ECC can be observed (see Section Discussion for details).

motor unit identification and data analysis were performed by utilizing the three channel decomposition technique computer algorithm, "Daisy" (Søgaard et al., 1996; Farina et al., 2001; Olsen et al., 2001). The MU classification was performed semi-automatically with a high degree of operator interaction.

The statistical analysis is limited to the isometric contractions, as the low number of subjects and analyzed units prevents statistical analysis for the dynamic contractions. The normality of distribution was tested with the Shapiro-Wilk test. In the data from the pooled motor units an analysis of variance (ANOVA) was used to compare the effects of contraction intensities. When a significant difference was detected, Bonferroni's method was used to locate the difference. The critical level of significance was $P < 0.05$. Descriptive statistics include mean and standard deviation.

RESULTS

The maximal voluntary isometric plantar flexion force was 149.2 ± 35.7 Nm. The MUDR analysis was based on a total of 2600 motor unit discharges 75 unique identified motor units. The number of subjects with decomposable units in each condition and the number of units can be seen in **Figure 2**. In ISO contractions, where force levels were explored up to 100% MVC, the MUDR increased significantly along with the increase in relative force level between 40 and 100% MVC (**Figure 2**). The increase followed a second order polynomial equation ($y = 0.6x^2 - 2.2x + 9.0$, $R^2 = 0.96$). Similarly the relative sEMG activity of Soleus and Gastrocnemius increased with every step increase in force after 20% MVC. At the three lowest force levels all three contraction types were measured and according to the descriptive analysis the MUDR (**Figure 2**) and the RMS of surface EMG (**Table 1**) were

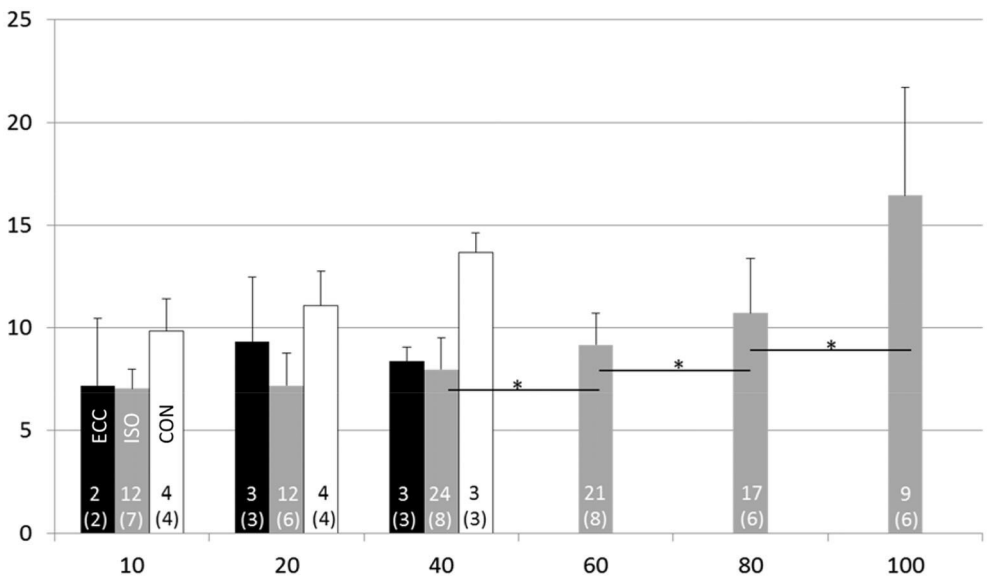

FIGURE 2 | Motor unit discharge rate (1/s; X ± SD) at different force levels in eccentric (ECC), isometric (ISO) and concentric (CON) contractions. Number of motor units and subjects (in parenthesis) for each contraction type and activation level are displayed at the bottom of each bar. * = difference between action levels ($p < 0.05$).

Table 1 | Relative surface EMG levels of soleus (SOL) and gastrocnemius medialis (GM) in different levels and contraction types, calculated relative to isometric MVC.

Type	Level(%)	SOL%	GM%
ECC	10	31.8 ± 10.1	39.7 ± 0.2
	20	39.5 ± 16.7	34.0 ± 13.5
	40	43.3 ± 6.0	70.1 ± 20.1
ISO	10	20.9 ± 7.5	15.8 ± 4.1
	20	31.2 ± 6.7*	23.3 ± 7.2*
	40	47.5 ± 7.7*	32.8 ± 14.7*
	60	63.6 ± 6.9*	50.5 ± 16.0*
	80	73.3 ± 6.2*	81.8 ± 20.3*
	100	96.9 ± 10.9*	128.4 ± 32.2*
CON	10	47.2 ± 19.6	18.4 ± 2.1
	20	61.8 ± 20.7	42.2 ± 5.1
	40	70 ± 12.6	53.1 ± 20.5

** Significantly higher than in lower force level. Statistics calculated only for ISO, see text for details.*

higher in concentric (CON) compared to isometric (ISO) and ECC.

DISCUSSION

The main finding in the present study is that the in elderly males CON contractions required a higher MUDR than ECC or isometric contractions to reach the same relative force level.

As stated in the methods section, the same study has been conducted with adult males, aged 20–30 years (Kallio et al., 2013). In comparison, the current results show that in ISO trials MUDR was significantly lower in the elderly in most measured force levels (10, 40, 60 and 80% MVC). Similarly, both SOL and GA s-EMG-levels were significantly lower in the elderly (10, 20, 40 and 60% MVC). The observed age-difference in MUDR confirms the results of our previous measurements in isometric conditions (Kallio et al., 2012, 2013), as well as in submaximal dynamic (Kallio et al., 2010) contractions matched by relative surface EMG instead of force. Dalton et al. (2009) found in isometric condition an age difference in soleus MUDR at 25 and 50% MVC, but not at the higher contraction levels. Like the current study Dalton also reported a force-related increase in MUDR that was most pronounced at the high intensities in the elderly. The present results for the soleus muscle support the findings in other muscles, showing the age-difference in MUDR to be largest at high force levels (Kamen et al., 1995; Patten and Kamen, 2000; Kamen and Knight, 2004; Barry et al., 2007). Overall the current knowledge seems to suggest that the age-related changes in on MUDR are similar for the plantar flexor and the ankle dorsal flexor tibialis anterior (Connelly et al., 1999; Klass et al., 2008), as well as the small hand muscles first dorsal interosseous (Kamen et al., 1995) and adductor digiti minimi (Nelson et al., 1984). The decreased MUDR in the elderly has been suggested to be an adaptation to the increased twitch duration to optimize force generation (Roos et al., 1997). Previous studies have confirmed that the triceps surae twitch duration actually increases with age, suggesting that tetanus may be achieved with lower discharge rates (Dalton et al., 2009, 2010; Kallio et al., 2012).

Unfortunately the number of identifiable motor units was very low in the dynamic contractions. This was unexpected, considering that in the same study protocol with eleven younger subjects the number of units was between 2 and 9 times higher. Measuring and analyzing single motor units in dynamic contractions in elderly is very challenging, especially in ankle joint that has quite a small range of motion. Accelerating and controlling the ankle extension velocity is not easy, and the combination of a good MU recording and a kinematically successful trial was rare. The lower number of kinematically successful trials we were able to measure with the elderly explains only partially the lower N in current study. Why we had so few clear intramuscular EMG-recordings compared to the previous study is not clear. It could be speculated, that the increase in subcutaneous body fat could cause some of the wire-electrodes to have been placed too superficially in the elderly. Although we did not measure the body fat in our subjects, the small difference in BMI (25 in young, 26 in elderly) would not support this hypothesis.

It can be questioned if the age-related deterioration in muscle performance, like decreasing maximal force and slowing of MUDR and force production, is just an inevitable part of ageing or if it can be partly explained by an age related change in everyday activity. In the present study all the elderly subjects were as active as the younger subjects in our previous study (Kallio et al., 2013). Still, the isometric MVC was 29.3% lower ($p < 0.01$) compared to adult males. However, the open-ended questionnaire also showed clear differences in the types of activities young and elderly engaged in. The young subjects were more active in sports requiring strength and speed (e.g., running, soccer, ice-hockey, weight-training, tennis), than the elderly who mainly were walking with and without poles, swimming and gardening).

The descriptive analysis showed the largest MUDR in CON. Since we controlled the weights so that the absolute plantarflexion forces in all intensities were equal between contraction types, the most likely reason for the difference is found in the cross-bridge physiology. It has been shown that the force production of the cross-bridge is decreased when producing movement in CON contractions (Joyce et al., 1969). For this reason, to generate an equal amount of force, more activity either in terms of more recruited motor units or higher discharge frequency is required in CON compared to ISO or ECC contractions, which was also apparent as an increased surface EMG.

The joint rotation velocity (10°/s) in the current study was quite slow compared to most natural movements. For example, in walking, the angular velocity in dorsiflexion is ∼30°/s and in plantarflexion ∼220°/s (estimated from Lichtwark et al., 2007). The next challenge for motor unit studies would be to see how the activation functions in normal locomotion.

To our knowledge, this is the first attempt to record the effects of aging on the MUDR in dynamic contractions of large leg extensors. Based on our limited data the increase in MUDR when performing CON contractions was found to be similar to that of younger men. In isometric contractions the MUDR of the elderly men was lower compared to younger men in most measured force levels.

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Delayed depolarization and firing behavior of human motoneurons during voluntary muscle contractions

Lydia P. Kudina* and Regina E. Andreeva

Department of Bioinformatics, Institute for Information Transmission Problems (Kharkevich Institute), Russian Academy of Sciences, Moscow, Russia
**Correspondence: kudina@iitp.ru*

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INTRODUCTION

The firing behavior of motoneurons is governed by the interaction between the intrinsic properties of motoneurons and the synaptic inputs that they receive. In particular, long-lasting after-hyperpolarization (AHP) following each motoneuron spike and thus decreasing motoneuron excitability immediately after spike was found to be a key mechanism in controlling of interspike interval (ISI) duration (Eccles et al., 1958; Kernell, 1965). As a result, motoneurons commonly fire single discharges at rather low firing rates, especially during gentle voluntary muscle contractions and postural tasks. However, some motoneurons, under conditions of weak synaptic drive, can fire double discharges (doublets) with very short intradoublet ISIs (up to a few ms). Each doublet is commonly followed by a prolonged post-doublet (interdoublet) ISI resulting from the AHP summation (Calvin and Schwindt, 1972). Two types of doublets have been observed: single and repetitive ones. Single doublets have been reported many times in cat and human motoneurons (e.g., Eccles and Hoff, 1932; Hoff and Grant, 1944; Denslow, 1948; Calvin and Schwindt, 1972; Kudina, 1974; Kudina and Churikova, 1990; Kirkwood and Munson, 1996; Piotrkiewicz et al., 2008; Stephenson and Maluf, 2010). Repetitive doublets have also been demonstrated in some of the reports above, but as a rule by the way; and only a few studies have directly addressed this topic (Bawa and Calancie, 1983; Kudina and Alexeeva, 1992; Kudina and Andreeva, 2010). However, a number of questions are still unanswered, in particular the question of whether there are certain muscles and (or) certain motoneurons for which repetitive doublet firing is likely to appear. This question will be taken up below based on an analysis of doublet firing in different limb and trunk muscles in healthy humans.

With regard to the origin of this surprising firing, currently, the short delayed depolarization (DD) appearing as a "hump" on the falling phase of an action potential, preceding the AHP (Granit et al., 1963; Kernell, 1964; Nelson and Burke, 1967; Kernell, 2006) and thus shortly increasing MN excitability is widely accepted as the mechanism responsible for *single* doublets in cat (Calvin and Schwindt, 1972; Kirkwood and Munson, 1996; see also Kernell, 2006), and human motoneurons (Kudina, 1974; Bawa and Calancie, 1983; Kudina and Alexeeva, 1992; Bawa and Lemon, 1993; Garland and Griffin, 1999; Piotrkiewicz et al., 2008; Stephenson and Maluf, 2010). As to the origin of *repetitive* doublets, the question still remains open. At first sight, one could assume that they have the same underlying mechanism like single doublets. However, as known from findings by Granit et al. (1963) and Calvin (1975), in cat motoneurons, the hump-DD changes systematically during rhythmic firing evoked by intracellular depolarization, becoming smaller with each successive spike. Therefore, as may be expected, in firing motoneurons the DD can reach the threshold only occasionally and generate single doublets only. Consequently, it might be suggested that the DD *alone* can hardly play a pivotal role in producing repetitive doublets.

In our recent studies (Kudina and Andreeva, 2010, 2013), we hypothesized that the mechanism underlying repetitive doublet firing in human motoneurons during voluntary muscle contraction is complex, including the hump-DD *as the primary determinant* that possibly becomes persistent due to a plateau potential probably activated in parallel with a common synaptic input. The assumption about a plateau potential role in repetitive doublet mechanisms was inferred from the comparison between essential characteristics of human motor unit (MU) repetitive doublet firing and properties of cat motoneuron firing in the presence of a plateau potential (e.g., Conway et al., 1988). If this assumption is true, then the question arises what are the DD characteristics which become persistent during repetitive doublet firing. The question will be considered below.

ANALYSIS OF MOTONEURON DOUBLET FIRING PROPERTIES

In order to analyze the firing behavior of human motoneurons, MU recordings during voluntary muscle contractions were used. Five healthy volunteers (aged 46–62 years) took part in this investigation. All subjects gave written informed consent for the experimental procedures, which were approved by the local Ethics Committee and conformed to the Declaration of Helsinki. Six muscles were investigated: the trapezius, latissimus dorsi, triceps brachii, flexor carpi ulnaris, first dorsal interosseous, and tibialis anterior.The detailed information on the methods is given earlier (Kudina and Andreeva, 2010). Briefly, the potentials of single MUs were recorded using a bipolar needle electrode, amplified by an electromyograph DISA and stored on the magnetic tape for off-line analysis. During the experiments, subjects were asked to recruit a few MUs by slowly developing a gentle voluntary contraction of a muscle investigated, to maintain MU firing for a few minutes (using MU potential feedback), and then to relax. These ramp-and-hold contractions were repeated many times throughout the experiment. Before the recordings, the subjects were trained for approximately

15-20 min to search for MUs capable of firing double discharges. The MU potentials were transferred to a computer by an A/D converter with the sampling rate of 10 kHz. Action potentials of each MU were identified on the basis of their amplitude and waveform shape. The results of the computer identification were verified by visual inspection of single MU records by an experienced operator.

A total of 228 MUs were identified; 96 MUs (42.1 %) displayed both single-spike and doublet firing, while the remaining ones were found to fire only more typical single discharges. Mean discharge rates of both MU groups were commonly in the range of 6–17 imp/s. Concerning the MU recruitment thresholds, note that all MUs were recorded at low-force contractions and, thus, belonged to the lowest-threshold part of motoneuron pools. Typical examples of single doublets are shown in **Figure 1A**. They were recorded at MU recruitment, among single-spike firing or at MU de-recruitment, i.e., the conditions of doublet occurrence in the muscle under study were consistent with previous observations in the literature. Repetitive doublets appeared typically in motoneurones at their recruitment, i.e., repetitive doublet trains were initiated in quiescent motoneurones rather than in firing ones (**Figure 1B**).

To obtain common characteristics of firing behavior of MUs with and without repetitive doublets, their ISI distributions were plotted (**Figure 1C**). MUs that did and did not express repetitive doublet firing showed similar ISI distributions during their single-spike firing. In contrast, during repetitive doublet firing, the MUs demonstrated ISI distributions in which the intradoublet ISIs formed a separate group of short ISIs. Interdoublet ISIs were typically in the range of the longest single-spike ISIs or even beyond them.

It was found that although single doublets were seen to occur in each of the six muscles investigated, repetitive doublets (discharge trains including of 3-110 doublets in succession, each of those followed by a prolonged interdoublet ISI) were observed in only three of them: in the trapezius, triceps brachii, and latissimus dorsi. As a result, these muscles exhibited the high doublet incidence estimated as the mean number of doublets per a doublet discharging MU. As to the flexor carpi ulnaris, first dorsal interosseous, and tibialis anterior none of these muscles demonstrated repetitive doublets and their doublet incidence was very low. This raises the question of whether there are any common traits for muscles firing or not firing repetitive doublets.

It appeared that repetitive doublets tended to be common in motoneurons from cervical spinal segments supplying the trapezius (C_2–C_4), the triceps brachii and latissimus dorsi (C_7–C_8), in contrast to those from more caudal spinal segments innervating the first dorsal interosseous and flexor carpi ulnaris (C_8, Th_1), and especially lumbar and sacral segments supplying the tibialis anterior (L_4–L_5, S_1), in which only single doublets were scarcely recorded.

Further we compared intradoublet ISIs in two groups of muscles - generating or not generating repetitive doublets (**Figure 1D**). Although the ranges of pooled intradoublet ISIs in both groups were rather close (3.3–30.0 and 5.2–27.3 ms), their mean ISI was found to be significantly shorter in the muscles that displayed repetitive doublets (6.1 ± 2.4 vs. 15.2 ± 7.5 ms; $P < 0.05$). Moreover, motoneurons with repetitive doublets displayed the marked mode in the intradoublet ISI distribution in the range of 4.0–6.0 ms. In this range, about 70 % of intradoublet ISIs have been occurred.

DISCUSSION

The present findings showed that among spinal motoneuronal pools a certain cranio-caudal gradient can be suggested in the capacity of motoneurons for repetitive doublets during voluntary muscle contractions. This suggestion is in line with previous data for the trapezius (Denslow, 1948; Kudina, 1974; Kudina and Alexeeva, 1992; Kudina and Andreeva, 2010; Stephenson and Maluf, 2010) as the muscle with frequent repetitive doublets, and the flexor carpi ulnaris (Kudina and Churikova, 1990), the first dorsal interosseous (Day et al., 1989) and the tibialis anterior (Andreassen and Rosenfalck, 1980), as the muscles without repetitive doublets. This point is in keeping with findings in other muscles: the flexor carpi radialis (C_6–C_7), the muscle in which long trains of repetitive doublets have been reported for the first time (Bawa and Calancie, 1983), in contrast to the rectus femoris (L_2–L_4) in which only occasional doublets have been observed (Kudina, 1974).

These differences in the prevalence of repetitive doublets between the motoneuron pools could be either due to differences in the properties of the DD and (or) to differences in motoneuron metabotropic inputs resulting in the activation of plateau potentials that is necessary, as was hypothesized, for keeping the sustained DD (Kudina and Andreeva, 2010).

Currently, in human experiments, there is no possibility of the reliable estimation of the strength of metabotropic inputs to a motoneuron pool. However, there is the possibility to estimate whether there are differences in properties of the DD; in particular *its effective duration* can be inferred from intradoublet ISI durations. According to the present data, mean intradoublet ISIs was found to be significantly shorter in the muscles that displayed repetitive doublets. Thus, the data allow concluding that the short duration of intradoublet ISIs can be used as a rather reliable *predictor* of whether or not a given motoneuron can fire repetitive doublets. Moreover, motoneurons firing repetitive doublets displayed the marked mode in the intradoublet ISI distribution (i.e., preferred intradoublet ISIs) in the range of 4.0–6.0 ms. It means that there were preferable moments of the appearance of extra-spikes creating doublets. Thus, it can be suggested that during repetitive doublets, the hump DD appears to be rather persistent. This stands in contrast to the hump disappearance during rhythmic firing found in acute animal experiments (see Introduction). Obviously, in humans, during a voluntary muscle contraction, the DD was sustained by a certain additional mechanism (possibly plateau potentials?).

CONCLUSION

During discussions of mechanisms underlying motoneuron firing behavior, the AHP is commonly considered as one of key factors, whereas the DD is mentioned only in passing, if any. However, phenomenon of repetitive doublet firing gives evidence that under certain conditions of natural motor control (for

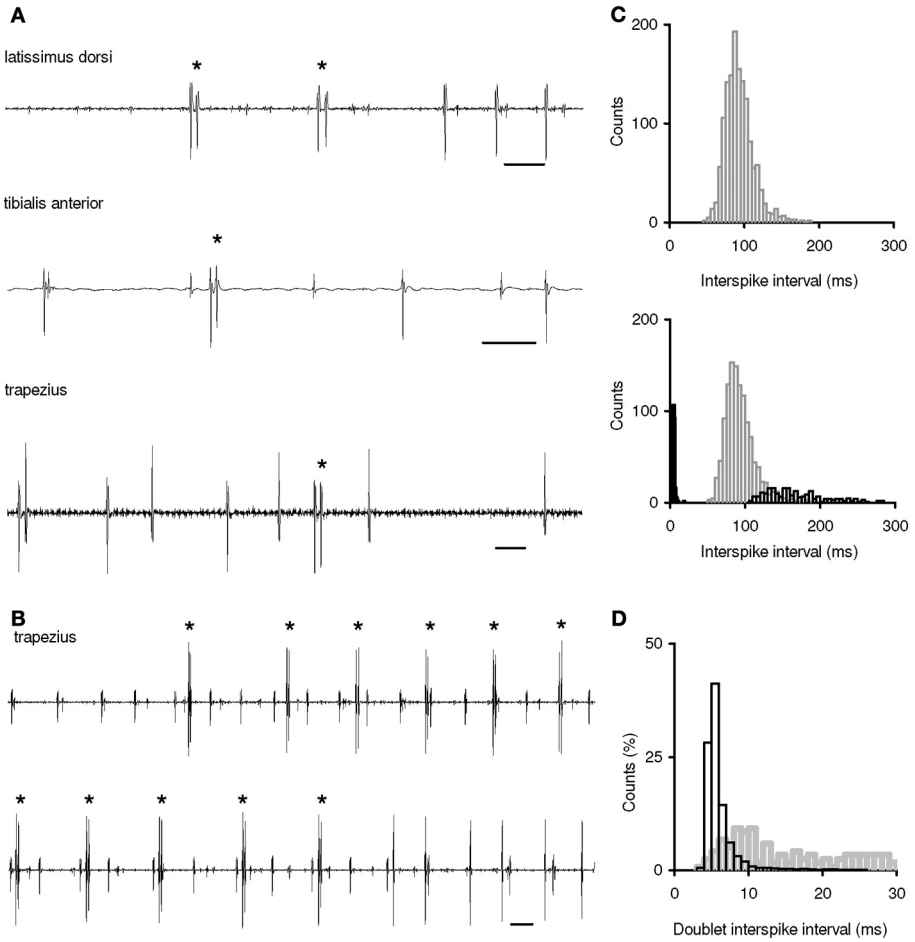

FIGURE 1 | Doublet firing in human motoneurons during voluntary muscle contractions. (A) Examples of MU single doublets. Top, two initial doublets at MU recruitment; middle, a doublet among single-spike firing; bottom, a doublet at MU de-recruitment. **(B)** Examples of repetitive doublets at MU recruitment (two records of MU potentials are in succession). Doublets are marked by asterisks. Time bar: 50 ms. **(C)** Interspike interval (ISI) distributions of two trapezius MUs from the same experiment. Top, an MU displaying only single-spike firing ($n = 1,418$). Bottom, an MU displaying both single-spike firing and doublet firing; gray bars: single-spike ISIs ($n = 1,193$), black filled bars: intradoublet ISIs ($n = 234$), black open bars: interdoublet ISIs ($n = 234$). Bin width for intradoublet ISIs, 1 ms; for the rest, 5 ms. **(D)** MU intradoublet ISIs in various muscles; black bars: single and repetitive doublets in the trapezius, triceps brachii, and latissimus dorsi ($n = 8,807$); gray bars: single doublets in the flexor carpi ulnaris, first dorsal interosseous, and tibialis anterior ($n = 107$). Bin width, 1 ms.

instance, during some postural tasks) the DD, in common with the AHP, can contribute to control of the motoneuron firing behavior in healthy humans. In summary, we like to emphasize that although the properties of repetitive doublets, their underlying mechanisms and functional significance need further investigation, but even at present the obtained results indicate that the analysis of repetitive doublet firing can be a promising approach to investigations of certain cellular mechanisms of diseased motoneurone pool as the doublet phenomenon is significantly enhanced in a number of motoneuron diseases (e.g., Partanen, 1978; Rowińska-Marciñska et al., 1999; Kleine et al., 2008; Piotrkiewicz et al., 2008; Weber et al., 2009).

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Double discharges in human soleus muscle

Maria Piotrkiewicz[1]*, Oğuz Sebik[2], Erdal Binboğa[3], Dariusz Młoźniak[1], Bożenna Kuraszkiewicz[1] and Kemal S. Türker[2]

[1] *Polish Academy of Sciences, Department of Engineering of Nervous and Muscular System, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland*
[2] *Laboratory of Neuromuscular Research, Koç University School of Medicine, Istanbul, Turkey*
[3] *Faculty of Medicine, Department of Biophysics, Ege University, Izmir, Turkey*

Edited by:
Parveen N. S. Bawa, Simon Fraser University, Canada

Reviewed by:
Hans Hultborn, University of Copenhagen, Denmark
Jennifer M. Jakobi, University of British Columbia Okanagan, Canada

****Correspondence:***
Maria Piotrkiewicz, Polish Academy of Sciences, Department of Engineering of Nervous and Muscular System, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Trojdena Street 4, 02-109 Warsaw, Poland
e-mail: masia@ibib.waw.pl

Double discharges (doublets) were recorded from human soleus (SOL), where they have never been reported before. The data analyzed in this study were collected from 12 healthy volunteers. The subjects were recruited for other studies, concerning: (1) estimation of motoneurons' (MNs) afterhyperpolarization (AHP) duration and (2) analysis of motor unit responses to nerve stimulation, and were not trained to voluntarily evoke doublets. The majority of intradoublet intervals fell into the commonly accepted range 2–20 ms. However, two SOL MNs from one presented exceptional doublets of intradoublet interval about 37 ms. This interval was virtually identical with the interval between second and third discharge in the few triplets recorded from another subject. It is hypothesized that triplets are generated by the delayed depolarization with the second narrow hump, which is the same as the hump responsible for exceptional doublets.

Keywords: soleus, double discharges, firing patterns, human motoneuron, delayed depolarization

INTRODUCTION

Motoneurons (MNs) during voluntary isometric contractions of a healthy human muscle discharge rhythmically with mean firing rates typically not exceeding 40/s. This type of discharge is controlled by afterhyperpolarization (AHP), which in MNs is longer than in many other types of nerve cell (Tripathy et al., 2012). However, some MNs are capable of firing double discharges (doublets) with short interspike intervals (ISIs) of a few milliseconds.

According to standard electromyographic terminology (AAEM, 2001), intradoublet ISIs should not exceed the range from 2 to 20 ms. However, detailed studies (Kudina, 1974; Halonen et al., 1977; Partanen and Lang, 1978; Bawa and Calancie, 1983; Kudina and Alexeeva, 1992b; Rowinska-Marcinska et al., 1999; Piotrkiewicz et al., 2008) have shown that the limits of this range can be sometimes exceeded and that each doublet is usually followed by prolonged ISI, which was considered a distinctive feature of doublets as early as in 1944 (Hoff and Grant). Moreover, in interval histograms of many doublet-firing MNs so-called "outsiders" can be seen, i.e., ISIs exceeding official intradoublet ISI range, but shorter than the lower limit of single ISI distribution.

Doublets are often recorded in non-physiological conditions, e.g., ischemia (Kugelberg, 1948) or neuromuscular disorders (Rowinska-Marcinska and Karwanska, 1994; Rowinska-Marcinska et al., 1999; Kostera-Pruszczyk et al., 2002; Piotrkiewicz et al., 2008) and therefore sometimes they have been considered to be a sign of MN dysfunction (Partanen and Lang, 1978). However, they can be also found in normal voluntary MN activity, although much more seldom (Denslow, 1948; Kudina, 1974; Andreassen and Rosenfalck, 1979; Bawa and Calancie, 1983). Single doublets are occasionally observed with MN recruitment, derecruitment or interspersed in rhythmic activity. Certain MNs are capable of firing series of intradoublet and interdoublet ISIs (repetitive doublets, Bawa and Calancie, 1983; Kudina and Churikova, 1990; Kirkwood and Munson, 1996; Kudina and Andreeva, 2010). The series may also be triggered through voluntary training (Bawa and Calancie, 1983; Kudina and Andreeva, 2010).

In animal studies, doublets were reported in cat (Calvin, 1974; Hoffer et al., 1987; Kirkwood and Munson, 1996) and rat (Gorassini et al., 2000) muscles. In the latter study, doublets were observed in fast hindlimb muscles (medial and lateral gastrocnemius and tibialis anterior), but not in the slow soleus (SOL). It should be mentioned, however, that the first evidence of doublets in animal experiment was obtained from the SOL of decerebrate cat (Eccles and Hoff, 1932; Hoff and Grant, 1944).

In human experiments, doublets were documented in flexor carpi radialis (Bawa and Calancie, 1983), flexor carpi ulnaris (Kudina and Churikova, 1990), extensor digitorum communis (Weber et al., 2009), palmaris longus (Bawa and Calancie, 1983), biceps brachii (Bawa and Calancie, 1983; Dengler et al., 1988; Piotrkiewicz et al., 2008), triceps brachii (Kudina and Andreeva, 2010), tibialis anterior (Andreassen and Rosenfalck, 1979, 1980), rectus femoris (Kudina, 1974) spinal extensors (Denslow, 1948), and trapezius (Denslow, 1948; Kudina and Alexeeva, 1992b; Kudina and Andreeva, 2010; Stephenson and Maluf, 2010). Trapezius and spinal extensors were reported to have the highest incidence of doublets (Denslow,

1948). To our knowledge, doublets have not been reported in human SOL.

The aim of this study was to document doublets observed in SOL muscle during long-lasting experiments that were designed for other purposes: investigation of AHP duration in human motoneurones (Piotrkiewicz et al., 2001) and study of responses to low-threshold stimulation of the tibial nerve (Binboğa et al., 2011).

METHODS

SUBJECTS

The data analyzed in this study were measured from previously recorded data sets (Piotrkiewicz et al., 2001; Binboğa et al., 2011). The 12 healthy volunteers, aged 21–58 (mean 38.3 years) were not trained to voluntarily evoke doublets and gave written informed consent to the experimental procedures that had gained Ethical Approval from the applicable institutional committees.

Experimental procedures

The detailed description of the experiments is given in previously published papers (Piotrkiewicz et al., 2001; Binboğa et al., 2011). Below, only the details relevant for the present study will be given.

Experiment 1. Data for the study of AHP duration were collected in the Kharkevich Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow. During this investigation subjects were comfortably seated in an armchair and instructed to perform a series of isometric muscle contractions of various strengths keeping motor units (MUs) firing steadily with the help of auditory and visual feedback of the MU discharges. During the experiment, 5–21 constant-force electromyogram (EMG) fragments of 50–100 s duration were recorded from the SOL muscle. Between consecutive recordings, 3–4 min rest was provided. MU potentials were picked up by a bipolar needle electrode (DISA, 9013K0822), amplified by an electromyograph DISA A/S (Denmark, type 14 A 30) at 200–500 mV/cm with filters set at 20–10000 Hz, and stored on the magnetic tape for off-line analysis.

Experiment 2. Data for the study of MU responses to low-threshold electrical stimulation were obtained in the Centre for Brain Research at Ege University, Izmir, Turkey. During this investigation the subject lay prone on a physiotherapy table with right foot fixed to a force plate. The ankle angle was positioned at 90°. Subjects were instructed to gently plantar flex to recruit a single motor unit in SOL muscle. A single experiment lasted for 1–2.5 h. MU potentials were picked up from the SOL muscle by intramuscular custom made disposable bipolar wire electrodes, amplified (500x), filtered with a 200–10000 Hz band-pass filter, and stored by Cambridge Electronic Design (CED; UK) acquisition system for off-line analysis.

Data analysis

All data were transferred to PCs by A/D converters with the sampling rates from 10 to 20 kHz, depending on the frequency content of the signal, so that there was no aliasing. Usually, potentials of a few MUs were recorded simultaneously in each experiment (**Figure 1**). Motor unit potential recordings from both

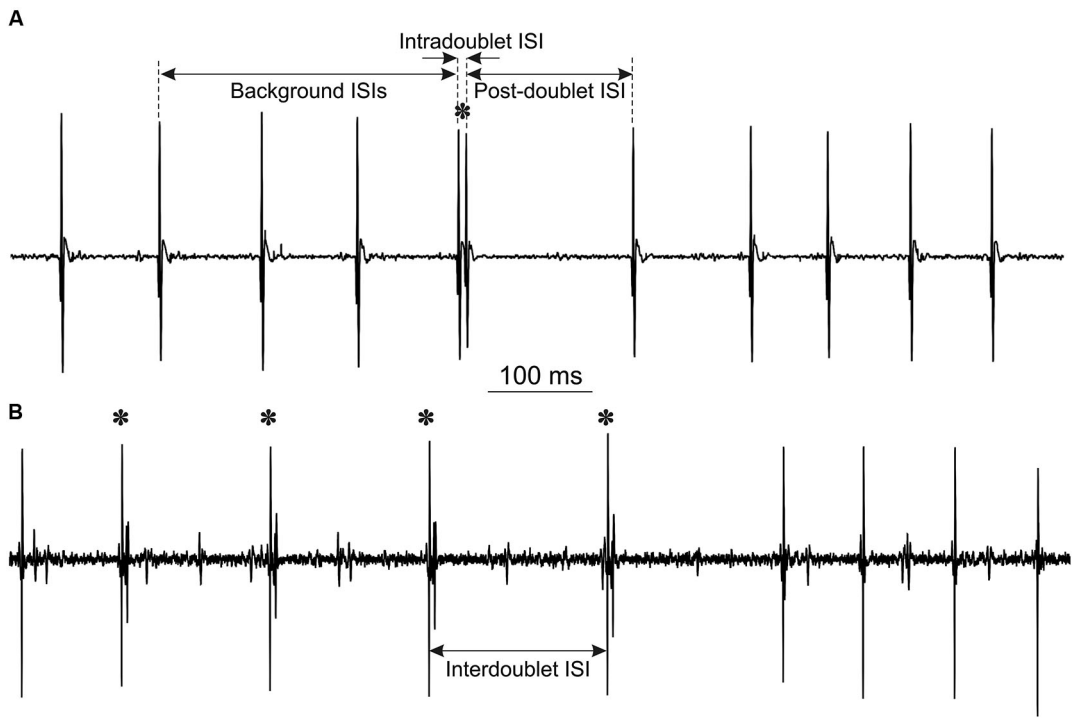

FIGURE 1 | Examples of MU potentials (doublets are marked by asterisks): (A) single doublet, indicated are intervals, measured for double discharge analysis. Note the prolonged post-doublet ISI, which is one of the distinguishing features of "true" doublets. **(B)** Repetitive doublets.

experiments were decomposed off-line into single MU potential trains by an operator-computer interactive method using custom software described elsewhere (Mazurkiewicz and Piotrkiewicz, 2004) and subjected to common analysis described below.

From the decomposed MU potential trains ISI histograms were constructed and those exhibiting bimodal distributions (see **Figure 2**) were searched for doublets. The search was based on the stereotyped firing pattern of doublets, which comprise "...two sequential firings of a motor unit action potential of the same form and nearly the same amplitude, occurring consistently in the same relationship to one another..." (AAEM, 2001) and are usually followed by a prolonged post-doublet ISI. The limits of intradoublet ISIs were determined from the histograms. Their upper limit sometimes exceeded 20 ms, specified by the official electromyographic terminology (AAEM, 2001).

The analysis included calculation of three ISIs for each doublet: mean background ISI from three consecutive intervals preceding the doublet, intradoublet ISI (between both doublet components) and post-doublet ISI (see **Figure 1A** for definitions). From these data the mean values and standard deviations of ISIs were calculated and histograms were plotted.

It was checked carefully if the doublets found in the data from the Experiment 2, were affected by the ongoing stimulation. This might have happened only if the second discharge had been synchronous with M- or H-response; however, such coincidences were not found.

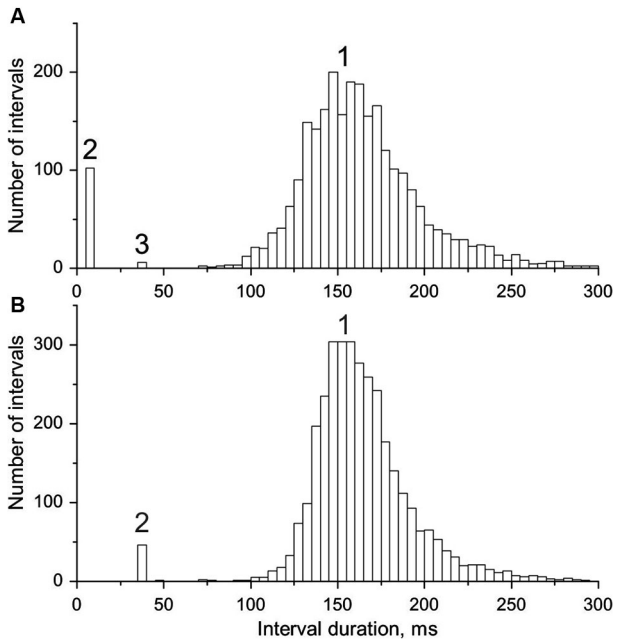

FIGURE 2 | Interval histograms of doublet-firing MUs. (A) MU from group R. **(B)** MU from group E (see text for the explanation of the acronyms); 1, single, 2, double, 3, triple discharges. Note the coincidence of timing of triplets in A with doublets in B and some outsiders in both distributions.

RESULTS

Altogether, 169 MUs were identified in the SOL muscle. Sixteen MUs from this population were capable of firing doublets (incidence 9.5%). The range of MU firing rates was 3.5–15 imp/s. Our experiments, not specifically designed for the investigation of doublets in SOL, observed an unexpected number of doublets generated spontaneously during sustained contractions.

Characteristic features of doublet-firing MUs are collected in **Table 1**. MUs were classified into three groups. MUs from group **S** fired occasional *single doublets* interspersed in regular discharge (see the example in **Figure 1A**); these doublets constituted less than 0.5 % of all discharges. MUs from the group **R** fired both *single* and *repetitive doublets*, i.e., the series of doublet and post-doublet discharges; in this group, the doublets constituted more than 6% of all discharges (see the example in **Figure 1B**). Group **E** comprised two MUs from one subject, firing *exceptional doublets* (about 1% of all discharges). This group will be described separately with more detail.

In **Figure 2** interval distributions of the two MUs with doublets are shown. Both have two separate maxima, one for regular discharges and the other for doublets. The histogram in **Figure 2A** has an additional maximum at around 36 ms, formed by six extremely long triplet intervals (35.9–36.5 ms; for more detailed description see the following section). **Figure 2B** presents the histogram of the MU with exceptional doublets. The duration of intradoublet ISI for this MU is within the limits 35.8–37.0 ms. Note striking similarity between both interval ranges (cf. also **Figure 5**).

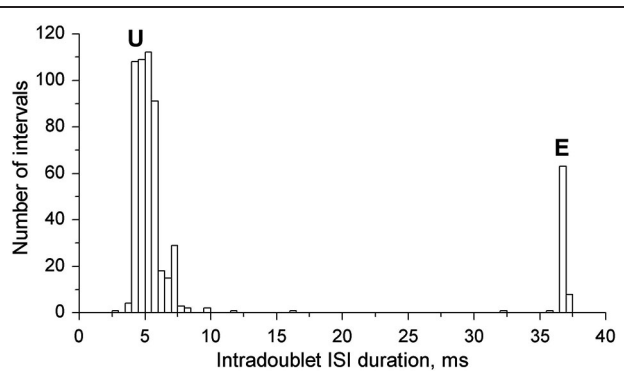

FIGURE 3 | Intradoublet ISI histogram. Note some outsiders between usual **(U)** and exceptional **(E)** ISIs.

In **Figure 3** the joint intradoublet ISI histogram for SOL MUs is presented with expanded scale. Exceptional doublets (**E**) create here a narrow maximum at about 37 ms, which differs considerably from the broader maximum of usual doublets (**U**).

UNUSUAL MULTIPLE DISCHARGES

As already mentioned above, in two MUs of one subject exceptional doublets were observed, whose intradoublet ISI considerably exceeded the usual limits of 2.5–20 ms (set by the standards of electrophysiological terminology (AAEE, 1987; AAEM, 2001). **Figure 4** illustrates a long section of the discharge of a MU with exceptional doublets. The unit was discharging

Table 1 | Firing characteristics of MU groups.

MU group	Number of MUs	Total number of MU spikes	Doublets			Triplets		
			Number	Incidence* [%]	Interval (mean ±SD) [ms]	Number	Incidence* [%]	Interval** (mean ±SD) [ms]
S, with single doublets only	12	49231	46	0.09	7.27±2.48			
R, with single and repetitive doublets	2	7193	445	6.19	5.05±0.64	6	0.08	36.28±0.22
E, with exceptional doublets	2	17462	186	1.07	36.88±0.38			

** Calculated with respect to total number of MU spikes.*

*** Interval between second and third discharge.*

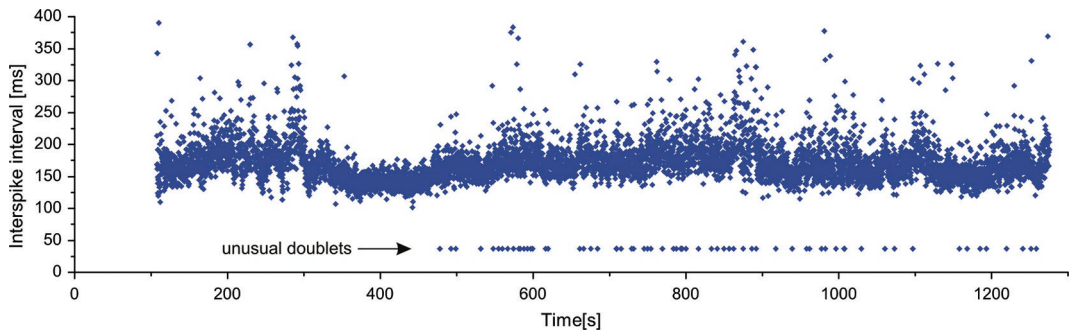

FIGURE 4 | Long sequence of consecutive discharges of a MU with exceptional doublets.

around 6.5 Hz and occasionally slowed down below 5 Hz. It began to fire doublets about 8 min after the start of the experiment and continued until its end.

The intervals of these doublets exhibited much less variability (coefficient of variation 1.04%) than those of the usual doublets (12.0% for repetitive and 21.4% for single doublets). They were also accompanied by the prolonged post-doublet ISI.

The special class of unusual multiple discharges are triplets, which are much more seldom than doublets (cf. **Table 1**). In our experimental data collected from SOL, we encountered only one MU firing triplets. Triplets presented the stereotyped firing pattern (**Figure 5**): the interval between second and third discharge (triplet ISI) was substantially longer than that between first and second discharge (intradoublet ISI).

Surprisingly, the triplet ISI duration was virtually equal that of the exceptional intradoublet ISI (**Figure 5**, cf. also **Figure 2** and **Table 1**). This coincidence is quite remarkable given that the exceptional doublets and the triplets were recorded from two different subjects.

DISCUSSION

This paper presents the doublets recorded from SOL muscle, which were found unexpectedly among single MU data collected for other purposes. SOL is the muscle perhaps most frequently investigated in human studies (e.g., Person and Kudina, 1972; Ashby and Labelle, 1977; Sabbahi and Sedgwick, 1987; Kudina, 1988; Kudina and Pantseva, 1988; Miles et al., 1989; Türker and Miles, 1991; Person and Kozhina, 1992; Kiehn and Eken, 1997; Türker et al., 1997). However, doublets have never been reported in this muscle. Even in the experiments testing the excitability of human MUs within the ISI none of the 141 SOL MUs was found to reveal any sign of increased excitability in the initial interval fragment (Sabbahi and Sedgwick, 1987; Kudina, 1988) in contrast to MUs from flexor carpi ulnaris capable of firing doublets (Kudina and Churikova, 1990). Thus, the occurrence of doublets in SOL must be a very rare phenomenon and the incidence of MNs firing doublets calculated in this study as 9.5%, is by no means severely overestimated.

The question which arises from these data is: why doublets were observed in SOL in these two series of experiments? Firstly, these observations were made in experiments of long duration (approximately 1–2.5 h) and never encountered at the beginning of the experiment (see **Figure 5**). This seems to have something in common with "warm-up" phenomenon, i.e., the decrease in the MU firing threshold during repeated or sustained contractions (e.g., Gorassini et al., 2002). This phenomenon has been shown to occur in MNs and was attributed to the facilitation of a voltage-dependent persistent inward current (Svirskis and

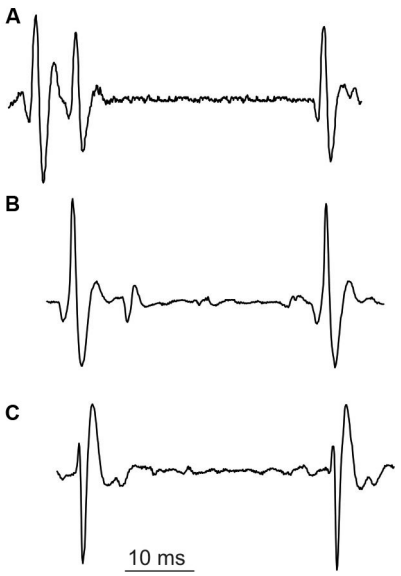

FIGURE 5 | Unusual discharges. (A) triplet, **(B)** and **(C)** exceptional doublets (first potential aligned with the second potential in **(A)**).

Hounsgaard, 1997; Bennett et al., 1998). Recently, similar mechanism was proposed as the explanation for repetitive doublet firing (Kudina and Andreeva, 2010). It may be hypothesized that such a "warm-up" is necessary also for initiation of single doublet generation. In many earlier SOL studies such long sequences might not have been recorded. Moreover, the doublets might often remain unnoticed, if the researcher was analyzing data with another purpose, and/or was not familiar with their specific firing pattern. It is also important to note that the second spike of the doublet usually has different amplitude and/or shape. Recent studies often rely on the automatic recognition software, which may not mark this discharge as belonging to the same MU.

In official EMG terminology (AAEM, 2001), two potentials of the same MU are classified as a doublet, if the interval between them is shorter than 20 ms. However, in this study we report two cases of MUs with intradoublet ISI above 30 ms. The very low variability of these intervals is remarkable, and even more so is their similarity to the triplet ISI, given that they were recorded from two different subjects.

These unusual phenomena are much more seldom than doublets in SOL *per se*, so there is little chance on their detailed investigation in future. We can only propose the explanation for the triplet interval (below), which is highly speculative and should be treated with caution.

It is commonly accepted that doublets recorded in healthy human muscles are generated in MNs exhibiting delayed depolarization (Kudina, 1974; Partanen, 1979; Bawa and Calancie, 1983; Kudina and Churikova, 1990; Kudina and Alexeeva, 1992a; Garland and Griffin, 1999; Piotrkiewicz et al., 2008; Kudina and Andreeva, 2010; Stephenson and Maluf, 2010), which has a shape of a prominent hump that may spontaneously cross the firing threshold, evoking an extra spike (Granit et al., 1963; Kernell et al., 1964; Calvin, 1974). It is possible that certain MNs possess the second hump situated further down the interspike voltage trajectory. This type of hump would be responsible for the third discharge in triplets and for the exceptional doublets, which are generated by MNs without initial hump responsible for usual doublets. The late hump should be very sharp to explain the extremely low variability of triplet and exceptional intradoublet ISIs.

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Afterhyperpolarization of human motoneurons firing double and triple discharges

Maria Piotrkiewicz* and Bożenna Kuraszkiewicz

Engineering of Nervous and Muscular System, Nałęcz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland
**Correspondence: masia@ibib.waw.pl*

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INTRODUCTION

During isometric voluntary contractions of a healthy human muscle, motoneurons (MNs) fire usually with low mean rates, rarely exceeding 25/s (e.g., Garland and Griffin, 1999). However, there are MNs, which sometimes fire double discharges (doublets) with interspike interval (ISI) of few ms. This is observed seldom in normal MNs (e.g., Denslow, 1948; Kudina, 1974; Bawa and Calancie, 1983) but does so more often in neuromuscular disorders (Partanen, 1978; Kostera-Pruszczyk et al., 2002; Piotrkiewicz et al., 2008), where it is considered to be an early sign of MN dysfunction (Rowinska-Marcinska et al., 1999).

Our special attention is paid to the so-called "true" doublets recorded in some MNs under conditions of constant synaptic drive, as opposed to those recorded during rapid contractions (Bawa and Calancie, 1983; Kudina and Andreeva, 2013a). Initial doublets, observed during repetitive movements such as locomotion (Zajac and Young, 1980; Hennig and Lomo, 1987) or respiration (Kirkwood and Munson, 1996), where the synaptic drive undergoes periodic changes, also cannot be classified as "true" doublets. For the sake of clarity, the adjective "true" will be omitted in the further text.

It has been observed that not each human MN is capable of firing doublets (Kudina, 1974; Bawa and Calancie, 1983). By the analogy to animal studies, it was hypothesized that doublets may be generated only in these MNs, which possess the delayed depolarization (DD, Granit et al., 1963; Kernell, 1964; Calvin, 1974) with a prominent hump that may spontaneously cross the firing threshold and evoke an extra spike (Nelson and Burke, 1967; Calvin, 1973). This hypothesis was verified by Kudina and Churikova (1990), who tested the changes in excitability of human motoneurons within ISI by studying the responses of single MNs to the stimulation of Ia afferents. In MNs capable of firing doublets they revealed the period of increased excitability during the first 15 ms after regular discharge, which corresponded well to the duration of DD observed in animal experiments (Granit et al., 1963; Kernell, 1964; Nelson and Burke, 1967; Calvin, 1973).

Nowadays, DD is widely accepted as the mechanism responsible for doublet generation in human MNs (e.g., Kudina, 1974; Bawa and Calancie, 1983; Kudina and Churikova, 1990; Garland and Griffin, 1999). However, it can explain only the origin of occasional doublets, interspersed in regular rhythmic MN activity. Repetitive doublets, i.e., series of doublet-postdoublet ISIs, require some additional mechanism, which would support the DD hump, shown to disappear during MN rhythmic firing (Granit et al., 1963; Calvin and Loeser, 1975). It was recently hypothesized that repetitive doublet firing may be related to plateau potentials (Kudina and Andreeva, 2010, 2013a).

It is still under debate, whether DD is related to other characteristics of a MN, measurable in human experiments. Kernell (1964) noted that DD with a definite hump had a tendency to be more common in MNs with short afterhyperpolarization (AHP), although it could occur with an AHP of any duration. Also in humans, MNs capable of firing doublets were more often found in the fast muscles (Bawa and Calancie, 1983; Kudina and Alexeeva, 1992) than in the slower ones (Andreassen and Rosenfalck, 1980; Kudina and Andreeva, 2013b). For the long time it was believed that the MNs supplying one of the most often investigated slow human muscle, the *soleus*, are devoid of DD. However, it has been recently shown that also MNs supplying human soleus may fire doublets (Piotrkiewicz et al., 2013), which seems to question Kernell's finding. In the present paper we will compare the estimates of AHP duration of MNs capable and not capable of firing double discharges and comment on this controversy. We will also propose a hypothesis on the AHP-related mechanism underlying firing of triplets, which can be observed in some doublet firing MNs.

AHP DURATION OF MNs CAPABLE AND NOT CAPABLE OF FIRING DOUBLE DISCHARGES

Our opinion is essentially based on the results of the experiments reported earlier (Piotrkiewicz et al., 2001), which were aimed toward investigation of AHP duration in slow (*soleus,* SOL) and fast *(biceps brachii,* BB) human muscle (Exp. 1). Within the motor unit potential trains, recorded in these experiments during steady-state voluntary isometric muscle contractions, we found also doublets and triplets (**Figure 1A**, Piotrkiewicz et al., 2008). Additionally, we included the results from the *triceps brachii* (TB) from another study, designed specifically for the investigation of double discharges (Kudina and Andreeva, 2010). In this study, the subjects were trained to search for doublet firing motor units. They were instructed to slowly develop a gentle voluntary isometric muscle contraction until a motor unit

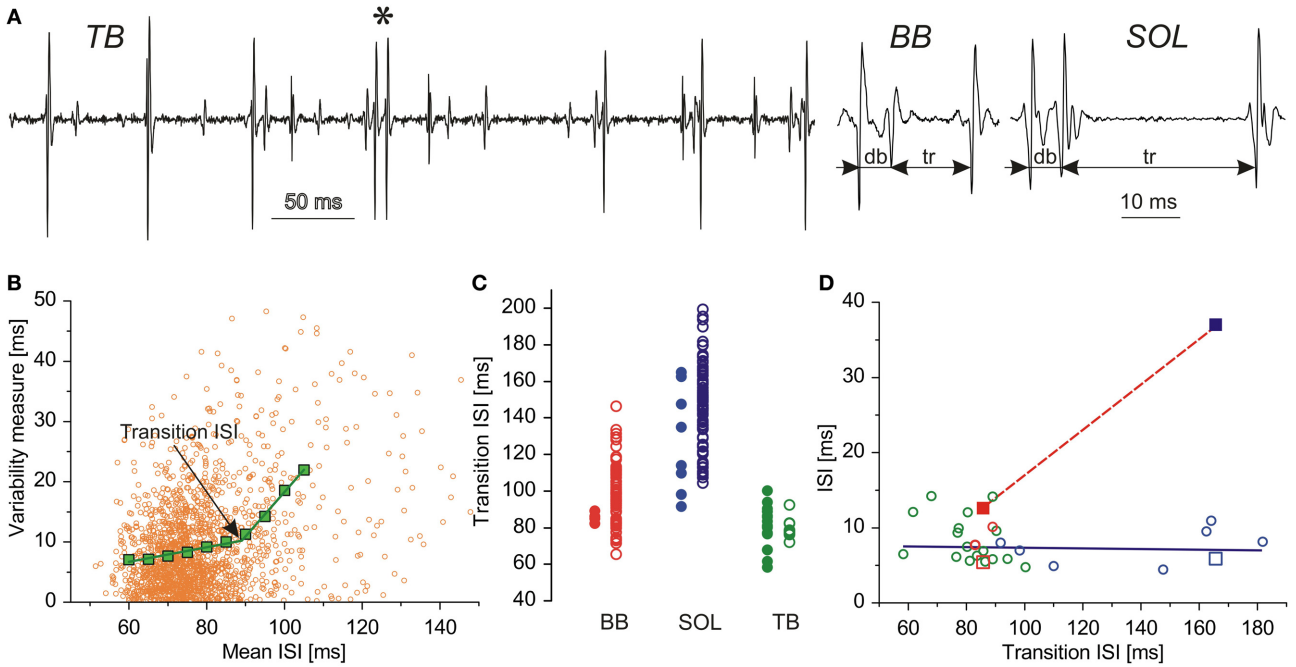

FIGURE 1 | Double discharges and AHP. (A) Examples of double and triple discharges, from left to right: single doublet from TB (marked with an asterisk) interspersed in rhythmic MN activity, triplet from BB (11 occurrences), and SOL (6 occurrences); db, doublet ISIs; tr, triplet ISIs; **(B)** Estimation of transition ISI. Ordinate, variability measures: orange dots, *CD*, green squares, CD_m; transition ISI marked by an arrow; abscissa: mean ISI; **(C)** Comparison of transition ISIs for MNs with (heavy circles) and without doublets (open circles); **(D)** Plots of doublet (open symbols) and triplet ISIs (filled symbols) vs. transition ISI: BB, red; TB, green; SOL, blue. Squares, two MNs capable of firing triplets; circles, remaining MNs.

started firing doublets, and then to keep contraction constant for a few minutes (Exp. 2).

It is commonly accepted that each motor unit discharge is equivalent to the discharge of its MN. Motoneuron AHP duration was estimated from single motor unit potential trains by the method based on the observation of Person and Kudina (1972) that the relationship between the standard deviation and mean value of ISI sharply changes after a certain transition value that was hypothesized to correlate with the AHP duration of the MN. The hypothesis was verified by computer simulations (Piotrkiewicz, 1999) and in the direct recordings from cat MNs (Powers and Binder, 2000), which confirmed this correlation. The AHP duration estimates, which this opinion is based upon, were obtained by the modification of the above method (Piotrkiewicz et al., 2012), using the ISI variability analysis proposed by Holt et al. (1996). The variability was measured as the absolute consecutive difference (CD) between two adjacent ISIs. It was plotted against mean ISI, calculated from the same two intervals (MISI). An example of the plot *CD* vs. MISI is shown in **Figure 1B** (orange dots). The mean *CD* values (CD_m, green squares) were calculated after grouping the MISIs in 10 ms bins with 5 ms overlap. After rejection of points calculated from insufficient number of *CD* values, the mean data were fit with two linear regression lines and the intersection of these lines used to determine transition ISI, being an estimate of AHP duration.

The AHP duration was estimated for 80 MNs from BB, 90 MNs from SOL, and 28 MNs from TB. From these, MNs firing doublets constituted 5% for BB (4 MNs), 9% for SOL (8 MNs), and 64% for TB (18 MNs). Two MNs recorded in Experiment 1, one from BB and one from SOL, fired also triplets that were much scarcer than doublets: for BB MN 11 triplets vs. 1592 doublets, for SOL MN 6 triplets vs. 342 doublets. **Figure 1A** (right) presents characteristic triplet firing pattern, which was similar in the two MNs: the ISI between first two components was equal to the doublet ISI, whereas triplet ISI measured between second and third spike was substantially longer (2.4 times for BB and 6.5 times for SOL).

Figure 1C shows the comparison of transition ISIs for MNs with (filled symbols) and without doublets (open symbols) from BB, SOL, and TB. The ranges of both clusters overlap, but the mean values of transition ISIs calculated in each of MN pools of BB and SOL separately are shorter for doublet firing MNs than for MNs without doublets: 85.70 vs. 99.39 and 128.01 vs. 148.04 ms, respectively. Thus, although the AHPs in doublet firing MNs supplying SOL are longer than in their counterparts supplying BB, within the pool of each muscle they tend to be shorter than those in the other MNs.

At the first sight, TB data do not seem to confirm this observation. The difference in mean values of transition ISIs between MNs with and without doublets is negligible: 80.40 vs. 80.00 ms and many of MNs from the former group present transition ISIs longer that those from the latter. However, there are striking differences between TB and BB in doublet

incidence and transition ISI ranges. Since both TB and BB are fast muscles, it could be presumed that the AHP duration ranges of their MNs should be comparable. Moreover, it should be noted that the contraction strengths in the Exp. 2 were lower than in the Exp. 1 (below 10 and 30% maximum voluntary contraction, respectively). Thus, the MNs recorded from TB could be expected to have the lower thresholds than those from BB. Consequently, according to the commonly known rules of the orderly recruitment (Milner-Brown et al., 1973a,b; Henneman et al., 1974) as well as to the match observed between motor unit twitch contraction time and the AHP duration of its MN (Kernell et al., 1999), one could expect that AHPs estimated for TB MNs would be rather longer than those of BB MNs. Despite these expectations, the transition ISIs in TB not only are shorter, but their range is much narrower than that of BB MNs.

The differences observed could be related to the differences in experimental protocols, specifically to the different MN sampling. Whereas the sampling in the Exp. 1 could be assumed to be random, in Exp. 2 it was influenced by the training for search of MNs with doublets. It is impossible to say, what happens with MNs during training. It must involve some changes in synaptic inflow, since the majority of doublets recorded in Exp. 2 were repetitive ones. However, it may also be presumed that due to this training the MN sample was limited to those cells, in which finding hump-like DD was most feasible, i.e., to the MNs with shortest AHPs. The narrow range of transition ISIs and the very high proportion of doublet firing MNs in TB sample (64%) are in favor of this presumption. Summing up, the results from all three muscles seem to be in line with the earlier observation of Kernell (1964).

INTERSPIKE INTERVALS OF DOUBLE AND TRIPLE DISCHARGES vs. AHP DURATION

Figure 1D presents the dependency of the intervals between spike components of doublets and triplets on the AHP duration. The doublet ISI duration of MNs supplying all muscles investigated was independent of transition ISI (open symbols). This result is in line with the suggestions of Granit et al. (1963) and Kernell (1964) that DD may not be the "true" afterpotential. The more recent studies on the ion channels involved in the generation of MN rhythmic firing confirm that the mechanisms responsible for AHP and DD differ from each other (Viana et al., 1993; Kobayashi et al., 1997).

In contrast to the doublet ISI, the triplet ISI measured for SOL (blue filled square) was substantially longer than that measured for BB (red filled square). This observation may suggest that its firing might not be caused by DD, but by the later hump in the time course of AHP conductance described by Baldissera and colleagues (Baldissera and Gustafsson, 1974; Baldissera and Parmiggiani, 1979). This hump has been shown to coincide with the AHP time-to-peak, which would be longer in MNs with longer AHPs. Moreover, AHP summation after short doublet ISI may enhance conductance hump. On the other hand, AHP summation would result in diminished DD (Granit et al., 1963), which also indicates that the firing of the third spike related to DD is hardly possible.

CONCLUDING REMARKS

Doublets are rarely recorded during the voluntary muscle activity in healthy human subjects. They are generated by the MNs with hump-like DD, whose characteristics, which could be measured in human experiments, are largely unknown. However, the knowledge of the properties of these MNs is important, since their incidence increases significantly in some neuromuscular diseases, where it is considered to be an early sign of MN dysfunction. We believe that although AHPs of doublet firing MNs supplying SOL are longer than those of BB and TB, in each MN pool the doublet firing human MNs belong to those with shortest AHPs, as do the cat MNs with hump-like DDs. We admit, however, that by now the evidence supporting this belief is rather weak.

Triplets are much more scarce than doublets. Thus, collecting bigger experimental material is virtually impossible. We decided to present this opinion hoping that it may inspire other researchers to look for these rare events in their experimental recordings. We are aware that our data are not sufficient to justify solid conclusions concerning mechanism(s) underlying triplet firing. Nevertheless the specificity of the opinion article encouraged us to formulate the above presented speculative hypothesis about the unusual, intriguing phenomenon of triplet firing.

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Testing the excitability of human motoneurons

Chris J. McNeil[1,2], Jane E. Butler[1,3], Janet L. Taylor[1,3] and Simon C. Gandevia[1,3]*

[1] Neuroscience Research Australia, Randwick, NSW, Australia
[2] School of Health and Exercise Sciences, University of British Columbia, Kelowna, BC, Canada
[3] Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia

Edited by:
Ulf Ziemann, Eberhard Karls University Tübingen, Germany

Reviewed by:
Ulf Ziemann, Eberhard Karls University Tübingen, Germany
Yoshikazu Ugawa, Fukushima Medical Universtiy, Japan

***Correspondence:**
Simon C. Gandevia, Neuroscience Research Australia, PO Box 1165, Randwick, NSW 2031, Australia.
e-mail: s.gandevia@neura.edu.au

The responsiveness of the human central nervous system can change profoundly with exercise, injury, disuse, or disease. Changes occur at both cortical and spinal levels but in most cases excitability of the motoneuron pool must be assessed to localize accurately the site of adaptation. Hence, it is critical to understand, and employ correctly, the methods to test motoneuron excitability in humans. Several techniques exist and each has its advantages and disadvantages. This review examines the most common techniques that use evoked compound muscle action potentials to test the excitability of the motoneuron pool and describes the merits and limitations of each. The techniques discussed are the H-reflex, F-wave, tendon jerk, V-wave, cervicomedullary motor evoked potential (CMEP), and motor evoked potential (MEP). A number of limitations with these techniques are presented.

Keywords: motoneuron, H-reflex, F-wave, tendon jerk, V-wave, CMEP, MEP

INTRODUCTION

The motoneuron was described as the "final common path" of the nervous system (Sherrington, 1906) and has been a focal point of neuroscience research for over a century. Since the introduction of the terminology, the designation of "final common path" has been frequently expanded to include not only the α-motoneuron but also the muscle fibers which it innervates (i.e., the motor unit) (e.g., Denslow and Hassett, 1942). This expanded definition is sensible from a functional perspective because movement requires contraction of muscle fibers and the properties of muscle fibers are largely dictated by the properties of the motoneuron which innervates them (see Burke, 1981; Henneman and Mendell, 1981; Binder et al., 1996 for reviews).

Motor unit properties have been directly studied in animals (e.g., Burke et al., 1971; Peter et al., 1972) and are directly related to the size of the motoneuron. In brief, motoneurons with a large soma (and hence a large axonal diameter) have a low input resistance, high firing threshold, brief after-hyperpolarization, fast conduction velocity, and their muscle fibers have a fast twitch contraction time, high twitch tension and a poor resistance to fatigue. As the size of motoneurons decreases, these responses gradually shift to the opposite ends of their spectra. Output properties of human motoneurons can be gleaned from whole-nerve stimulation, spike-triggered averaging (e.g., Milner-Brown et al., 1973; Thomas et al., 1990) or intraneural stimulation (e.g., Thomas et al., 1990). Measurement of somato-dendritic properties is more difficult and frequently relies on reflex and antidromic inputs to the motoneurons.

Like the assessment of motor unit properties, tests of motoneuron pool excitability in humans are necessarily indirect. Before we discuss the most common methods used to test excitability of the human motoneuron pool, it is first necessary to define our use of the term "excitability" in this review. For our purposes, the term "excitability" is a relative one. That is, if the same input is delivered to the motoneuron pool before and after an intervention (e.g., muscle fatigue), do more or fewer motoneurons generate action potentials after the intervention? A bigger or smaller output would represent a net increase or decrease in motoneuron excitability, respectively. The change in excitability will reflect the balance of inhibition and facilitation but it is difficult to determine the mechanism in a given situation; e.g., a decrease in excitability could be due to either an increase in inhibition or a decrease in facilitation. Further, any change in "excitability" may not apply uniformly across the whole motoneuron pool.

Regardless of the methodology or the intervention studied (e.g., acute vs. chronic), the goal of testing human motoneuron excitability is the same: to know more about the status of the motoneuron pool. The aim of this review is to discuss briefly the existing methodologies which test motoneuron excitability by evoking compound muscle action potentials with particular focus on the benefits and limitations of each. Discussion of the H-reflex, F-wave, tendon jerk, V-wave, cervicomedullary motor evoked potential (CMEP) and motor evoked potential (MEP) is included below and summarized in **Table 1**. There are a number of single motor unit approaches with their own advantages and disadvantages but these methods are largely beyond the scope of this review. However, it deserves emphasis that these approaches provide useful information but usually only about a limited number of motoneurons which have a low-threshold in a voluntary contraction.

H-REFLEX

Stimulation of a peripheral nerve can evoke a reflex response termed the Hoffmann or H-reflex (Magladery and McDougal, 1950) because it was first described in the soleus muscle by Hoffmann (1918). The H-reflex reflects the response of the motoneuron pool to a volley from large-diameter primary muscle

Table 1 | Brief summary of the methodologies used to test excitability of motoneurons in humans.

Response	Key information	Advantages/ recommendations	Disadvantages/caveats
H-reflex	Method: submaximal stimulation of a peripheral motor nerve. Volley: single in group I muscle afferents (and other afferents). Potential: motoneurons activated by Ia excitation. Note: motoneurons recruited according to size principle.	Potentially painless. Possible to test in relaxation or weak contraction.	Not entirely monosynaptic. Limited to soleus and a few other motoneuron pools in relaxation. Test conditions must be painstakingly maintained. Subject to presynaptic inhibition. Subject to post-activation (homosynaptic) depression. Affected by changes in axonal excitability.
F-wave	Method: supramaximal stimulation of a peripheral motor nerve. Volley: single antidromic in motor axons. Potential: motoneurons activated by antidromic excitation. Note: only a small number of motoneurons backfire; in muscles with an H-reflex at rest, the response occurs preferentially in large motoneurons because the antidromic volley collides with the H-reflex impulse in small motoneurons.	A direct method which does not rely on afferents. Interpretation is clearest when tested in relaxation.	Relatively insensitive to motoneuron excitability. Necessary to average or measure many responses. Limited to distal muscles. Can be painful. Contaminated by H-reflex when recorded during weak contraction.
Tendon jerk	Method: tendon taps with a reflex hammer. Volley: multiple from muscle spindle primary endings (and other afferents). Potential: motoneurons activated by Ia excitation.	Painless. Simple to administer. May be the only test available for a muscle. Tested in relaxation.	Not entirely monosynaptic. Difficult to replicate mechanics of tendon tap across trials and conditions. Thixotropic state of intrafusal fibers needs to be controlled.
V-wave	Method: supramaximal stimulation of a peripheral motor nerve during a voluntary contraction. Volley: single in both muscle afferents and motor axons. Potential: motoneurons activated by Ia excitation. Note: only motoneurons whose axons are first cleared by collision of descending volitional and antidromic impulses contribute.	Best for strong (maximal) contractions.	Difficult to identify which elements of the motor system are responsible for any change seen in the response. Will vary with motoneuron firing rate. Can be painful.
CMEP	Method: submaximal stimulation at the level of the pyramidal decussation. Volley: single descending in the corticospinal tract. Potential: motoneurons activated by corticospinal excitation. Note: onset latency must be monitored to avoid root stimulation.	Not subject to conventional presynaptic inhibition. Largely monosynaptic. Unnecessary to average many responses. Possible to test in relaxation and contraction.	Painful. Not entirely monosynaptic. Difficult to obtain in some subjects. Difficult to obtain in some motoneuron pools.
MEP	Method: submaximal transcranial magnetic stimulation of the motor cortex. Volley: multiple descending in the corticospinal tract. Potential: motoneurons activated by corticospinal excitation. Note: some motoneurons can discharge more than once.	Painless. Large proportion of the motoneuron pool can be accessed under appropriate conditions. Possible to test in relaxation and contraction.	Affected by both cortical and spinal excitability and hence cannot measure either in isolation. Not entirely monosynaptic. Although not painful, can be unsettling at high stimulus intensities.

Note: Submaximal/supramaximal refers to the stimulus strength relative to the current required to evoke the maximal compound muscle action potential (M_{max}).

spindle afferents (**Figure 1A**). Most commonly recorded as a multi-unit response from surface electromyographic activity, it is possible to record an H-reflex in single motor units (e.g., Trontelj, 1968; Ashby and Zilm, 1982; Burke et al., 1984; Miles et al., 1989). Similar to the descending input during voluntary contractions, the synaptic Ia input will recruit motoneurons in an orderly fashion from smallest to largest (slow to fast motor units; Buchthal and Schmalbruch, 1970) according to the Henneman

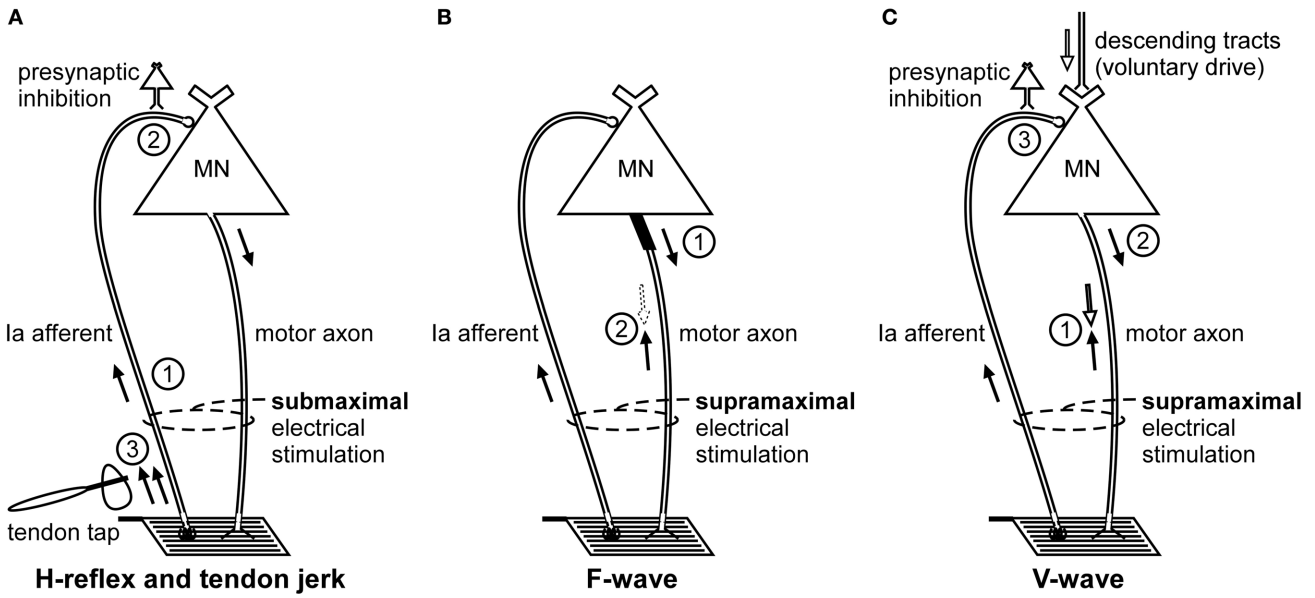

FIGURE 1 | Schematic representation of the volleys and pathways involved in the production of the H-reflex, tendon jerk, F-wave, and V-wave. Only the most critical elements are labeled so see text for a complete description of the factors which can influence each response. **(A)**, #1—electrical stimulus evokes a single afferent volley which recruits motoneurons for the H-reflex according to the size principle; #2—presynaptic inhibition can influence afferent input to the motoneuron; #3—tendon tap evokes multiple volleys which arrive at the motoneuron over 25 ms. **(B)**, #1—a small number of motoneurons may discharge to produce F-waves after antidromic impulses reach their soma; #2—at rest, F-waves are likely to be limited to large motoneurons due to reflex activation of smaller motoneurons and collision with the antidromic volley prior to the soma. **(C)**, #1—in motor axons conducting orthodromic impulses of voluntary drive, voluntary and antidromic impulses will collide; #2—reflex response which travels along motor axons cleared by the collision described in point #1 will contribute to the V-wave; #3—presynaptic inhibition can influence afferent input to the motoneuron.

size principle (e.g., Henneman and Mendell, 1981). Delivery of a series of progressively stronger stimuli generates a recruitment curve of the H-reflex and the muscle compound action potential (M-wave). In brief, H-reflex size increases with stimulus intensity until it reaches a maximum. This point can occur when further increases in intensity do not result in further increases in the net excitatory input to the motoneurons. Alternatively, a maximum can be reached because further increases in intensity reduce H-reflex size due to collision of afferent-evoked orthodromic impulses with antidromic impulses evoked in the motor axons that contribute to the growing M-wave. The H-reflex recorded at the tipping point is referred to as H_{max}. Comparison of H-reflex size to the maximal M-wave enables an estimate of the motoneuron pool involved in the H-reflex. For the soleus muscle in most subjects the percentage of involved motoneurons is ∼50% (Taborikova and Sax, 1968). A recruitment curve provides additional parameters (e.g., H-reflex threshold, slope of the ascending limb of the recruitment curve) which give insight into H-reflex input/output relationship (e.g., Zehr, 2002; Klimstra and Zehr, 2008). Finally, data about recruitment curves improve the validity of comparisons of data across time or experimental conditions.

Initially believed to be a purely monosynaptic Ia reflex (Hoffmann, 1922; Magladery et al., 1951; Paillard, 1955), it has since been established that the relatively long rise time of the compound excitatory postsynaptic potential (EPSP) (1.9–2.1 ms in soleus motoneurons; Birnbaum and Ashby, 1982; Burke et al., 1983) enables disynaptic (and possibly oligosynaptic) Ia pathways and Ib afferents to exert an influence on the H-reflex (Burke et al., 1983, 1984). Because the threshold to electrical stimulation and the conduction velocity of the fastest Ia and Ib afferents are probably not greatly different, both afferents feature prominently in the initial volley which arrives at the spinal cord (Pierrot-Deseilligny et al., 1981). Ib afferents acting via an inhibitory interneuron do not prevent the monosynaptic Ia EPSPs which initiate the H-reflex but do terminate the EPSPs with inhibitory postsynaptic potentials (IPSPs) at an interval as brief as 1 ms (Pierrot-Deseilligny et al., 1981). The first experimental, rather than theoretical, evidence of this non-reciprocal group I inhibition was a disynaptic limitation of the size of the quadriceps H-reflex (Marchand-Pauvert et al., 2002). Although this inhibition limits the size of the H-reflex (Burke et al., 1984), the majority of the response recorded under most conditions reflects monosynaptic Ia afferent input to the motoneuron pool. The influence of disynaptic or oligosynaptic input on motoneuron recruitment is determined by the size of the compound monosynaptic EPSP relative to the recruitment threshold of each motoneuron. That is, early recruited units in the response will be recruited by monosynaptic input but the discharge of the last recruited motoneurons will reflect the balance between monosynaptic excitation and di- or oligosynaptic inhibition and/or excitation. Hence, a change in H-reflex size occurs primarily through a change in this balance. This is true regardless of the size of the test H-reflex.

For more than 50 years, the H-reflex has been widely used as a test of the excitability of the human motoneuron pool. However, there are a number of caveats for this test which are commonly ignored or under-appreciated, despite a wealth of experimental data (e.g., Paillard, 1955) and detailed discussion of the technique (e.g., Schieppati, 1987; Pierrot-Deseilligny and Mazevet, 2000; Zehr, 2002; Pierrot-Deseilligny and Burke, 2005). A major mechanism that has long been known to alter the size of the H-reflex is the degree of presynaptic inhibition of Ia terminals (e.g., Frank and Fuortes, 1957; Eccles et al., 1961; Hultborn et al., 1987; see Rudomin and Schmidt, 1999 for review). Some other key mechanisms which can influence the size of the H-reflex include post-activation depression or homosynaptic depression (e.g., Magladery and McDougal, 1950; Crone and Nielsen, 1989; see Hultborn and Nielsen, 1998 for review) and contributions of oligosynaptic pathways (e.g., Pierrot-Deseilligny et al., 1981; Burke et al., 1983, 1984). Finally, in testing the H-reflex, it is difficult to be sure that the afferent volley itself is constant because activity leads to axonal hyperpolarization and reduced excitability in sensory and motor axons (e.g., Kiernan et al., 1997; Vagg et al., 1998) such that the same stimulus intensity is likely to activate fewer afferent axons after a voluntary contraction. See Pierrot-Deseilligny and Burke (2005) for a detailed discussion of these and other mechanisms.

The size of an H-reflex is sensitive to changes in subject posture (Hugon, 1973) and attention (Bathien and Morin, 1972), so it is critical that these factors vary as little as possible when collecting H-reflexes. Further, these factors make day-to-day comparisons of the H-reflex particularly difficult. The H-reflex is also strongly influenced by the frequency of stimulation as post-activation depression reduces the size of a second response elicited within 10 s of the first. To avoid this reflex attenuation, the stimulation frequency of repeated H-reflexes would ideally not exceed 0.1 Hz. However, collection of the requisite large number of responses at this rate is time-consuming and so a faster stimulation rate is more practical even if some post-activation depression remains. As the decay of post-activation depression is curvilinear (Magladery and McDougal, 1950), stimulating at 0.2–0.3 Hz strikes an acceptable balance between the level of depression and the time required to collect the responses (Pierrot-Deseilligny and Mazevet, 2000). The frequency of stimulation can be increased as high as 4 Hz during voluntary contraction because the post-activation depression seen in relaxed muscle is greatly attenuated or abolished (Burke et al., 1989; see also Stein et al., 2007) possibly because the extra impulse evoked by the electrical stimulus will have negligible impact on transmitter release from Ia afferents which are already discharging (Stein and Thompson, 2006). Although the size of the effect was relatively small, another factor to consider is the regularity of the stimuli. An interstimulus interval which varied between 0.5 and 1.5 s (mean of 1 s) evoked a larger H-reflex compared to stimulation at a constant interval of 1 s (Hoehler et al., 1981).

A practical limitation of H-reflexes is that they can only be obtained consistently from a small number of muscles during relaxation (typically soleus, flexor carpi radialis, quadriceps). However, this list of muscles expands greatly if stimulation is given while the subject performs a weak voluntary contraction. This ensures some motoneurons are discharging repetitively, brings others closer to threshold and thus increases motoneuron excitability. Burke and colleagues (1989) identified a number of other benefits to testing the H-reflex during voluntary contraction which include: the abolition of post-activation depression (homosynaptic depression); larger response sizes with lower stimulus intensities and hence a clearer distinction between the end of the M-wave and the onset of the H-reflex; a focus of the reflex response to the active motoneuron pool so that specific reflex arcs can be studied. Additional benefits are a reduction in the levels of homonymous (Fournier et al., 1983) and heteronymous Ib inhibition (Pierrot-Deseilligny and Fournier, 1986). Presynaptic inhibition is reported to be the same at rest and during steady contraction (Meunier and Pierrot-Deseilligny, 1989; Nielsen and Kagamihara, 1993) and so appears to represent neither an advantage nor a disadvantage to testing the H-reflex during voluntary contraction.

Thus, far, this section has described homonymous connections, that is from the stimulated Ia afferents to the motoneuron pool of the same muscle. However, there are also heteronymous links between Ia afferents of one nerve to the motoneuron pools of muscles supplied by a different nerve. For example, stimulation of the femoral nerve delivers monosynaptic Ia excitation not only to the quadriceps motoneurons but also to the soleus motoneuron pool (e.g., Bergmans et al., 1978; Meunier et al., 1990). Hence, appropriately-timed stimulation of the femoral nerve facilitates the soleus H-reflex. Heteronymous Ia monosynaptic excitation has been demonstrated in both the upper (e.g., Cavallari and Katz, 1989; Marchand-Pauvert et al., 2000) and lower limbs (e.g., Meunier et al., 1993) of humans. The utility of these connections is the ability to test the excitability of a motoneuron pool without the serious effect of activity-dependent changes in the excitability of the homonymous afferents.

F-WAVE

Although not termed the F-wave until 1950 (Magladery and McDougal, 1950), this late response to stimulation of a peripheral nerve was first described by Eccles and Pritchard (1937). Described as a recurrent discharge (e.g., Eccles, 1955), the F-wave reflects backfiring of a small number of motoneurons which are reactivated by antidromic impulses following supramaximal stimulation of a peripheral nerve (**Figure 1B**). Because F-waves are small (often less than 0.5 mV) and inconsistent in both size and shape, large numbers of responses are collected for averaging (Lin and Floeter, 2004). The variability of onset latency and morphology would cause considerable phase cancellation if raw F-waves were averaged online so potentials must be measured individually or full-wave rectified prior to averaging (Espiritu et al., 2003). In a clinical setting, rather than calculating the average response to a large number of stimuli, persistence (the percentage of stimuli evoking a response) and the difference in latency between the onset of the slowest and fastest single motor unit potentials are used as objective measures of properties of the motoneuron pool.

The production of an F-wave by a given motoneuron is believed to depend on the excitability of the axon initial segment (Eccles, 1955), and perhaps also the first node of Ranvier

(Gogan et al., 1984). The passage of the antidromic impulse to the soma will make these sites transiently refractory. If the axon initial segment remains refractory when the antidromic impulse evokes a somato-dendritic action potential, an orthodromic action potential will not be initiated in the axon to be propagated to the periphery and recorded as an F-wave. Intraneural stimulation of single thenar motor axons indicates that generation of an F-wave in an individual motoneuron is probabilistic and occurs rarely (after <2% of stimuli). Further, their incidence is unrelated to motor axon conduction velocity or twitch force (Thomas et al., 2002). This suggests a contribution from a mixed population of motoneurons. However, in more common experimental and clinical testing, the stimulus intensity is higher and the motoneurons which generate F-waves are likely to be limited to large motoneurons due to reflex activation of smaller motoneurons and collision with the antidromic volley prior to the soma (Espiritu et al., 2003).

Interpretation of the F-wave is simplest when the muscle is relaxed at the time of stimulation but responses can be recorded during voluntary contraction (e.g., Giesebrecht et al., 2011). The disadvantage to collecting responses during contraction is that a collision between voluntary orthodromic impulses with antidromic impulses will leave some motor axons clear to transmit an H-reflex to the muscle and thereby obscure the F-wave. This problem is exacerbated as the strength of contraction increases because a greater proportion of motor axons will see their antidromic impulse obliterated before it reaches the soma. During strong voluntary contractions, few antidromic impulses will reach the soma because of collision with the orthodromic voluntary and reflex action potentials. In these circumstances, any recorded potential would almost certainly be a V-wave (see V-wave section which follows).

It has been suggested that F-waves are a useful and direct measure of motoneuron excitability (e.g., Fisher, 1992) but subsequent reports suggest that F-waves only offer a flawed measurement of motoneuron excitability (Hultborn and Nielsen, 1995; Espiritu et al., 2003; Lin and Floeter, 2004; Pierrot-Deseilligny and Burke, 2005). Chief among the limitations is the relative insensitivity of F-waves to changes in motoneuron excitability. Nonetheless, they are potently depressed following fatiguing voluntary contractions (Khan et al., 2012; Rossi et al., 2012). A reduction in F-waves can be caused not only by inhibition of the motoneuron pool but by facilitation as well (Eccles, 1955; Hultborn and Nielsen, 1995). In a strongly facilitated motoneuron pool, the antidromic impulse which invades the soma will be followed by a somato-dendritic action potential at such a short interval that the axon initial segment is still refractory (Eccles, 1955). However, this may not be a practical limitation in human studies based on results with voluntary contractions (Giesebrecht et al., 2011). The other key criticisms of the F-wave concern the practice of comparing H-reflexes and F-waves in an effort to separate events at the level of the motoneurons; that is, to gain insight into changes in presynaptic inhibition versus changes in motoneuron excitability (e.g., Leis et al., 1995). Hultborn and Nielsen (1995) questioned the validity of such a comparison on three theoretical bases. The first relates to the collision between antidromic impulses and H-reflex discharges in slowly conducting motor axons. An enhancement of motoneuron excitability (e.g., by voluntary contraction) will increase the size of the H-reflex and thereby actually decrease the number of motoneurons capable of producing an F-wave because of a greater number of collisions. Second, the motor unit populations involved in the two potentials differ. The H-reflex involves small motor units with slowly conducting axons whereas, for reasons described above, the F-wave is likely to involve large motor units with fast axons. The third point relates to the mode of activation of the two responses (afferent vs. antidromic). To illustrate their concerns, Hultborn and Nielsen (1995) conducted a simple experiment which showed that F-waves could be an order of magnitude less sensitive than H-reflexes to changes in motoneuron excitability (although both responses were facilitated by a conditioning stimulus to the femoral nerve).

TENDON JERK

For limb muscles in which an H-reflex is not easily obtained, an alternative method of testing motoneuron excitability is a tendon jerk reflex. A tendon tap with a reflex hammer or more controlled means will create a stretch-induced barrage of discharges from muscle spindle primary endings and other afferents (**Figure 1A**). The size of the muscle spindle afferent volley depends on the mechanics of the tap and the sensitivity of the sensory endings. This sensitivity can be changed by contraction of the intrafusal muscle fibers on which the endings are located. Like the electrically-induced H-reflex, the mechanically-induced tendon jerk cannot be considered purely monosynaptic (e.g., Burke et al., 1983). However, unlike the relatively synchronous volley of the H-reflex, the afferent volley of the tendon jerk includes multiple discharges of a single Ia afferent and lasts for 25 ms (Burke et al., 1983, 1984). As a result, the afferent volley which arrives at the motoneurons is more dispersed for the tendon jerk than the H-reflex. This is one of several differences between the two reflexes which invalidate a comparison of the two responses as a surrogate measure of efferent drive to the muscle spindles (i.e., fusimotor activity) (Burke et al., 1983).

While ongoing fusimotor drive must potentially alter the size of the afferent volley evoked by a tendon tap, the history of prior fusimotor drive exerts a potent effect on the volley (Polus et al., 1991)due to the "thixotropic" behavior of the intrafusal muscle fibers (see Proske et al., 1993 for review). If a muscle is undisturbed or lengthened slowly following fusimotor activation, intrafusal fibers remain taut as actin-myosin cross bridges are maintained or re-established in a more stretched position. Conversely, passive shortening after fusimotor activation will maintain the cross bridges but cause the intrafusal fibers to become slack (Polus et al., 1991). This can produce large changes in background spindle firing rates. When spindles are held taut as a result of prior activity, the response to a tendon tap is increased. Note that this will have the opposite effect on the H-reflex as the increased background firing will lead to increased presynaptic inhibition (Polus et al., 1991).

The long held view that background fusimotor drive is necessary to elicit a tendon jerk from relaxed muscle is false (Burke et al., 1981). The relative ease of obtaining a tendon jerk in one muscle compared to another is likely to depend on

intrinsic spinal mechanisms. While the tendon jerk has the advantage of being simple to administer, changes in its size depend on many factors, beginning with the mechanical status of the tendon and transmission of the transient lengthening to the receptors.

V-WAVE

First described by Upton and colleagues (1971), the volitional wave (V-wave) is a variation of the H-reflex which is recorded during a voluntary contraction. In contrast to the submaximal stimulation used to evoke an H-reflex (see H-reflex section), a V-wave is evoked by supramaximal stimulation of a peripheral mixed nerve (**Figure 1C**). The supramaximal stimulus generates antidromic impulses in all motor axons as well as impulses in all group Ia afferents provided the stimulus intensity is $\geq 4\times$ motor threshold (Gracies et al., 1994). In motor axons involved in the voluntary contraction, there is a collision between voluntary orthodromic and the evoked antidromic impulses which leaves these axons clear to transmit a reflex response to the muscle. Conversely, motor axons not involved in the contraction, do not contribute to the production of the V-wave because any reflex response will either collide with the antidromic impulse or the antidromic impulse will reach the soma first and leave these motoneurons refractory when the afferent volley arrives.

The V-wave is influenced by many factors (e.g., strength of voluntary contraction and the range of maximal firing rates within a motoneuron pool) and the consequent difficulty in interpreting a change to the response raises questions about its usefulness as an independent measure of motoneuron excitability. During a maximal voluntary contraction, the size of the V-wave is proposed to indicate the level of descending voluntary drive conveyed by the motoneurons (Aagaard et al., 2002). According to this proposal, an increase in V-wave size indicates increased motoneuron discharge rates or recruitment (Aagaard et al., 2002) which may reflect increased supraspinal input to the motoneuron pool. However, it is important to recall that motoneuron discharge rate reflects not only supraspinal input to the motoneuron but the response to all inputs which arrive at the motoneuron so the origin of an increase in V-wave size is uncertain. The possible sensitivity of the V-wave to supraspinal input is unlike that of the H-reflex which is largely dependent on events at a spinal level (see H-reflex section). This distinction has led to the recent application whereby changes in the V-wave are compared to those in the H-reflex before and after training to distinguish between supraspinal and spinal neural adaptations (Aagaard et al., 2002; Vila-Cha et al., 2012).

CMEP

Non-invasive electrical or magnetic stimulation of spinal tracts can evoke large, short-latency responses in arm and leg muscles (Ugawa et al., 1991, 1994; Gandevia et al., 1999; Martin et al., 2008; see Taylor and Gandevia, 2004 for review). To evoke responses in arm muscles, axons are activated at the level of the cervicomedullary junction near the pyramidal decussation (**Figure 2**). Consequently, such a response is generally termed a CMEP. Electrical stimulation is accomplished by passing a brief high-voltage pulse between electrodes fixed near the mastoid processes. For magnetic stimulation, a double-cone coil is placed at the back of the head, with the center of the coil near the inion. Several terms describe these forms of stimulation and they are often used interchangeably. These include cervicomedullary, transmastoid, brainstem, or corticospinal tract stimulation. However, because the stimulus site can vary, not all the terms are actually synonymous. For example, responses with corticospinal components can be obtained in leg muscles with electrical stimulation over the cervical or thoracic spine (Martin et al., 2008). Responses produced by stimulation over the thoracic spine, are referred to as thoracic spine MEPs (TMEPs; Martin et al., 2008) rather than CMEPs.

Descending motor pathways other than the corticospinal tract (as well as ascending pathways) will be activated by the stimulus, but there is strong evidence that the CMEP is primarily the result of motoneuron activation by a single descending volley elicited by excitation of corticospinal axons (Berardelli et al., 1991; Ugawa et al., 1991; Gandevia et al., 1999; Taylor et al., 2002). The singular

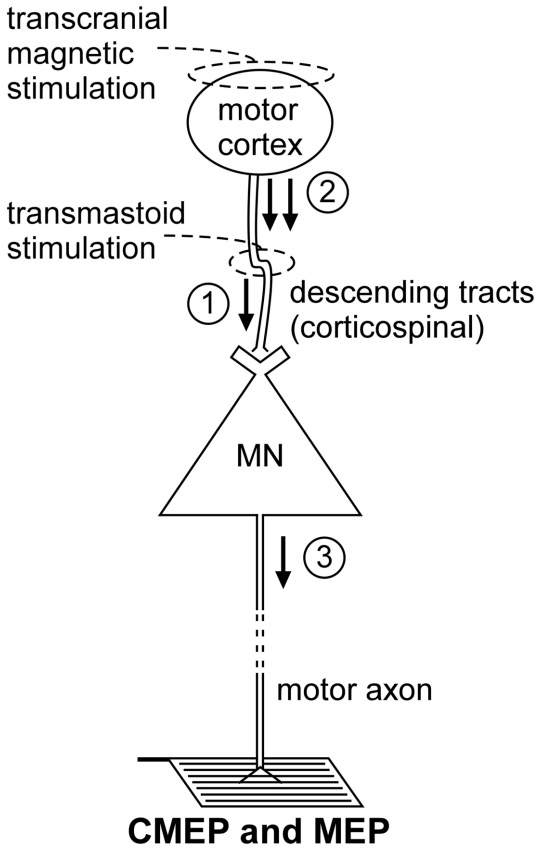

FIGURE 2 | Schematic representation of the volleys and pathways involved in the production of the CMEP and MEP. Only the most critical elements are labeled so see text for a complete description of the factors which can influence each response. #1—transmastoid stimulation evokes a single volley which is not subject to conventional presynaptic inhibition; #2—transcranial magnetic stimulation evokes multiple descending volleys;#3—transcranial magnetic stimulation can cause multiple discharges of a single motoneuron so that MEP size can exceed that of the maximal compound muscle action potential (M_{max}).

nature of the descending volley was confirmed by epidural recording in anaesthetized patients (Rothwell et al., 1994). In awake subjects, a collision experiment in which an ulnar nerve stimulus given before the brainstem stimulus (Berardelli et al., 1991) caused complete occlusion of the CMEP in abductor digiti minimi. In contrast, an ulnar nerve stimulus did not fully occlude the response to transcranial electric (Day et al., 1987) or magnetic stimulation (Hess et al., 1987) of the motor cortex which induce multiple corticofugal volleys and multiple discharges from some motoneurons. Additional collision experiments demonstrate that the CMEP is largely conducted via the large-diameter axons of the corticospinal tract. Brainstem stimulation given at appropriate times relative to electrical (Ugawa et al., 1991) or magnetic stimulation of the motor cortex (Berardelli et al., 1991; Gandevia et al., 1999; Taylor et al., 2002) largely occluded the motor cortical evoked potential (MEP) which suggests that the two stimuli activate many of the same corticospinal axons.

There are two important attributes of CMEPs which make this stimulation technique the most direct method to test motoneuron excitability to synaptic input in conscious humans (Martin et al., 2008). First, there is evidence that they have a large monosynaptic component in the upper limb (Petersen et al., 2002) and probably in the lower limb (Martin et al., 2008). Second, the descending tracts are not subject to conventional presynaptic inhibition due to primary afferent depolarization (Nielsen and Petersen, 1994; Jackson et al., 2006). This latter feature is in contrast to the H-reflex pathway (see Rudomin and Schmidt, 1999 for review). However, CMEPs must also be influenced by non-monosynaptic inputs although these have not been identified, and changes in CMEPs after strong voluntary contractions are postulated to reflect some presynaptic mechanism other than conventional presynaptic inhibition (Gandevia et al., 1999). Another advantage of the CMEP is that large responses can be evoked and hence averaging of large numbers of responses is not usually necessary.

Despite the advantages of corticospinal stimulation, like all stimulation techniques, it has limitations. A practical disadvantage is the discomfort produced by the stimulus. Most subjects will tolerate the stimuli but some find them prohibitively painful. The issue of pain is particularly relevant for stimuli delivered while the subject is relaxed because the discomfort is much less during muscle contraction and decreases as the level of voluntary effort increases. Apart from the issue of greater transient discomfort for the subject, the pain of stimulation when the subject is relaxed can indirectly affect the data. The size of the CMEP is sensitive to motoneuron excitability and so data collected in relaxation can be contaminated by weak inadvertent contraction if the subject instinctively "tenses up" in anticipation of the stimulus.

Another disadvantage of corticospinal stimulation is the inability or difficulty in obtaining responses of sufficient size in some subjects and some motoneuron pools. Even in subjects who tolerate stimuli within the normal range of intensities, it may not be possible to activate motoneurons via descending tract stimulation and record a valid CMEP. This occurs when the stimulus intensity required to evoke a response also activates nerve roots distal to the motoneuron soma. Such direct activation of the motor axon will mean that the "CMEP" is contaminated by a direct motor response and may not reflect motoneuron excitability accurately. The presence of nerve root stimulation can be identified in two ways: an abrupt ∼1–2 ms reduction in onset latency of the CMEP with an increase in stimulus intensity; or the absence of a large increase in CMEP size (relative to the CMEP recorded in relaxation) if a given stimulus is delivered during a weak voluntary contraction.

Corticospinal (transmastoid) stimulation has recently been paired with transcranial magnetic stimulation (TMS) as a novel means to test motoneuron responsiveness during ongoing muscle activity and fatigue without the confounding influence of unknown levels of descending voluntary drive (McNeil et al., 2009, 2011a,b,c). With this technique, a corticospinal stimulus is delivered 100 ms after a strong conditioning TMS pulse which transiently (∼200 ms) silences descending drive. The interruption of descending drive briefly stops motoneuron output so the excitability of motoneurons can be tested in a state of artificial relaxation (McNeil et al., 2009) without stopping the task and thereby altering the progression of fatigue.

MEP

TMS of the motor cortex (**Figure 2**) is widely used to test "motor cortical" excitability but is included here because of the profound impact of motoneuron excitability on the size of the MEP. This effect is best demonstrated by the comparison of MEPs recorded from a muscle during relaxation and voluntary contraction. Regardless of stimulus intensity (e.g., Di Lazzaro et al., 1998; McNeil et al., 2011a), MEP size increases markedly from relaxation to a weak contraction and the principal mechanism for this shift is enhanced motoneuron excitability (Hess et al., 1987; Taylor et al., 1997; Di Lazzaro et al., 1998). Hence, researchers must exercise caution when interpreting changes in MEP size as changes in "cortical" excitability. To make this claim, a valid test of motoneuron excitability must be performed to eliminate the possibility that the change in MEP size is mediated at a motoneuronal level. Even then, changes in MEP size may not represent changes in "cortical" excitability as there are significant non-monosynaptic contributions to MEPs so that changes at premotoneuronal sites can modify MEP size. For example, for muscles of the upper limb other than intrinsic hand muscles, significant excitation occurs through the C3–4 propriospinal system (Gracies et al., 1991).

CONCLUSIONS

One element of testing motoneuron excitability which needs further investigation is the matter of specificity. That is, if an increase or decrease in excitability is noted with one method (e.g., CMEP), is the same change evident when other methods (e.g., H-reflex) are applied? We recently compared the effects of fatigue on CMEPs (TMEPs) and F-waves (Giesebrecht et al., 2011) and there are the previously described comparisons between H-reflexes and F-waves (Hultborn and Nielsen, 1995) and H-reflexes and V-waves (Aagaard et al., 2002; Vila-Cha et al., 2012). However, additional comparisons of this nature would increase our understanding of the mechanisms involved and provide insight into the validity of different methods under various conditions.

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I_H activity is increased in populations of slow versus fast motor axons of the rat

***Chad Lorenz*[1] *and Kelvin E. Jones*[1,2] ***

[1] *Faculty of Physical Education and Recreation, University of Alberta, Edmonton, AB, Canada*
[2] *Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada*

Edited by:
Annie Schmied, National Center for Scientific Research, France

Reviewed by:
Penelope A. McNulty, Neuroscience Research Australia, Australia
Karl Ng, University of Sydney, Australia

***Correspondence:**
Kelvin E. Jones, Faculty of Physical Education and Recreation, University of Alberta, Edmonton, AB T6G 2H9, Canada
e-mail: kejones@ualberta.ca

Much is known about the electrophysiological variation in motoneuron somata across different motor units. However, comparatively less is known about electrophysiological variation in motor axons and how this could impact function or electrodiagnosis in healthy or diseased states. We performed nerve excitability testing on two groups of motor axons in Sprague–Dawley rats that are known to differ significantly in their chronic daily activity patterns and in the relative proportion of motor unit types: one group innervating the soleus ("slow motor axons") and the other group innervating the tibialis anterior ("fast motor axons") muscles. We found that slow motor axons have significantly larger accommodation compared to fast motor axons upon application of a 100 ms hyperpolarizing conditioning stimulus that is 40% of axon threshold ($Z = 3.24$, $p = 0.001$) or 20% of axon threshold ($Z = 2.67$, $p = 0.008$). Slow motor axons had larger accommodation to hyperpolarizing currents in the current-threshold measurement (−80% $Z = 3.07$, $p = 0.002$; −90% $Z = 2.98$, $p = 0.003$). In addition, we found that slow motor axons have a significantly smaller rheobase than fast motor axons ($Z = -1.99$, $p = 0.047$) accompanied by a lower threshold in stimulus-response curves. The results provide evidence that slow motor axons have greater activity of the hyperpolarization-activated inwardly rectifying cation conductance (I_H) than fast motor axons. It is possible that this difference between fast and slow axons is caused by an adaptation to their chronic differences in daily activity patterns, and that this adaptation might have a functional effect on the motor unit. Moreover, these findings indicate that slow and fast motor axons may react differently to pathological conditions.

Keywords: axon physiology, ion channels, hyperpolarization-activated inwardly rectifying cation conductance, nerve excitability test

INTRODUCTION

Many studies have demonstrated that changes in chronic daily activity patterns alter the electrophysiological properties of cat and rodent motor neurons measured from the soma: after exercise (Beaumont and Gardiner, 2002, 2003; Gardiner et al., 2005), chronic stimulation (Munson et al., 1997), and hindlimb unweighting or spinal transections (Hochman and McCrea, 1994; Cormery et al., 2005). Muscle contractile properties also change in response to different activity levels like chronic stimulation and exercise (Kernell et al., 1987; Westgaard and Lomo, 1988; Gordon et al., 1997) or decreased activity levels through spinal transection (Cope et al., 1986; Munson et al., 1986). However, there is comparatively little known about the association between the electrophysiological properties of motor axons and motor unit type, or whether the axons change in response to altered chronic activity levels that induce plasticity of motor unit phenotype. The primary exception is conduction velocity (CV), which is altered in response to chronic changes in daily activity patterns of the motor units (Carp and Wolpaw, 1994; Munson et al., 1997; Beaumont and Gardiner, 2002). However, it has been acknowledgd for some time that conventional nerve CV provides limited physiological insight and additional measures from nerve excitability studies are warranted (Krarup and Moldovan, 2009; Kiernan and Lin, 2012).

Nerve excitability studies in humans have demonstrated that electrophysiology is different between axons innervating different muscles (Kuwabara et al., 2000, 2001; Krishnan et al., 2004; Bae et al., 2009; Jankelowitz and Burke, 2009) and between motor axons innervating the same muscle but activated at different percutaneous stimulation thresholds (Trevillion et al., 2010). Similar studies in rodents suggested that motor axons activated at different stimulation thresholds vary moderately in measures of nerve excitability (Mori et al., 2010; Nodera and Rutkove, 2012). These studies suggest that motor axon conductances such as hyperpolarization-activated inwardly rectifying cation conductance (I_H) and slow potassium conductance (I_{Ks}) may vary across muscles and axons of different threshold. However, none of these studies have examined populations of axons from muscles with distinct distributions of motor unit types. The previous research suggests that the major differences in motor axons are a result of differences in conductances rather than simply a consequence of different axon diameters.

The purpose of this study is to compare the electrophysiology of axons innervating the soleus muscle (SOL) to those innervating

the tibialis anterior (TA) muscle in the rat. SOL axons were considered to represent a population of *slow*- and TA axons *fast*- motor axons. About 94% of rat TA motor units are classified as a fast (fast fatigable or fast fatigue-resistant) motor unit type while 6% are slow, based on a modified version of Burke's criteria originally used to distinguish motor units in the cat hindlimb (Totosy de Zepetnek et al., 1992). Note the motor unit distribution in the human TA has a much larger contribution from slow motor units (Johnson et al., 1973). On the other hand, about 80% of the motor units in rat SOL are classified as slow motor unit type while 20% are the fast motor unit type (Gillespie et al., 1987). Rat TA and SOL motor units have dramatically different activity patterns in locomotion (Gorassini et al., 2000) and presumably much different overall daily activity patterns (e.g., Hennig and Lomo, 1985). Thus, by using TA and SOL axons in this study, we analyzed axons of different motor unit types as well as axons with different chronic daily firing patterns.

MATERIALS AND METHODS

SURGERY AND RECORDINGS

A total of 14 female Sprague–Dawley rats, weighing 280 ± 50 g (mean ± SD), were used in this study. These weights in female Sprague–Dawley rats correspond to an age of approximately 90 days, which represents young but sexually mature females (Yang et al., 2000; George and Bostock, 2007). Rats were housed in pairs in a 12:12 h light–dark cycle, environmentally controlled (22–24°C, 40–70% humidity) room. Water and rat chow (Lab Diet 5001, PMI Nutrition, Brentwood, MO, USA) were provided *ad libitum*. All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee: Health Sciences for the University of Alberta.

Anesthesia was induced by intraperitoneal injection of a mixed dose of 60 mg/kg ketamine and 7.2 mg/kg xylazine (KX) with additional KX dosages to maintain a surgical plane of anesthesia for the duration of the experiment. A rectal thermometer monitored internal body temperature, which was maintained between 34.5 and 38.5°C by a heating lamp. Upon completion of all experiments, animals were euthanized by an overdose of KX followed by cervical dislocation.

An electrical clipper was used to initially remove hair over the lower back and legs followed by a depilatory cream to remove the remaining hair on the lower back and hip areas. Percutaneous electrical stimulation was delivered via Ag/AgCl 3M Red Dot electrodes (10 mm diameter), with the active electrode placed over the sciatic notch and the return electrode placed over the lumbar vertebrae at the midline of the back (**Figure 1A**). Electromyographic (EMG) recordings were made using Teflon-coated stainless steel monofilament wire (Cooner Wire 765–40; 40G) with approximately 3 mm of Teflon removed from the end of the wire. The wire was threaded through a hypodermic needle (TA 26G, SOL 27G) with ∼2 mm extending from the tip and twisted to form a hook. To record a bipolar intramuscular compound muscle action potential (CMAP) signal in TA the first needle was inserted near the motor endplate on the proximal 1/3 of the muscle, and a second needle was inserted about 2.5 mm distally. Insertion of the hypodermic needles in SOL was similar except they were placed about 5 mm apart because of the orientation of the motor endplate and muscle fibers in SOL. After the stainless steel recording electrodes were securely placed in each muscle, the needles were removed. To minimize EMG from other muscles and contamination of the target CMAP during the threshold tracking procedure, extensor digitorum longus, flexor digitorum longus, medial gastrocnemius, and plantaris were denervated. The distal 3/5 of lateral gastrocnemius (LG) was excised, rather than denervated, because SOL axons are intertwined with LG axons in a shared nerve branch that is not easily separated in the rat (**Figure 1B**).

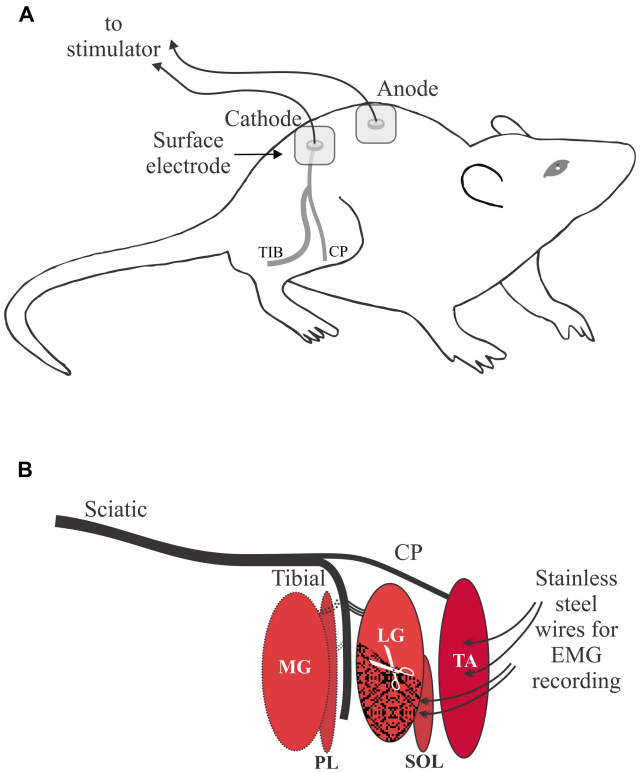

FIGURE 1 | Overview of the experimental setup. (A) Percutaneous electrical stimulation of the sciatic nerve with the cathode over the sciatic notch illustrated for the right leg. Proper skin preparation was essential under all stimulating electrodes. **(B)** Schematic of the branching of the sciatic nerve to the two target muscles: tibialis anterior (TA) and soleus (SOL). Other muscles of the lower leg were denervated: medial gastrocnemius (MG), plantaris (PL). The distal portion of the lateral gastrocnemius (LG) muscle was excised to reduced artifact from LG into electromyographic (EMG) recordings from SOL.

Compound muscle action potentials were amplified (to cover one-half to two-thirds of the A/D range), filtered (10–1000 Hz) and digitized by a 12-bit A/D board (National Instruments DAQ-6062e, Austin, TX, USA) at a rate of 10 kHz. Noise introduced from nearby power sources was removed in real time using a Hum Bug 50/60 Hz Noise Eliminator (Quest Scientific Instruments, North Vancouver, BC, Canada). In cases of excessive levels of noise which occurred in six rats, a 60 Hz notch filter was also implemented. The time required from anesthetic induction to complete all measures in an animal was typically 1 h. In five

animals the electrodes were repositioned and the tests repeated as a result of signal contamination. The time required in these cases was approximately 1.5 h. Arterial blood gases and acid base status were not monitored but are well maintained at rest in this preparation over this time period (Jendzjowsky and DeLorey, 2013).

NERVE EXCITABILITY TESTING

Multiple excitability measures of TA and SOL axons were made from the right leg. Stimuli were delivered using QTRAC software (Institute of Neurology, Queen Square, London, UK) and an isolated bipolar constant current stimulator (Digitimer DS5, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). The QTRAC protocol "TRONDCMW" consists of five nerve excitability indices: threshold electrotonus (TE), current-threshold (I/V), recovery cycle (RC), rheobase, and strength-duration time constant (SDTC). The primary outcome measure for each of these indices is the change in the amplitude of the test stimulus required to produce a target CMAP: in this case 40% of the maximum with a tolerance of ±7.5%. The duration of the test stimulus was 1.0 ms except during rheobase and SDTC in which the duration varies between 0.2 and 1.0 ms. Krishnan et al. (2009) has a more detailed description of this validated methodology.

For TE, a 100 ms sub-threshold conditioning stimulus with an amplitude expressed as a percentage (±40 and ±20%) of the test stimulus is applied and the change in test stimulus amplitude is measured at multiple delays relative to the start of the conditioning stimulus. The I/V is a similar test with a 200 ms sub-threshold conditioning stimulus and the change in test stimulus amplitude is measured at the end of the conditioning stimulus. The amplitude of the conditioning stimulus for I/V measurements varies from +50 to −100% of the unconditioned test stimulus. In RC, a supramaximal conditioning stimulus is applied to the nerve, and the test stimulus is applied at delays ranging from 2 to 200 ms. Rheobase and SDTC are determined via linear regression of the charge-duration data obtained through five test stimuli that range in duration from 0.2 to 1.0 ms.

ANALYSIS

All statistical analysis was done in SPSS version 21. Normality was assessed by the Shapiro–Wilk test and it was found that most data groups (groups within each of the five nerve excitability indices) *violated* the normality assumption at an alpha level of 0.05. Specifically, the hypothesis of a normal distribution was rejected for all data groups except TE +20% and depolarizing I/V measures. Therefore the non-parametric Wilcoxon signed-rank test was used for paired comparison of TA and SOL axons within each individual rat. For comparisons involving TE, paired comparison of TA and SOL axons were made at three delays for each of the four conditioning stimulus levels, i.e., ±40 and ±20%. For comparisons involving I/V, there were five data groups analyzed from I/V +50 to +10% and 10 groups analyzed from I/V 10 to −100%, all spaced by increments of 10%. In RC, all 18 delays ranging between 2.0 and 200 ms delay were analyzed. Finally, rheobase and SDTC were also used to compare TA and SOL axons. In order to control for type I statistical errors, we divided the alpha level (0.05) by the number of data groups within each respective nerve excitability measure. Therefore, the adjusted alpha level (α_a) for determining statistical significance was 0.017 in depolarizing and hyperpolarizing TE, 0.01 in depolarizing I/V, 0.005 in hyperpolarizing I/V, 0.006 in hyperpolarizing I/V Slope, 0.003 in RC, and 0.05 in rheobase and SDTC. The whiskers in each box-and-whisker plot represent 5 and 95% confidence intervals, while the box represents the interquartile range and the line the median. Line plots include the median value and error bars are the 95% confidence interval.

RESULTS

THRESHOLD ELECTROTONUS

Previous studies using nerve excitability testing have indicated that particular testing delays within the TE measurement are of greater interest since these are where properties diverge (Schwarz et al., 2006; George and Bostock, 2007; Sittl et al., 2011). These delays are 20–30, 100–109, and 120–150 ms and constitute the planned contrasts for statistical analysis (**Figure 2A**, gray areas). We averaged all the data within each of these delay ranges and used these values for comparisons between TA and SOL axons. **Figure 2A** displays the averaged data from TA and SOL axons for depolarizing TE +40% and hyperpolarizing TE −40%.

The initial fast phase, which is proportional to the applied current, was the same for TA and SOL axons for all depolarizing and hyperpolarizing conditioning currents. During depolarizing TE +40% there was no statistical difference between TA and SOL axons. In hyperpolarizing TE −40%, TA axons had a significantly greater threshold increase than SOL axons at 20–30 ms ($Z = 3.24$, $p = 0.001$) and 100–109 ms ($Z = 3.24$, $p = 0.001$), and a significantly smaller threshold decrease at 120–150 ms ($Z = -2.61$, $p = 0.009$; **Figure 2B**). For the TE −20% condition at 100–109 ms, TA axons had a significantly greater threshold increase than SOL axons ($Z = 2.67$, $p = 0.008$; **Figure 2C**). The data for these measures are given in **Table 1**.

CURRENT-THRESHOLD

There was no significant difference between TA and SOL axons at any of the five depolarizing conditioning strengths. At hyperpolarizing conditioning strengths, however, we found that TA axons had significantly greater threshold increases at I/V −80% ($Z = 3.07$, $p = 0.002$) and −90% ($Z = 2.98$, $p = 0.003$; **Figure 3**).

The slopes of the TA and SOL I/V curves were calculated at hyperpolarizing conditioning strengths, and the slope of the TA axon line was found to be significantly smaller than the SOL axon line at I/V −40% ($Z = 2.79$, $p = 0.045$) and −50% ($Z = 3.10$, $p = 0.018$). The values for these I/V measurements are given in **Table 1**.

RECOVERY CYCLE

There were no significant differences between TA and SOL axons in RC (**Figure 4**; **Table 1**) using the adjusted alpha value of 0.003 for multiple comparisons. Comparison at delays of 2–5 ms showed that TA axons had smaller increases in threshold compared to SOL axons (p-values less than 0.05), but these differences did not meet criteria for rejection of the null-hypothesis given the multiple comparisons.

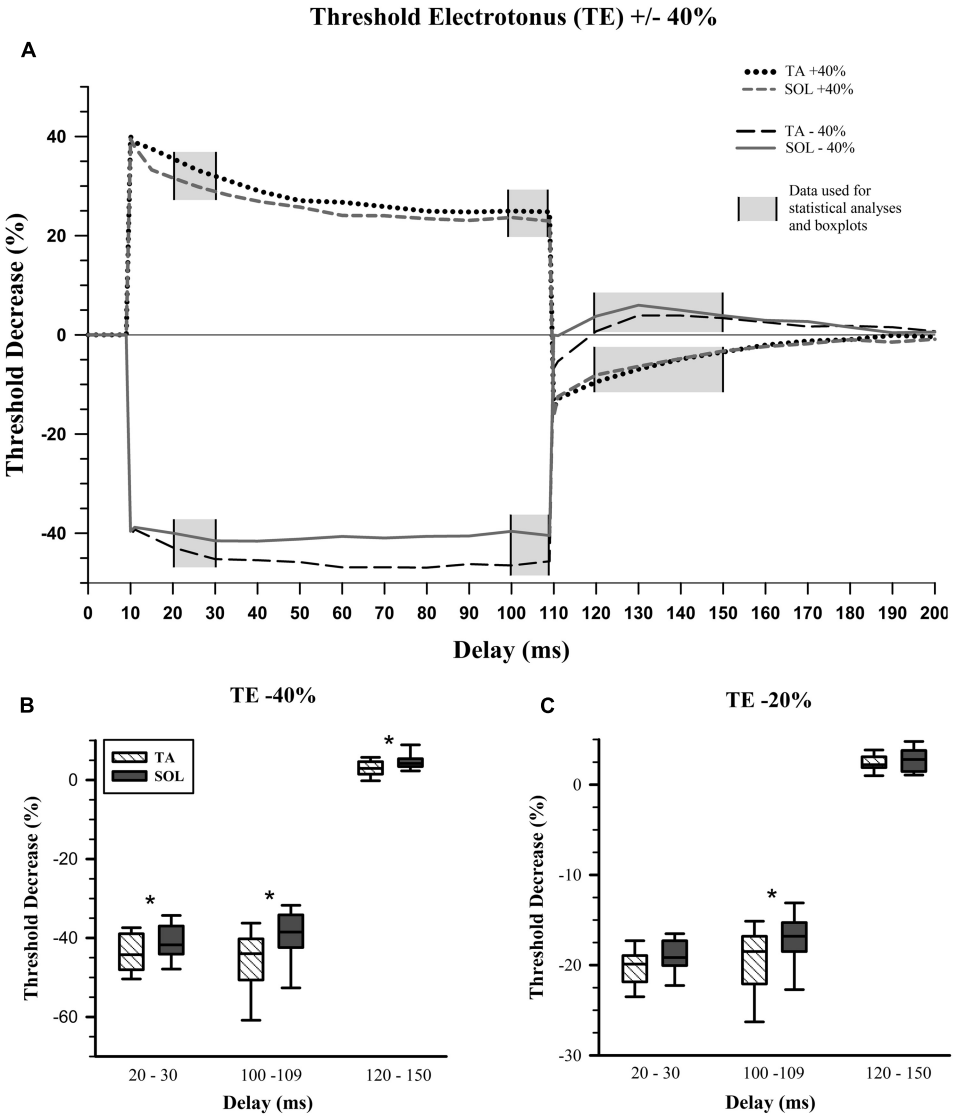

FIGURE 2 | Threshold electrotonus. (A) Average values for depolarizing and hyperpolarizing conditioning pulses with 40% amplitude. Planned statistical comparisons of TA and SOL axons were done at delay ranges illustrated with a gray background. **(B)** Percent change in threshold for the hyperpolarizing 40% threshold electrotonus (TE) condition. The whiskers in each box-and-whisker plot represent 5 and 95% confidence intervals, while the box represents the interquartile range and the midline the median. TA data in hatched boxes, SOL in gray boxes. Wilcoxon signed-rank tests, which are paired comparisons, show a statistically significant increase in median "Threshold Decrease (%)" for SOL compared to TA axons at all delays. **(C)** Percent change in threshold for the hyperpolarizing 20% TE condition. There is a statistically significant increase in median threshold change for SOL compared to TA axons, only at the end of the hyperpolarizing condition pulse, i.e., 100–109 ms. $*p < 0.017$.

STIMULUS-RESPONSE CURVES, RHEOBASE, AND STRENGTH-DURATION TIME CONSTANT

The stimulus-response curves showed a rightward shift for the TA axons indicating a higher threshold (**Figure 5A**). The normalized stimulus-response curve for TA was less steep compared to that for the SOL axons (**Figure 5B**). Paired comparisons found that TA axon rheobase was modestly but significantly larger than SOL axon rheobase ($Z = 1.99$, $p = 0.047$; **Figure 5C**). The higher rheobase for TA axons is consistent with the rightward shift of the stimulus-response curve. The SDTC was not different between the two axon groups ($Z = 1.36$, $p = 0.17$; **Figure 5D**).

DISCUSSION

The present findings show that many measures of nerve excitability are indistinguishable for slow soleus and fast TA motor axons in the rat. The exception to this general finding is for measures of accommodation to prolonged hyperpolarization during the TE and I/V tests. The most parsimonious explanation for the differences is that voltage-gated inwardly rectifying current I_H is greater in slow compared to fast axons. However, some of the differences can not be explained by differences in I_H: stimulus-response curves and rheobase, as well as differences in −40% TE at the early delay of 20–30 ms.

Table 1 | Mean values and 95% confidence intervals for the dependent variables generated by nerve excitability testing (NET) of *fast* TA versus *slow* SOL motor axons.

NET measure	TA axons (mean ± 95% CI)	SOL axons (mean ± 95% CI)	*p*-value $<\alpha_a$
Threshold electrotonus (%)			
TE +40% at 20–30 ms	34.35 ± 2.53	30.76 ± 3.09	
TE +40% at 100–109 ms	24.89 ± 2.59	23.32 ± 2.20	
TE +40% at 120–150 ms	−6.22 ± 0.92	−5.63 ± 1.20	
TE −40% at 20–30 ms	−44.04 ± 2.62	−40.75 ± 2.67	*
TE −40% at 100–109 ms	−46.07 ± 4.60	−40.02 ± 3.83	*
TE −40% at 120–150 ms	2.98 ± 1.16	4.65 ± 1.12	*
TE −20% at 20–30 ms	−20.20 ± 1.15	−19.14 ± 1.05	
TE −20% at 100–109 ms	−19.54 ± 2.23	−17.18 ± 1.71	*
TE −20% at 120–150 ms	2.35 ± 0.54	2.66 ± 0.71	
Current-threshold (%)			
I/V −80%	−135.15 ± 19.82	−112.89 ± 14.90	*
I/V −90%	−161.12 ± 21.68	−135.32 ± 17.37	*
I/V slope at −40%	0.66 ± 0.091	0.79 ± 0.12	*
I/V slope at −50%	0.56 ± 0.076	0.67 ± 0.095	*
Recovery cycle (%)			
RC at 2.0 ms	60.07 ± 10.42	96.78 ± 33.34	
RC at 2.5 ms	39.06 ± 6.95	58.46 ± 17.10	
RC at 3.2 ms	22.56 ± 5.22	31.54 ± 14.68	
RC at 4.0 ms	8.66 ± 5.40	19.49 ± 9.25	
RC at 5.0 ms	6.46 ± 3.54	10.78 ± 3.46	
RC at 6.3 ms	5.64 ± 2.82	8.06 ± 2.37	
Rheobase (mA) and strength-duration time constant (ms)			
Rheobase	1.45 ± 0.20	1.39 ± 0.34	*
SDTC	0.25 ± 0.032	0.28 ± 0.040	

The alpha criterion was adjusted (α_a) for multiple comparisons for each of the main outcome measures. TE = 0.017, depolarizing I/V = 0.01, hyperpolarizing I/V = 0.005, I/V slope = 0.006, RC = 0.003, and 0.05 for rheobase and SDTC.
** indicates p-values less than these adjusted alpha.*

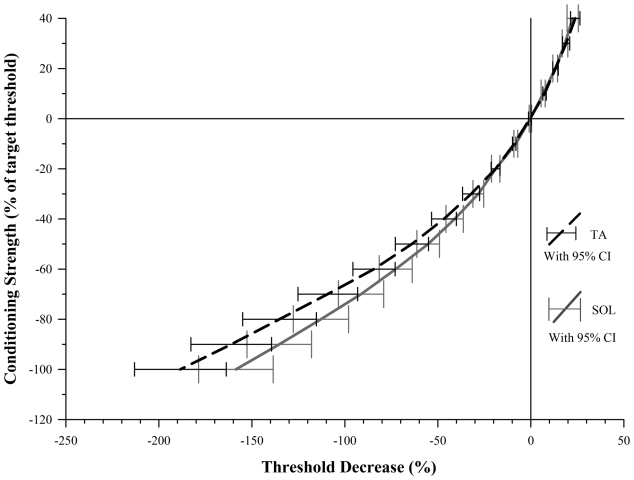

FIGURE 3 | Conditioning current versus threshold. For conditioning strengths of −80 and −90% there is a significantly smaller decrease in threshold for SOL compared to TA axons indicative of stronger depolarization current activated by the hyperpolarization for SOL axons. The difference between percent threshold decrease of SOL and TA axons at −80% was 22.3, 25.8 at −90% and 29.8 at a conditioning strength of −100%. Increased variance at −100% likely contributed to the failure to meet the threshold alpha of 0.006 to reject the null hypothesis.

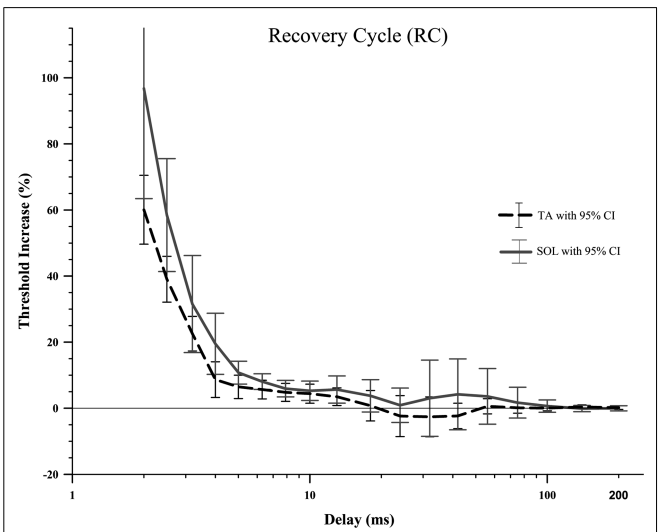

FIGURE 4 | The recovery cycle in the rat TA and SOL motor axons did not show a period of superexcitability followed by subexcitability, just a slowly decaying relative refractory period. A Wilcoxon signed-rank test did not find any significant differences between TA and SOL axons at any of the 18 delays. It should be noted that the large number of delays (18) that were compared created a very small adjusted alpha level, 0.003. Therefore there is an increased risk of type 2 error.

The most direct evidence implicating the channel responsible for I_H, hyperpolarization-activated cyclic nucleotide-gated (HCN) channel, in TE and I/V nerve excitability measures comes from direct *in vitro* voltage recording of rat spinal root myelinated axons (Baker et al., 1987). HCN channels are blocked by cesium (Biel et al., 2009) and Baker et al. (1987) showed that the late accommodation in measures similar to TE and I/V was antagonized by cesium. Yang et al. (2000) used cesium chloride to decrease accommodation to hyperpolarizing TE measures using an *in vivo* rat, tail motor axon preparation similar to the current study. For TE −20 and −40% conditioning pulses the threshold reduction at delays of 100–109 ms was greater after cesium administration. Thus reducing I_H in mixed tail motor axons generates responses that are similar to the data from fast TA motor axons reported here (**Figure 2**). This supports our inference that fast motor axons have weaker I_H activity compared to slow motor axons.

This is not the first study to suggest that I_H may vary across a population of motor axons, but it is the first to test two clearly distinct populations. A previous study investigated motor axons in

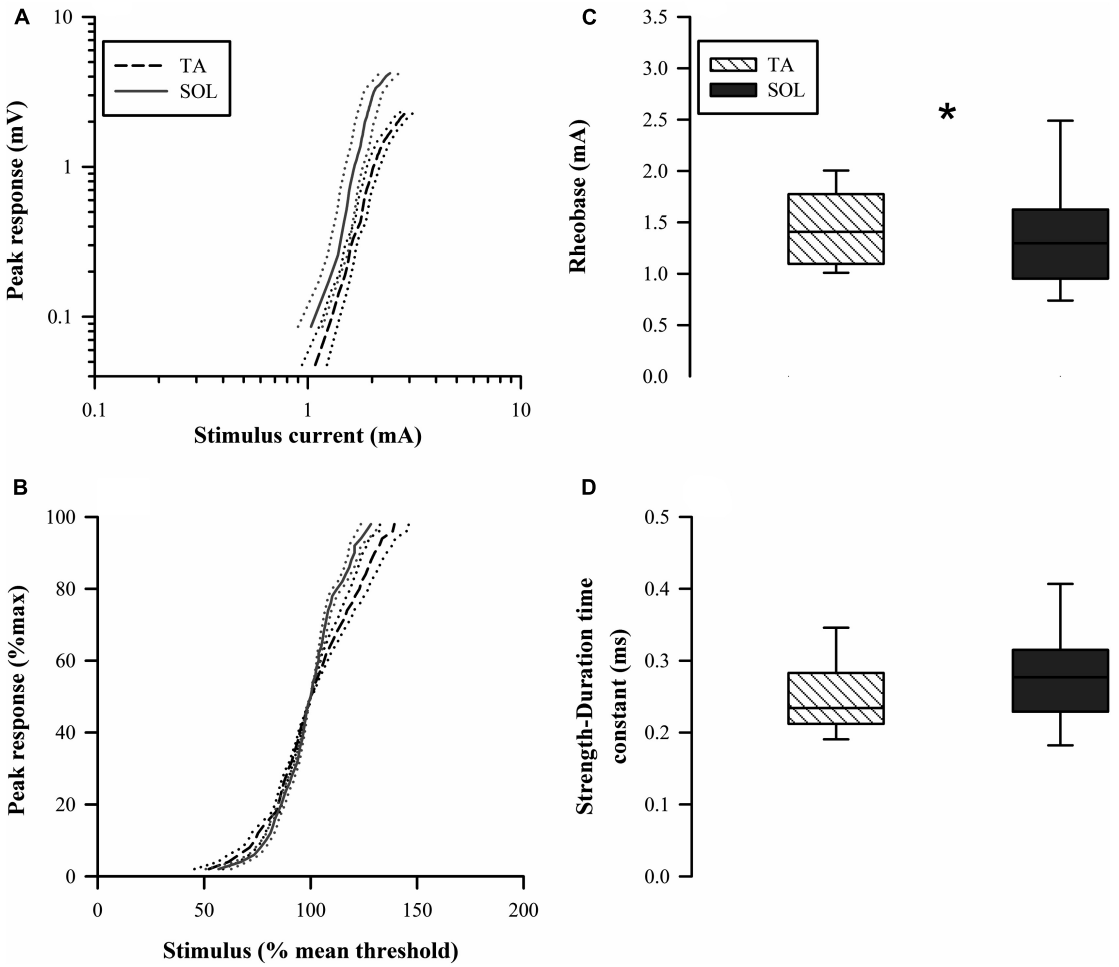

FIGURE 5 | Raw stimulus-response curves (A) are shifted to the right for TA axons indicating higher threshold. The normalized stimulus-response curves **(B)** indicated that the relative slope is less for TA axons. **(C)** Rheobase for TA axons (median 1.45 mA) was significantly larger in paired comparisons to SOL axons (median 1.39 mA). **(D)** The strength-duration time constant (SDTC) was not significantly different for the two types of motor axons.

human median nerve to conclude that the lowest threshold axons had different electrophysiological properties compared to higher threshold axons (Trevillion et al., 2010). The best explanation for the unique properties of low-threshold motor axons was that they had a greater level of I_H activity, and this inference was based on computer modeling. The authors used *F*-wave latency to characterize ten single motor units (45% of their total sample) as having a faster CV and therefore larger diameter compared to the average motor axon in the population responses. Their conclusion was that axons of a larger diameter and faster CV had greater I_H activity. This is the opposite of the findings in the present study that found greater I_H activity in the slower soleus motor axons compared to fast TA motor axons. It is not clear at this time why there is a clear discrepancy in the two findings other than the obvious species difference and use of anesthetic.

There are four isoforms of the HCN channel, HCN1–HCN4, all of which allow for passage of a mixed inward cationic current of both K^+ and Na^+ ions. HCN channels are found in all three major neuron compartments: the soma, dendritic tree, and the axon proper (Baker et al., 1987; Takigawa et al., 1998; Robinson and Siegelbaum, 2003; Biel et al., 2009). In addition to being voltage dependent, HCN channels have been found to be influenced by acidic lipids (i.e., PIP2), protons, extracellular K^+ concentration, and cytosolic proteins, and usually most significantly by cAMP (Biel et al., 2009). HCN1 has the fastest time constant of activation which can range anywhere from 30 to 200 ms at −140 to −95 mV. HCN2 is the next fastest isoform with the time constant of activation ranging from 150 ms to 1 s (Biel et al., 2009). Two recent nerve excitability studies comparing nerve excitability properties of motor and sensory axons have discussed the hypothesis that differences in I_H between these two axon groups are due to sensory axons having either greater expression of faster HCN isoforms and/or a different availability of HCN ligands that renders greater I_H in sensory compared to motor axons (Howells et al., 2012; Nodera and Rutkove, 2012). A higher density of channels on slow axons, the expression of different isoforms, or cAMP dependent phosphorylation status generating a difference in half-activation

voltages could explain the increased I_H in slow motor axons in the present study. There was no *a priori* hypothesis that I_H current would dominate the difference between slow and fast axons. Future studies should use a modified nerve excitability testing protocol with additional stronger and more prolonged hyperpolarizing conditioning stimuli to further characterize the difference in I_H between fast and slow axons (Tomlinson et al., 2010).

Thus, it appears I_H is a plastic ionic conductance that varies according to the type of motor axon as well as between motor and sensory axons. As the type of activity patterns are different between most motor and sensory axons, as well as for lower and higher threshold axons, this points to the possibility that I_H expression depends on impulse activity patterns. This is the interpretation given for the finding of reduced I_H in human motor axons on the affected side for individuals experiencing hemiparesis subsequent to stroke (Jankelowitz et al., 2007). In these individuals the motor axons on the affected side are expected to have a chronic reduction in impulse activity, if spasticity is not excessive. The reduced chronic activity leads to an adaptive change in I_H that is consistent with our finding of less accommodation to hyperpolarization in the rat TA motor axons. Axons innervating the rat TA are mostly from fast motor units that fire less often but at higher frequencies than the axons of SOL, which are mostly from slow motor units (Gillespie et al., 1987; Totosy de Zepetnek et al., 1992; Gorassini et al., 2000). Activity-dependent hyperpolarization of axons is known to correlate positively with firing frequency as well as the number of impulses in a train (Erlanger and Gasser, 1973; Raymond, 1979; Morita et al., 1993; Kiernan et al., 2004), although the magnitude of hyperpolarization saturates at frequencies around 20–50 Hz in lizards (Morita et al., 1993) and possibly at 20–30 Hz in humans (Kiernan et al., 2004). Although the relative importance of firing frequency and impulse load in creating activity-dependent hyperpolarization in rat TA and SOL axons is unclear, (Raymond, 1979) has shown that in the frog, average firing frequencies as low as 1.25 Hz can cause axon threshold depression and therefore probably activity dependent hyperpolarization (see Figure 9 in Raymond, 1979). Bursts in rat SOL motor units fire at an average frequency of 20 Hz for roughly 30% of the day (Hennig and Lomo, 1985), and so it seems likely that SOL axons would generally experience a greater tendency toward activity-dependent hyperpolarization than TA axons due to greater activation by the Na^+–K^+ pump. It is possible that greater I_H expression in SOL axons offsets a tendency for their hyperpolarization caused by higher levels of activity.

While many of the differences in nerve excitability indices are consistent with the interpretation that I_H is greater in SOL axons, this mechanism may not explain differences in the stimulus-response curves, rheobase and early differences in hyperpolarizing TE. The finding that TA axons have a greater rheobase and have a rightward shift in the stimulus-response curve indicates that stronger stimuli were required to activate these axons. The magnitude of the differences was small and should be considered when assessing the importance of this finding. For example, rheobase differed on average by 0.06 mA that was much smaller than the SDs for this measure, which were 0.20 and 0.34 mA for TA and SOL axons respectively. Typically differences in rheobase have not been interpreted as resulting from I_H. Similarly, the rate of activation of HCN channels is typically considered too slow to contribute to differences in hyperpolarizing TE at the 20–30 ms delay (Tomlinson et al., 2010). It is important to emphasize that the difference reported in **Figure 2B** was not a result of different extents of the fast change in threshold at the onset of the conditioning stimuli. The typical delay for overt expression of the effects of I_H is closer to 100 ms in human studies but can be much earlier if the membrane potential is depolarized (Bostock et al., 1998). There is some evidence from nerve excitability studies in mice that axons in the sciatic nerve may appear to be depolarized, even if there is no reason to suppose this to be the case (Boerio et al., 2009, 2010). In any case, it can reasonably be argued that if there is membrane depolarization, it should affect the TA and SOL axons equally and facilitate the measurement of greater I_H in SOL axons at earlier delays. Alternatively, differences in tissue impedance between rat and human experiments may facilitate earlier onset of I_H in rat experiments.

In conclusion, while for many nerve excitability measures we were not able to distinguish between slow and fast motor axons, they were significantly different during strong 100–200 ms hyperpolarizing conditioning stimuli. The axon threshold in these conditions is strongly affected by hyperpolarization-activated current I_H and the findings suggest a greater level of I_H in slow motor axons. This finding has implications for the use of nerve excitability testing in neurodegenerative conditions like amyotrophic lateral sclerosis (ALS). In the SOD1-G93A mouse model of ALS there is a clear motor unit type dependent vulnerability with fast-fatiguing motor units being most susceptible (Frey et al., 2000; Pun et al., 2006; Hegedus et al., 2008; Deforges et al., 2009; Kanning et al., 2010). As the vulnerable motor axons become denervated early in the disease the population of axons generating the nerve excitability measures will change. Longitudinal changes in nerve excitability measures (e.g., Kanai et al., 2006) may be confounded by the change in population of heterogeneous motor axons that contribute to the measure.

AUTHOR CONTRIBUTIONS

Chad Lorenz contributed to the acquisition, analysis and interpretation of the data, drafted the initial version of the manuscript, derived from a Master's thesis, and gave final approval for the submitted publication. Kelvin E. Jones conceived the study and contributed to acquisition, analysis, and interpretation of the data. The final manuscript was revised for publication by Kelvin E. Jones.

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Asynchronous recruitment of low-threshold motor units during repetitive, low-current stimulation of the human tibial nerve

Jesse C. Dean[1], Joanna M. Clair-Auger[2], Olle Lagerquist[3] and David F. Collins[2]*

[1] Division of Physical Therapy, Medical University of South Carolina, Charleston, South Carolina, USA
[2] Faculty of Physical Education and Recreation and Centre for Neuroscience, University of Alberta, Edmonton, AB, Canada
[3] Northern Alberta Institute of Technology, Edmonton, AB, Canada

Edited by:
Parveen N. S. Bawa, Simon Fraser University, Canada

Reviewed by:
Kevin Power, Memorial University, Canada
Stephan Riek, The University of Queensland, Australia

****Correspondence:***
David F. Collins, Faculty of Physical Education and Recreation and Centre for Neuroscience, University of Alberta, E-488 Van Vliet Centre, Edmonton, AB, T6G 2H9 Canada
e-mail: dave.collins@ualberta.ca

Motoneurons receive a barrage of inputs from descending and reflex pathways. Much of our understanding about how these inputs are transformed into motor output in humans has come from recordings of single motor units during voluntary contractions. This approach, however, is limited because the input is ill-defined. Herein, we quantify the discharge of soleus motor units in response to well-defined trains of afferent input delivered at physiologically-relevant frequencies. Constant frequency stimulation of the tibial nerve (10–100 Hz for 30 s), below threshold for eliciting M-waves or H-reflexes with a single pulse, recruited motor units in 7/9 subjects. All 25 motor units recruited during stimulation were also recruited during weak (<10% MVC) voluntary contractions. Higher frequencies recruited more units (n = 3/25 at 10 Hz; n = 25/25 at 100 Hz) at shorter latencies (19.4 ± 9.4 s at 10 Hz; 4.1 ± 4.0 s at 100 Hz) than lower frequencies. When a second unit was recruited, the discharge of the already active unit did not change, suggesting that recruitment was not due to increased synaptic drive. After recruitment, mean discharge rate during stimulation at 20 Hz (7.8 Hz) was lower than during 30 Hz (8.6 Hz) and 40 Hz (8.4 Hz) stimulation. Discharge was largely asynchronous from the stimulus pulses with "time-locked" discharge occurring at an H-reflex latency with only a 24% probability. Motor units continued to discharge after cessation of the stimulation in 89% of trials, although at a lower rate (5.8 Hz) than during the stimulation (7.9 Hz). This work supports the idea that the afferent volley evoked by repetitive stimulation recruits motor units through the integration of synaptic drive and intrinsic properties of motoneurons, resulting in "physiological" recruitment which adheres to Henneman's size principle and results in relatively low discharge rates and asynchronous firing.

Keywords: motoneuron, electrical stimulation, reflex, sensorimotor integration, motor unit

INTRODUCTION

Understanding how motoneurons transform synaptic input into motor output is fundamental to understanding their role in the neural control of human movement. During voluntary contractions, motoneurons receive synaptic inputs from descending and reflex pathways. The present thinking is that the currents that drive motoneuron discharge come from synaptic inputs, intrinsic properties of the neurons themselves (e.g., persistent inward currents; PICs) and metabotropic mechanisms that regulate the strength of these currents over a wide range. These ideas about motoneuron discharge (see Heckman and Enoka, 2012 for review) are based on recordings from motoneurons in reduced animal models (Schwindt and Crill, 1980; Bennett et al., 1998b; Heckman and Lee, 2001), from motor units in humans (Kiehn and Eken, 1997; Gorassini et al., 1998, 2002a) and from computational models (Elbasiouny et al., 2006; Powers et al., 2012). A limitation of studying how motoneurons transform synaptic input into motor output during voluntary contractions in humans is that the temporal characteristics of the synaptic input are inherently ill-defined. One way to circumvent this problem is to study motor unit discharge in response to trains of electrically-evoked afferent impulses. In this way, the temporal characteristics of the synaptic drive are relatively well-defined and the relationship between synaptic drive and motor output can be quantified (Kudina, 1988; Jones and Bawa, 1995; Bawa and Chalmers, 2008; Binboğa et al., 2011). The purpose of the present experiments was to characterize the recruitment and ongoing discharge of human motoneurons when they receive trains of afferent impulses over a range of physiologically relevant frequencies.

Presently, we deliver trains of impulses to human motoneurons by stimulating afferents in the tibial nerve at different frequencies and measure the output by recording the discharge of soleus motor units. The afferent volley evoked by a single suprathreshold pulse delivered to the tibial nerve comprises activity in axons from muscle spindles, Golgi tendon organs and cutaneous receptors

(Burke et al., 1983) and traverses mono- and polysynaptic pathways to motoneurons (Burke et al., 1984). The first impulses reach the motor pool in ∼15 ms, with impulses traveling along slower axons and/or through polysynaptic pathways arriving ∼6–10 ms later (Burke et al., 1984). Although the effects of the afferent volley on motoneurons can be both excitatory and inhibitory (Burke et al., 1983; Marchand-Pauvert et al., 2002; Binboğa et al., 2011), the net result is a relatively synchronous discharge of the motor pool known as an H-reflex (Hoffmann, 1918), which is primarily due to inputs from Ia afferents acting through predominantly monosynaptic pathways (Pierrot-Deseilligny and Mazevet, 2000). During low-intensity repetitive stimulation of the tibial nerve, motor unit activity can develop gradually and has been qualitatively reported to be asynchronous from the stimulation pulses (Lang and Vallbo, 1967; Burke and Schiller, 1976; Collins et al., 2001). Similar contractions develop when vibration is applied over a tendon or muscle belly, a phenomenon known as the tonic vibration reflex (TVR; De Gail et al., 1966; Hagbarth and Eklund, 1966; Burke and Schiller, 1976). In contrast to during electrical stimulation, however, motor unit activity during the TVR has been reported to be phase-locked to the mechanical stimulus (Burke and Schiller, 1976). More recently, we have shown that during repetitive stimulation of the tibial nerve motor unit discharge can be both synchronous with each stimulus pulse, as an H-reflex (Klakowicz et al., 2006; Bergquist et al., 2011; Clair et al., 2011), and "asynchronous" from each stimulus pulse (Collins et al., 2001; Bergquist et al., 2011). The current study represents a quantitative analysis of how such trains of afferent input are transformed into motor output.

We delivered electrical stimulation to the tibial nerve at a low current, below the threshold at which a single pulse elicited a measurable soleus M-wave, H-reflex or ankle extensor torque. Stimulating at such a low current minimized the number of motor units recruited during repetitive stimulation and thus reduced the chances of more than one motor unit discharging simultaneously, at an H-reflex latency, enabling us to more easily quantify motor unit discharge patterns. Low current stimulation also avoided participant discomfort and thus the potential of participants "tensing-up" and/or producing descending commands that contribute to motor unit recruitment. Our working hypothesis is that the recruitment and ongoing discharge of motor units during repetitive electrical stimulation in humans reflects the integration of currents that arise from synaptic drive and intrinsic properties of the neurons themselves, resulting in motor unit discharge that can be either "time-locked" to each stimulus pulse (i.e., H-reflexes) or "asynchronous" from the stimulus pulses. We predicted that low-current electrical stimulation will recruit motor units that are recruited during weak voluntary contractions (Sypert and Munson, 1981), consistent with the synaptic source of recruitment which follows Henneman's size principle (Henneman et al., 1965; Calancie and Bawa, 1984), and that recruitment will occur over relatively long time periods (on the order of seconds), consistent with the amplification of synaptic inputs by PICs in motoneurons (Spielmann et al., 1993; Bennett et al., 1998a; Heckman and Lee, 2001; Gorassini et al., 2002b). We also predicted that recruitment will occur with no measurable increase in synaptic drive, as quantified by a modified version of the paired motor unit technique. The results of the detailed analyses of motor unit discharge during repetitive stimulation described herein provide insight into how sensory input is transformed into motor output in humans which may prove to be useful for investigating pathophysiological aspects of sensorimotor integration after injury or disease.

METHODS

Experiments were conducted on nine healthy adult volunteers (7 male, 2 female) ranging in age from 22 to 44. All subjects provided written informed consent before participation in the study. The experiments were approved by the University of Alberta Health Research Ethics Board and were conducted in accordance with the Declaration of Helsinki. Low-current electrical stimulation was delivered over the tibial nerve while soleus motor unit activity and ankle extensor torque were recorded. Motor unit recruitment latencies and discharge patterns were compared for a range of stimulation frequencies.

EXPERIMENTAL SETUP

To record isometric ankle extensor torque, subjects were seated on the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Inc, Shirley, NY, USA) with the right hip flexed to 110°, the right knee flexed to 90°, the right ankle at 90°, the right lateral malleolus aligned with the axis of the dynamometer, and the foot strapped to the Biodex footplate. The subject's trunk was at an approximate angle of 20° reclined from the vertical. Surface EMG was collected using self-adhesive electrodes (1" × 1", Disposable A10043 Gel Electrodes; Vermed Inc. Bells Falls, VT, USA) placed over the soleus.

Subjects completed 1–3 submaximal contractions of the ankle extensors to warm up prior to the collection of maximum isometric voluntary contraction (MVC) data. They were instructed to push down as if they were pressing a gas pedal, rapidly increase force to a maximum and hold this contraction for 5 s. Following the practice trials, each subject completed between 2 and 4 MVCs, separated by one minute of rest, until the MVC torques varied by less than 10% for two successive contractions. The MVC was quantified as the maximum torque achieved in a single trial during the time period beginning 1 s after the start of the contraction.

After the MVCs were completed, fine wires (0.002 inch diameter, stainless steel; A-M Systems Inc. Carlsborg, WA, USA) were inserted into the soleus muscle belly using a 23-gauge needle to record the activity of single motor units. After insertion, subjects held a weak voluntary contraction (<10% MVC) while the fine wires were slowly retracted until a clear individual motor unit was detected. All EMG data were band-pass filtered between 30 and 5000 Hz. All data were sampled at 10,000 Hz and stored for subsequent analysis.

ELECTRICAL STIMULATION

Surface electrodes (1" × 1", Disposable A10043 Gel Electrodes; Vermed Inc. Bells Falls, VT, USA) were placed behind the knee in the popliteal fossa to stimulate the tibial nerve. Rectangular electrical pulses (1 ms duration) were delivered using a Digitimer constant current stimulator (DS7A, Welwyn Garden City, Hertfordshire, England) under computer control. Before each trial, the stimulation current was varied in 0.05 mA increments to find the

highest current at which single pulses were sub-threshold for both M-waves and H-reflexes in both the surface and fine wire motor unit EMG and did not produce any detectable ankle extensor torque. Subjects were instructed to remain relaxed during the stimulation, and none reported any discomfort.

Prior to the electrical stimulation trials, subjects performed a voluntary contraction in which they increased ankle extensor torque up to approximately 10% of their MVC, an estimate of the expected maximum torque during the electrical stimulation trials, and then decreased the contraction back to rest. Subjects were instructed to increase and decrease the strength of their contraction as slowly as possible. At the beginning of each electrical stimulation trial, subjects received 3 single pulses of stimulation separated by 5 s to confirm that the stimulation current was sub-threshold. If an M-wave, H-reflex, or torque response was present, the stimulation intensity was reduced and the trial was restarted. Five seconds after the last single pulse, a 30 s stimulation train was delivered at one of seven frequencies: 10, 20, 30, 40, 60, 80, or 100 Hz. The order of these frequencies was randomized for each subject. In many trials soleus EMG activity and ankle extensor torque remained after the stimulation was turned off, in which case subjects were instructed to "relax completely" (>1 s after the end of stimulation), which has been shown to terminate any involuntary sustained activity (Collins et al., 2002). After the seven electrically stimulated trials, one for each frequency, another voluntary contraction with slowly increasing and decreasing torque was performed, with torque increased to the maximum torque level produced during the electrically stimulated trials. The trials for voluntary contractions were performed to determine the torque level at which motor units were first recruited voluntarily and to ensure that the same motor units were recorded during the different electrical stimulation trials. Two minutes of rest separated each trial.

Upon completion of these trials, the fine wire electrodes were slowly retracted while the subjects maintained a weak voluntary contraction. The electrodes were moved until the original motor unit was no longer detectable and a new motor unit was identified. The procedure described in the paragraph above was then repeated. This series of steps was performed until either: (1) the fine wire electrode was pulled out of the muscle belly; (2) four hours had elapsed from the beginning of the experiment; or (3) the subject expressed discomfort from sitting in the same position for the duration of the experiment.

MOTOR UNIT DETECTION

Single motor unit data were analyzed using Spike 2 software (Cambridge Electronic Design Limited; Cambridge, UK). Individual motor units were discriminated using the template matching function of the software and validated by visual inspection. Discriminating individual motor units during electrical stimulation can be complicated by the simultaneous firing of multiple motor units at M-wave or H-reflex latencies, particularly when the stimulation is suprathreshold for M-waves and/or H-reflexes. At the low stimulation currents used for the majority of the trials in this study, motor unit discharge was often unrelated to the timing of each stimulation pulse. This, along with the relatively small number of motor units recruited by the low-current stimulation, allowed easier discrimination of individual motor units.

In the present experiments, we compared the discharge patterns of motor units recruited by a range of stimulation frequencies, and ensured that we were analyzing the same motor unit during successive trials using *post-hoc* template matching. To confirm that the fine wire electrodes did not change location during the electrical stimulation trials, we compared the motor units recruited by a voluntary contraction before these trials to the motor units recruited voluntarily after the electrical stimulation trials. If the same motor units were not activated, the electrical stimulation data were not used.

DATA ANALYSIS AND STATISTICS

We were interested in the effect of different stimulation frequencies on the recruitment and firing patterns of an individual motor unit. Therefore, we performed detailed analysis only on motor units that were recruited by at least 3 stimulation frequencies; motor units had to be recruited with a stimulation frequency at or below 60 Hz. With this recruitment criterion, we analyzed data collected from a total of 25 distinct motor units.

To address the predictions derived from our working hypothesis, we quantified the following characteristics of motor unit discharge, which are described in more detail in the subsequent paragraphs: (1) ankle extensor torque at recruitment; (2) number of recruited motor units and recruitment latency; (3) temporal relationship between stimulation pulses and the discharge of single motor units; (4) instantaneous discharge rate after electrical stimulation; and (5) discharge rate at the time of recruitment of additional motor units. For each of the statistical tests, *post-hoc* tests were performed as appropriate when significance ($p < 0.05$) was found. *Post-hoc* Tukey comparisons were performed following ANOVAs, and *post-hoc* reduced Chi-square comparisons with a Bonferroni correction were performed following Chi-square tests. All descriptive statistics are reported as mean ± standard deviation.

ANKLE EXTENSOR TORQUE AT RECRUITMENT

We predicted that low-current stimulation would recruit low threshold motor units. To test this, we calculated the ankle extensor torque at the time of motor unit recruitment during both electrically stimulated and voluntary contractions, normalized by the subject's MVC ankle extensor torque. Motor unit recruitment was defined as the beginning of continuous motor unit firing. In some cases, motor units discharged once or twice, but then went silent again before beginning to fire continuously. Therefore, the beginning of continuous firing was calculated as the first motor unit discharge for which all subsequent interspike intervals were shorter than 600 ms. We used the criteria of 600 ms to identify "continuous" firing as was used previously by Gorassini et al. (2002b) based on the work of Matthews (1996) who identified 300 ms as the longest inter-spike interval for continuously firing soleus motor units. By using a criteria that was double this minimum value we were confident that we were not including in our analyses periods of isolated or sporadic motor unit activity.

NUMBER OF MOTOR UNITS RECRUITED AND RECRUITMENT LATENCY

Based on our working hypothesis, we expected higher stimulation frequencies to recruit more motor units at shorter latencies. To determine whether stimulation frequency had a significant effect on the number of motor units recruited (out of 25) we performed a Pearson Chi-square analysis. For each trial in which a motor unit was recruited by electrical stimulation, we calculated the recruitment latency as the time between the start of stimulation (i.e., the first stimulus pulse in the train) and the onset of continuous firing as described above. A repeated measures one-way ANOVA was performed to determine whether stimulation frequency had a significant effect on recruitment latency.

TEMPORAL RELATIONSHIP BETWEEN STIMULATION PULSES AND SUBSEQUENT DISCHARGES

To determine whether motor unit firing was synchronous or asynchronous from the stimulation pulses, we generated post-stimulus time histograms (PSTHs; bin width = 1 ms) of the motor unit discharges following each stimulation pulse for all trials in which the stimulation rate was 10 or 20 Hz. At higher stimulation frequencies, the interval between stimulation pulses was too short ($\leq$33.3 ms) to reliably detect motor units firing at an H-reflex latency (typically $\sim$35 ms). For all trials at 10 and 20 Hz, we calculated the mean and standard deviation of the number of motor unit discharges for each bin of the PSTH. We judged synchronous firing to occur when the number of motor unit discharges at a given latency exceeded the mean plus two times the standard deviation. For all trials, there was a single peak of synchronous firing that was 1–2 ms in duration and occurred between 35 and 44 ms after the stimulation pulse. These peaks were defined to be due to motor units firing at an H-reflex latency. Similar peaks at an M-wave or any other latency were never seen.

We predicted that motor units would fire at a rate within a narrow range, largely asynchronous from the stimulation pulses. The instantaneous discharge rate was calculated for all motor units recruited by 10–40 Hz electrical stimulation. At higher stimulation frequencies more stimulation artifacts were present per unit time and more motor units were recruited, making individual motor units more likely to become obscured. Peak discharge rate was defined as the instantaneous discharge rate values during the 5 s period with the highest average value. We performed repeated measures one-way ANOVAs to identify significant differences in the onset period and the peak discharge rate during 20, 30, and 40 Hz stimulation. These comparisons were performed for the nine motor units that were recruited at each of these stimulation frequencies.

Motor units that fired at an H-reflex latency after a stimulation pulse were assumed to fire as a result of the electrically-evoked afferent input. Therefore, we quantified the time between each stimulation pulse and the previous motor unit discharge, hereby termed "after firing period", and calculated the probability of a stimulation pulse delivered at this time causing a motor unit to fire at an H-reflex latency. We considered this probability an indicator of motor unit excitability, as a motor unit closer to its firing threshold would be more likely to fire in response to the electrically-evoked afferent volley.

INSTANTANEOUS DISCHARGE RATE AFTER CESSATION OF ELECTRICAL STIMULATION

If, as hypothesized, motor unit discharge was at least partially driven by activation of PICs, we would expect firing to be sustained beyond the end of stimulation. Sustained firing was deemed to be present if a motor unit continued to fire for at least one second after the end of electrical stimulation. To determine whether the electrically-evoked afferent volley influenced motor unit discharge rate, we performed a repeated measures one-way ANOVA to compare the discharge rate during the last second of stimulation to that during the first second of sustained firing after stimulation ended. This comparison was performed for the nine motor units that were recruited at stimulation frequencies of 20, 30, and 40 Hz.

DISCHARGE AT THE TIME OF RECRUITMENT OF ADDITIONAL MOTOR UNITS

Finally, we predicted that motor unit recruitment would occur without an increase in synaptic drive. We tested this using data from trials in which multiple motor units were recruited during the 30 s of electrical stimulation. In a modified version of the "paired motor unit recording" technique (Kiehn and Eken, 1997; Gorassini et al., 1998, 2002a,b), we monitored the discharge rate of the first recruited motor unit (the "control unit") at the time when an additional motor unit (the "test unit") was recruited. This approach relies on the assumption that there is a linear relationship between the synaptic drive received by the control and test motor units. If the discharge rate of the control unit was constant, this was taken as an indication that the synaptic drive to the motor pool was also approximately constant. To identify any changes in discharge rate, our surrogate measure of synaptic drive, we performed a repeated measures one-way ANOVA to determine whether the control unit instantaneous discharge rate changed significantly from the 1 s prior to the recruitment of the test unit to the 1 s period following this recruitment. We also tested whether discharge rates were affected by the afferent volley produced by the electrical stimulation by performing a repeated measures one-way ANOVA comparing discharge rate during the last second of stimulation and the first second of sustained firing for both control and test units. These comparisons were performed for the ten instances in which two distinct motor units were recruited by 10, 20, 30, or 40 Hz stimulation.

RESULTS

Electrical stimulation was delivered at a current below the threshold required for a single pulse to elicit either an M-wave or an H-reflex, or produce any ankle extensor torque (**Figure 1A**). When this stimulation, which ranged from 2.5–10 mA across participants, was delivered at a constant frequency (10–100 Hz), soleus motor unit activity and ankle extensor torque developed gradually in seven of the nine subjects and thus analyses were performed only on data from those seven subjects. **Figure 1** shows data from a single subject in whom stimulation at 60 Hz (panel B) resulted in the gradual development of torque ($\sim$4% MVC) and the recruitment of 2 distinct motor units whose discharge was sustained after the stimulation ended. Data from 25 motor

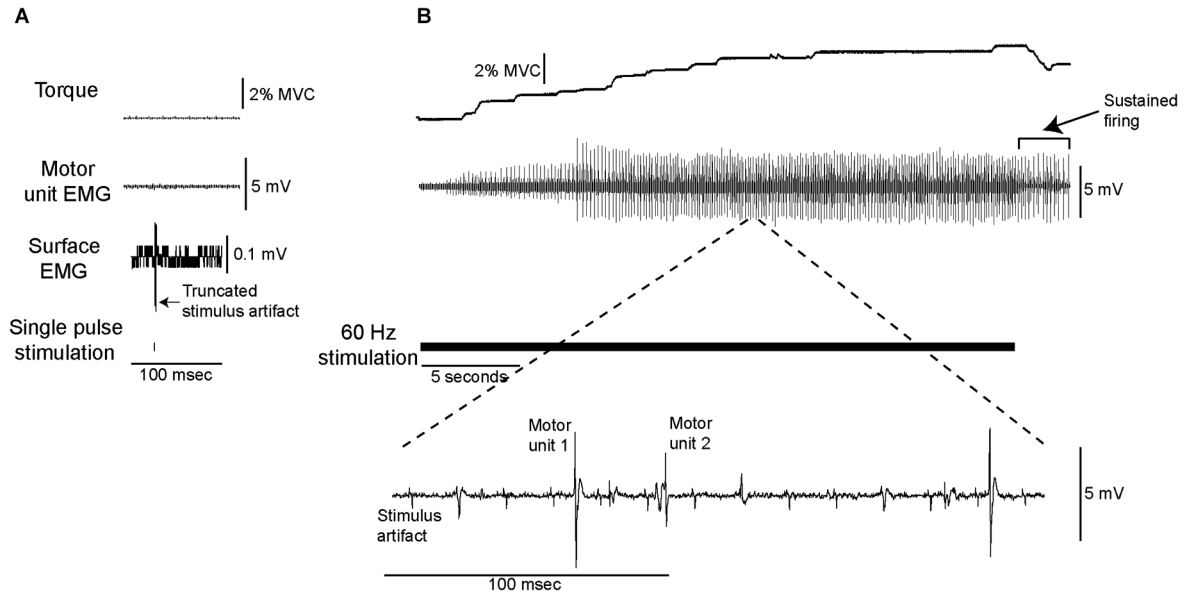

FIGURE 1 | Data from a single subject showing torque and motor unit activity evoked by low-current electrical stimulation. (A) The stimulation was delivered at a current at which a single pulse did not elicit any measurable change in ankle extensor torque, surface or intramuscular motor unit EMG. **(B)** Delivering this low-current stimulation for 30 s at 60 Hz (as shown by the solid horizontal line) resulted in the gradual development of motor unit activity and ankle extensor torque.

units that fit our recruitment criterion (see Methods) were analyzed.

ANKLE EXTENSOR TORQUE AT RECRUITMENT

Each of the motor units recruited during low-current electrical stimulation was also recruited during a voluntary contraction of similar strength. An example of data recorded from a single subject is shown in **Figure 2**. All 25 motor units recruited during electrical stimulation were recruited with a relatively weak (<10% MVC) voluntary contraction.

NUMBER OF MOTOR UNITS RECRUITED AND RECRUITMENT LATENCY

During 30 s of constant frequency low-current stimulation, motor units did not respond to the first stimulation pulse, but rather were silent for a given latency before being recruited (**Figure 3A**), or were not recruited at all. Higher stimulation frequencies recruited significantly more motor units within the 30 s of stimulation (Chi-square, $p < 0.05$; **Figure 3B**). A motor unit recruited by a given stimulation frequency was always recruited by each of the higher stimulation frequencies. For example, the 3 motor units recruited at 10 Hz stimulation were also recruited at all of the other stimulation frequencies.

Recruitment latencies spanned almost the entire course of the electrical stimulation, ranging from 0.5 to 29.6 s (**Figure 3C**). Several of the motor units were not recruited for a relatively long time, as 22 of the motor units had a recruitment latency longer than 10 s in at least one trial, and 9 of the motor units had a recruitment latency longer than 20 s in at least one trial. Higher stimulation frequencies recruited motor units at significantly shorter latencies than low frequencies (ANOVA, $p < 0.05$).

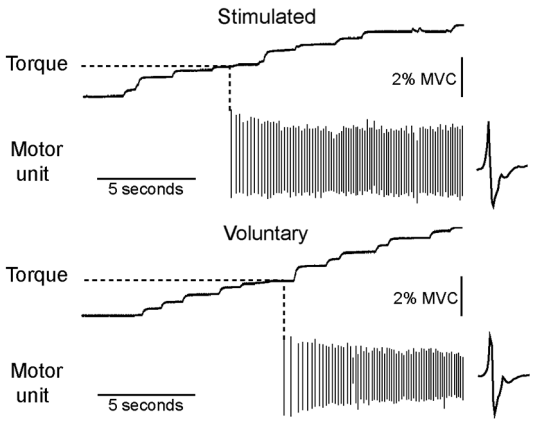

FIGURE 2 | All motor units recruited during low-current stimulation were also recruited during weak voluntary contractions. The torque level at which a motor unit first fired was measured for both electrically stimulated and voluntary contractions, as illustrated for a motor unit recorded from a single subject.

This is illustrated in **Figure 3D**, in which the 25 motor units are grouped into populations based on the minimum stimulation frequency at which they were recruited. For example, the line labeled 10 Hz illustrates the average recruitment latency across stimulation frequencies for the 3 motor units recruited during 10 Hz stimulation. The effect of increasing stimulation frequency on decreasing recruitment latency is evident in each of these populations.

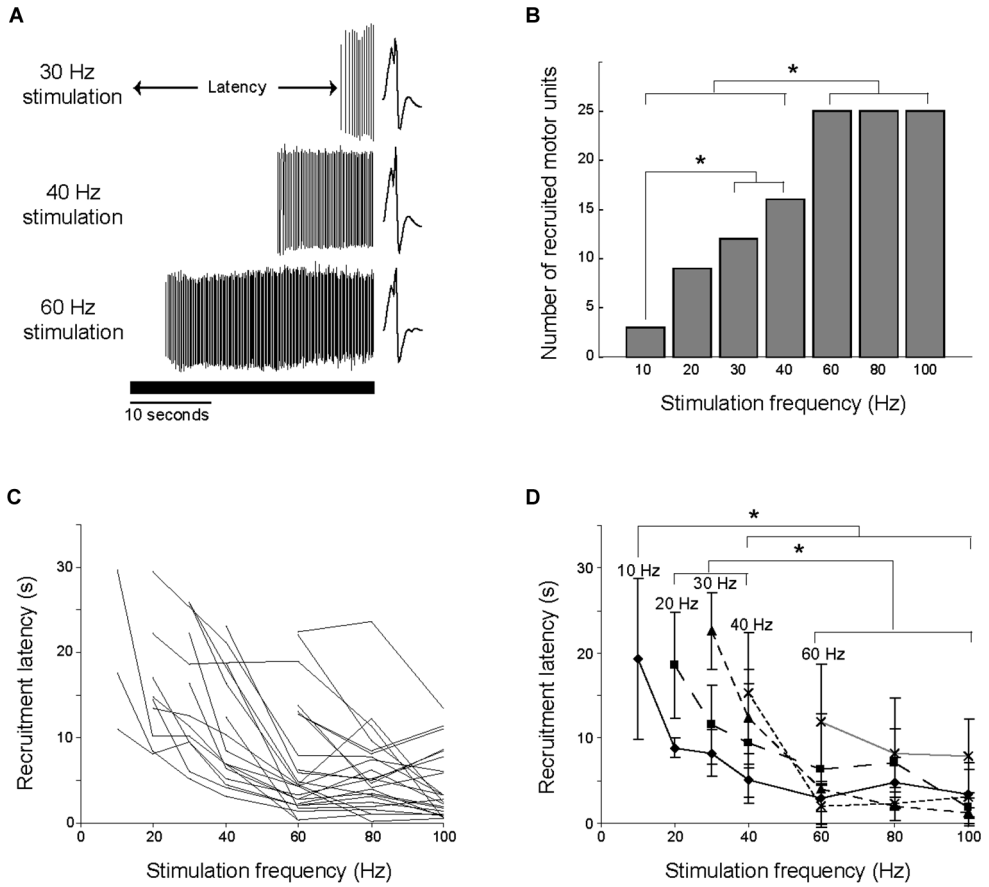

FIGURE 3 | Number of units recruited and latency of discharge onset were dependent on stimulation frequency. (A) The time between the beginning of stimulation and the onset of motor unit firing is illustrated for a single motor unit at three stimulation frequencies. The solid horizontal line shows the duration of the stimulation. **(B)** Higher stimulation frequencies recruited more motor units, with significant differences (*$p < 0.05$) between the indicated stimulation frequencies. **(C)** The change in recruitment latency with stimulation frequency is illustrated for each of the 25 motor units. The minimal stimulation frequency required to recruit each motor unit varied. **(D)** The 25 motor units were grouped by the minimal stimulation frequency required for their recruitment, and the average recruitment latencies were calculated for each group. Higher stimulation frequencies recruited motor units at shorter latencies, with significant differences (*$p < 0.05$) between the indicated stimulation frequencies.

TEMPORAL RELATIONSHIP BETWEEN STIMULATION PULSES AND SUBSEQUENT DISCHARGES

Once motor units were recruited, their discharge rate increased until a steady-state was reached, as shown for a single motor unit in **Figure 4A**. Motor unit discharge rate was not proportional to stimulation frequency, as the discharge rate reached approximately the same peak level regardless of stimulation frequency. Peak motor unit discharge rates were compared for the 9 motor units that were recruited during 20, 30 and 40 Hz stimulation. Peak discharge rate was significantly lower, albeit only slightly, with a stimulation frequency of 20 Hz (motor unit discharge rate = 7.8 ± 1.1 Hz) than with stimulation frequencies of 30 Hz (8.6 ± 1.1 Hz) or 40 Hz (8.4 ± 1.1 Hz), which were not significantly different (**Figure 4B**).

Qualitatively, motor units appeared to fire asynchronously from the stimulation pulses, as the latency between stimulus pulses and motor unit action potentials was not constant (**Figure 5A**, see also **Figure 1B**). PSTH analysis revealed that motor units fired at an H-reflex latency (1–2 ms peak ∼35–44 ms after a stimulation pulse) more often than would be expected from a completely asynchronous distribution, as illustrated in **Figure 5B** for a single motor unit. This analysis was performed for all trials in which a motor unit was recruited by a stimulation frequency of 10 or 20 Hz. In these 12 trials ($n = 9$ motor units), 24% (range 18–58%) of the motor unit discharges occurred at an H-reflex latency after a stimulation pulse, compared to the ∼2–4% of the time that would indicate true asynchronous firing.

The probability of a stimulation pulse causing a motor unit to fire at an H-reflex latency was dependent upon the timing of the pulse. The "after firing period", the time between the stimulation pulse and the previous motor unit discharge, was calculated for all motor units recruited by 10 or 20 Hz stimulation (12 trials, 9 motor units), as illustrated for a single motor unit in **Figure 6A**. Averaged across motor units, stimulation pulses delivered within 50 ms after a motor unit discharge (after firing period <50 ms) never caused the motor unit to fire at an H-reflex

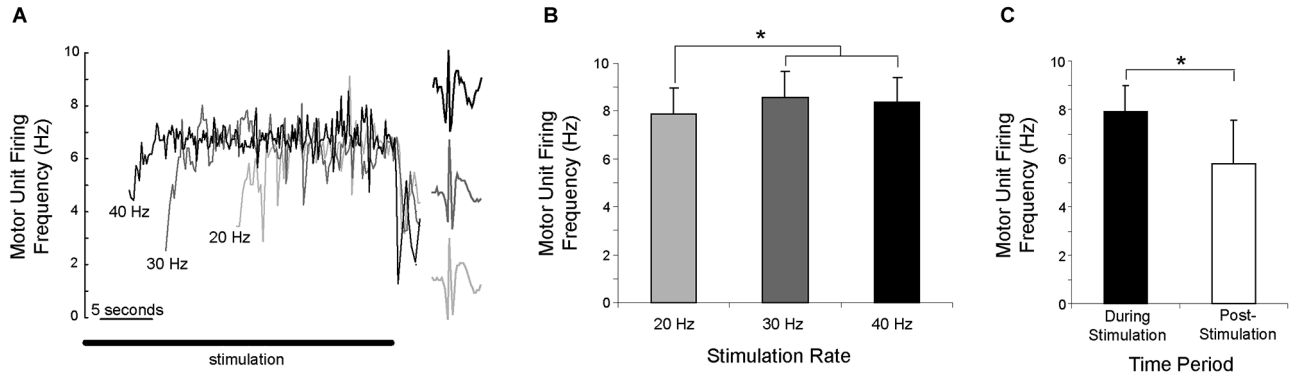

FIGURE 4 | Motor unit discharge rate was not proportional to stimulation frequency. (A) As illustrated for a single motor unit activated by three stimulation frequencies, there is a gradual increase in discharge rate, which then reaches a steady-state. The solid horizontal line shows the duration of the stimulation. **(B)** Averaged across subjects, the peak discharge rate was significantly (*$p < 0.05$) lower during 20 Hz stimulation than during 30 or 40 Hz stimulation. **(C)** Averaged across subjects and stimulation frequency, motor unit discharge rate during the first second of sustained firing was significantly (*$p < 0.05$) lower than the discharge rate during the last second of stimulation.

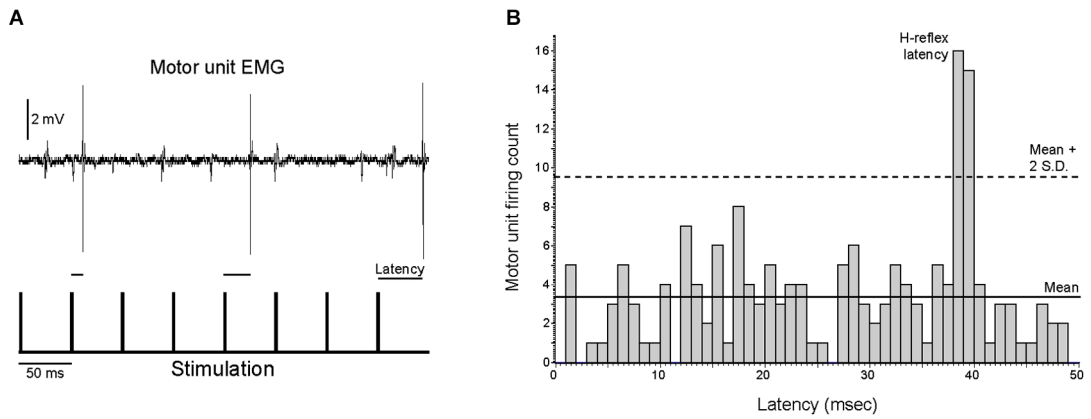

FIGURE 5 | Motor unit activity is only partially asynchronous from the stimulation pulses. (A) The latencies between motor unit discharges and the previous stimulation pulses were quantified for the nine motor units that were recruited with a stimulation frequency of ≤20 Hz, as illustrated for an excerpt of activity for a single motor unit. **(B)** Motor units fired at an H-reflex latency more often than would be expected from random chance, as illustrated for a single motor unit that fired at an H-reflex latency 18% of the time. For all analyzed motor units, this H-reflex latency peak exceeded the mean plus two standard deviations of the number of discharges per bin.

latency (**Figure 6B**). As the after firing period grew longer, the probability of the motor unit firing at an H-reflex latency after a stimulation pulse increased up to a maximum of 0.63 with an after firing period of 92 ms. Again, this analysis was performed for the 12 trials in which a motor unit was recruited by a stimulation frequency of 10 or 20 Hz.

INSTANTANEOUS DISCHARGE RATE AFTER CESSATION OF ELECTRICAL STIMULATION

Motor units often continued to fire after stimulation had ended, with sustained firing for at least one second occurring in 102 of 115 trials (89%). For the 9 motor units recruited during 20, 30 and 40 Hz stimulation, the discharge rate during the last second of stimulation (7.9 ± 1.1 Hz) was significantly higher than the discharge rate during the first second of sustained firing (5.8 ± 1.8 Hz) after stimulation ended (**Figure 4C**). The duration of the sustained activity was not quantified, as subjects were instructed to relax completely in order to allow 2 min of rest between trials and limit experiment duration. Although subjects stated that they were relaxed and were not voluntarily contracting, instructing them to "relax completely" terminated the motor unit activity.

DISCHARGE AT THE TIME OF RECRUITMENT OF ADDITIONAL MOTOR UNITS

In 10 trials, two clearly distinguishable motor units were recruited during the stimulation, as illustrated in **Figure 7A**. The discharge rate of the first motor unit recruited ("control unit") followed the pattern described above, with an initial increase followed by a steady-state in discharge rate. This discharge rate then remained constant even as additional motor units ("test units") were subsequently recruited. In no trials did the discharge rate of a control unit increase as a test unit was recruited. Across the ten

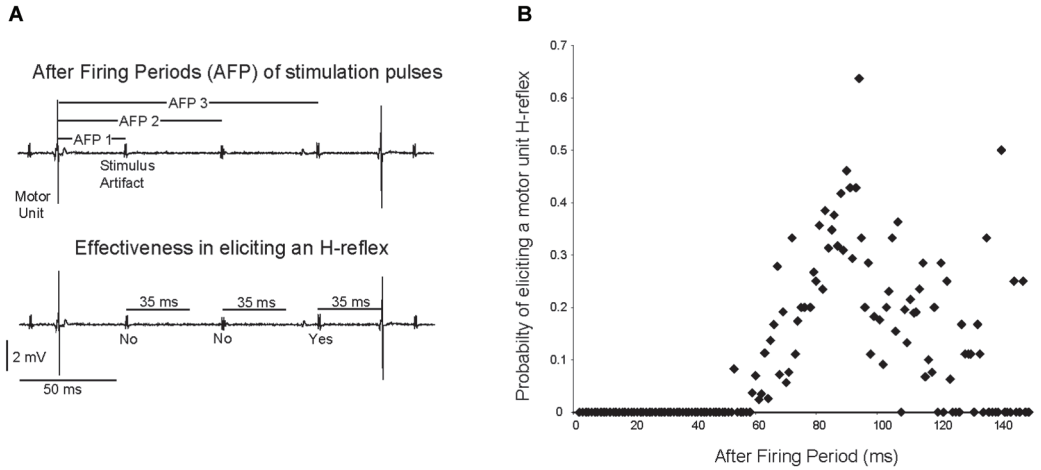

FIGURE 6 | The probability of a stimulation pulse eliciting an H-reflex depends on the time since the motor unit last fired. **(A)** As shown for a single motor unit, the time since the previous motor unit discharge was measured for each stimulation pulse, and termed the "after firing period". It was then determined whether this stimulation pulse elicited an H-reflex. **(B)** Across subjects, the probability of a stimulation pulse eliciting a motor unit H-reflex was calculated for each 1 ms after firing period increment.

analyzed paired motor unit recordings, the control unit discharge rate during the 1 s period immediately prior to the recruitment of the test unit was not significantly different than the control unit discharge rate during the subsequent 1 s period (ANOVA, $p > 0.05$; **Figure 7B**). The discharge rates of both the control and test units decreased significantly once the stimulation ended (ANOVA, $p < 0.05$; **Figure 7C**).

DISCUSSION

Repetitive electrical stimulation of the tibial nerve, delivered at an intensity that was below threshold for producing H-reflexes (or M-waves) with a single pulse, resulted in the gradual recruitment of low threshold soleus motor units. A wide range of relatively high electrical stimulation frequencies resulted in a narrow range of relatively low motor unit discharge frequencies. The electrically-evoked afferent volley influenced motoneuron discharge "directly", resulting in motor unit discharge that was time-locked to the stimulus pulses as H-reflexes, and "indirectly", as reflected by motor unit discharge that was temporally uncoupled from the stimulation. Accordingly, the discharge of recruited motor units was consistent with the physiological activity that occurs during voluntary contractions, including recruitment according to Henneman's size principle, relatively low discharge rates and asynchronous firing.

ELECTRICAL STIMULATION RECRUITED LOW THRESHOLD MOTOR UNITS

Electrical stimulation of afferents in the tibial nerve recruited a population of motor units with a low threshold for voluntary activation. Each of the 25 motor units recruited by the electrically-evoked afferent volley was also recruited during a weak voluntary contraction, indicating that this method of activating motor units follows Henneman's size principle as has been shown previously (Henneman et al., 1965; Calancie and Bawa, 1984). This contrasts with the random recruitment order that occurs when motor units are recruited as M-waves by the depolarization of motor axons when the stimulation is delivered at higher current stimulation than was used presently (Bickel et al., 2011). These differences in how motor units are recruited by "central" and "peripheral" pathways has implications for using electrical stimulation to generate contractions for rehabilitation (see Implications, below).

STIMULATION EFFICACY INCREASED OVER TIME

The effectiveness of the stimulation in recruiting motor units increased during the 30 s stimulus trains. Motor units were recruited at latencies ranging from 0.5 to 29.6 s, with the upper bound limited by stimulation duration. Recruitment latencies were longest when stimulation frequencies were lowest (10–40 Hz). Temporal summation of EPSPs does not occur when excitatory volleys are greater than 50 ms apart (frequencies <20 Hz) in either reduced animal models (Curtis and Eccles, 1960) or humans (Táboríková and Sax, 1969; Pierrot-Deseilligny et al., 1976; Ashby and Zilm, 1982; Powers and Turker, 2010). Therefore, the reported long recruitment latencies, particularly during stimulation at 10 Hz, cannot be caused solely by temporal summation of synaptic inputs, but must require an increase in either synaptic drive or excitability of the motor pool. These relatively long recruitment latencies are similar to previously reported motor unit recruitment with vibration (Kiehn and Eken, 1997), electrical stimulation of a peripheral nerve (Lang and Vallbo, 1967), extracellular activation (Spielmann et al., 1993) and intracellular current injection (Heckman and Lee, 2001).

MOTOR UNITS DISCHARGED "ASYNCHRONOUSLY" AND AT RELATIVELY LOW FREQUENCIES

Motor units did not discharge one-to-one with the stimulation frequency, but remained within a relatively narrow range

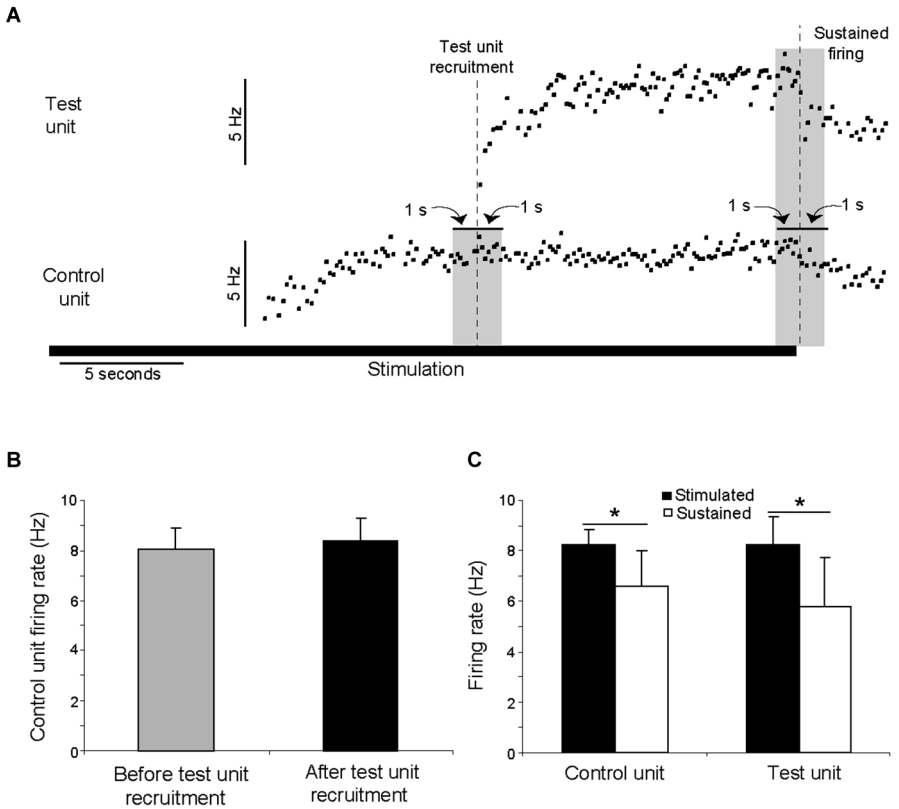

FIGURE 7 | The recruitment of additional motor units during electrical stimulation was not associated with an increase in the discharge rate of already active motor units. (A) A motor unit (control unit) was recruited by electrical stimulation, followed after approximately 8 s by the recruitment of an additional (test) motor unit. The control unit discharge rate did not increase when the test unit was recruited. These data are from a single trial in which the stimulation was delivered at 20 Hz for 30 s. The duration of the stimulation is shown by the solid horizontal line. The discharge rates of both units decreased when stimulation ended. **(B)** In ten instances, a control unit fired at its steady-state frequency for at least one second before a test unit was recruited. The discharge rate of the control unit during the 1 s period prior to the recruitment of the test unit was not significantly different than the discharge rate during subsequent 1 s period ($p > 0.05$). **(C)** For the same ten instances, the discharge rate of both the control and test unit decreased significantly ($p < 0.05$) once the stimulation ended.

(7.8–8.6 Hz) as stimulation frequency doubled from 20 to 40 Hz. This finding contradicts the qualitative results of Lang and Vallbo (1967), who reported that the mean firing of a motor unit increased as stimulation frequency increased, but is consistent with the "preferred" firing range of motor units, in which discharge rate does not scale linearly with synaptic input (Hornby et al., 2002). Motor units continued to discharge even after electrical stimulation ended, albeit at a significantly slower rate, indicating that afferent drive contributed to motor unit discharge, but was not solely responsible for the observed firing rate.

Motor unit discharge was largely, but not completely, asynchronous from the stimulation pulses, providing more evidence that discharge was influenced both directly, by the afferent volley (i.e., H-reflexes), and indirectly, by mechanisms intrinsic to the motoneurons themselves (i.e., asynchronous firing). The majority (76 %) of motor unit discharges did not occur at an M-wave or H-reflex latency after a stimulation pulse, eliminating an increase in neurotransmitter release associated with post-tetanic potentiation (Lloyd, 1949; Hultborn et al., 1996), or activity dependent increases in either motor or sensory axon responsiveness to electrical stimulation following repeated pulses (Burke et al., 2001) as possible explanations for the gradual development of motor unit activity. Conversely, if motor unit firing was not influenced by the afferent volley, motor unit discharge would be completely asynchronous from the stimulation, as previously reported qualitatively (Lang and Vallbo, 1967; Burke and Schiller, 1976; Collins et al., 2001). The present quantitative results demonstrate that when appropriately timed (at least 50 ms after the previous motor unit discharge), the electrically-evoked afferent volley was often sufficient to cause motor units to discharge. As the time since the last discharge increased, the probability of a stimulation pulse causing a response at an H-reflex latency increased, up to a maximum of 0.63 for pulses delivered 92 ms after a prior discharge. A similar recovery time course was found for soleus motor units during voluntary contractions (Jones and Bawa, 1995); this recovery during voluntary contractions depended on motor unit firing rates and was consistent with the predicted membrane voltage trajectories during the interspike interval.

POSSIBLE MECHANISMS

The amplification of synaptic input by PICs in spinal neurons (Lee and Heckman, 2000; Heckman and Lee, 2001; Heckman and Enoka, 2012) or the neuromodulatory facilitation of PICs (Perrier et al., 2002) could produce the type of motor unit discharge seen in the present experiments. Consistent with this idea, the paired motor unit recordings provide evidence that the gradual recruitment presently observed was not due to an increase in synaptic drive but rather was due to an increase in excitability of the motor pool. This technique has been used previously to monitor synaptic drive to the motor pool at a time when new motor units are being recruited (Kiehn and Eken, 1997; Gorassini et al., 1998, 2002a,b). In the present study, control motor units were sensitive to changes in synaptic drive; they fired at approximately 8 Hz, well below the maximal discharge rate of 20 Hz of voluntarily recruited soleus motor units (Bellemare et al., 1983). Additionally, motor unit discharge rates increased significantly when stimulation frequency increased from 20 to 30 Hz and discharge rates of both control and test units decreased significantly when stimulation ended. Thus, although "control" units were sensitive to changes in the electrically-evoked afferent volley, their discharge rate did not increase significantly at the time of recruitment of "test" units. This result suggests that recruitment of the additional motor unit was due to an increase in current provided by a post-synaptic mechanism such as activation of PICs in the motoneuron, not a pre-synaptic mechanism such as increased synaptic drive (Tokuno et al., 2003). We acknowledge that the control unit discharge rate may not exactly reflect changes in synaptic drive (Fuglevand et al., 2006), particularly at higher stimulation frequencies. However, previous paired motor unit results identify motoneurons as the most likely location of PIC activation (Powers et al., 2008; Vandenberk and Kalmar, 2014).

Increases in descending inputs or the magnitude of the electrically-evoked afferent volley cannot be ruled out as having influenced the observed motor unit recruitment. For example, ascending input to the brainstem may have prompted the release of monoamines such as serotonin from the raphe nucleus (Alvarez et al., 1998) or norepinephrine from the locus coeruleus (Lai et al., 1989). To directly address this possibility, the experiments described herein should be repeated in patients with complete spinal cord injuries, in whom the electrical stimulation of peripheral nerves would not be expected to have supraspinal effects. While our paired motor unit recordings suggest that it is unlikely that increased synaptic drive to the motor pool contributed to the observed contractions, we cannot rule out the possibility that increases in the descending input or the electrically-evoked afferent volley contributed to motor unit recruitment but was too small to significantly increase the discharge of our control unit. However, similar gradually-developing contractions and sustained firing have been observed in individuals with complete spinal cord injuries (Nickolls et al., 2004), suggesting that descending drive is not the primary contributor to the motor unit discharge behavior described in the present experiments. Further, repetitive activation of axons typically results in an activity-dependent hyperpolarisation, thus increasing axonal thresholds to electrical stimulation, which may have reduced and not increased the magnitude of the electrically-evoked afferent volley over the course of a stimulus train.

IMPLICATIONS

The present results describe how motoneurons transform afferent feedback into motor output in individuals with no neurological impairments. While such transformation may be a key contributor to voluntary contractions, afferent feedback during voluntary movements would be more temporally diffuse than the relatively synchronous activation of sensory axons that occurs during electrical stimulation. The synchronous nature of the electrically-evoked afferent volley is a limitation of the present study as it decreases the physiological relevance of our findings, however, it is a strength in that it allowed us to characterize how motoneurons respond to discrete excitatory volleys which would not be possible with vibration or voluntary contractions. We suggest that utilizing this "low-current stimulation" approach on individuals with neurological impairments may provide novel insight into how sensorimotor transformation is affected by injury or disease.

These findings also have implications for understanding how motor units are recruited during neuromuscular electrical stimulation. Electrical stimulation is used for rehabilitation after an injury or disease to prevent muscle atrophy, generate functional movements, or preserve motor unit types. Electrical stimulation, however, recruits motor units in a non-physiological order (Sheffler and Chae, 2007; Bickel et al., 2011) and leaves slow fatigue-resistant motor units, which are the most likely to develop disuse atrophy, (Burnham et al., 1997), relatively inactive. In contrast, generating muscle contractions through afferent feedback recruits motor units synaptically (Henneman et al., 1965; Bennett et al., 1998a), thereby first activating the fatigue-resistant muscle fibers (Sypert and Munson, 1981) with relatively weak stimulation. Thus, electrical stimulation delivered to recruit motor units by the electrically-evoked afferent volley may prove to be beneficial to reduce muscle atrophy and generate fatigue-resistant contractions. Such synaptic recruitment could also help maintain the biophysical properties of motoneurons, which are sensitive to changes in synaptic drive (see Gardiner et al., 2005 for review). In addition, modulating the excitability of a motor pool with low-current electrical stimulation of peripheral nerves may facilitate residual descending drive to augment voluntary commands and generate muscle contractions.

CONCLUSIONS

Presently we show that human motoneurons transform a wide range of synaptic input frequencies into a relatively narrow range of low output frequencies. Motor unit discharge was strongly influenced by properties intrinsic to the motoneurons themselves and was not solely driven by processes temporally-coupled with the synaptic input. This work supports the idea that sensory input evoked during electrical stimulation activates PICs in motoneurons, and describes a method of studying sensorimotor integration in humans with a more clearly defined input to spinal neurons than those produced by vibration or voluntary contraction.

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Single motor unit firing behavior in the right trapezius muscle during rapid movement of right or left index finger

Karen Søgaard[1]*, Henrik B. Olsen[1], Anne K. Blangsted[2] and Gisela Sjøgaard[1]

[1] *Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark*
[2] *Outcome Europe, St-Prex, Switzerland*

Edited by:
Maria Piotrkiewicz, Nalecz Institute of Biocybernetics and Biomedical Engineering – Polish Academy of Sciences, Poland

Reviewed by:
S. Jayne Garland, University of British Columbia, Canada
Katrina Shaun Maluf, University of Colorado, USA

****Correspondence:***
Karen Søgaard, Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark
e-mail: ksogaard@health.sdu.dk

Background: Computer work is associated with low level sustained activity in the trapezius muscle that may cause development of trapezius myalgia. Such a low level activity may be attention related or alternatively, be part of a general multi joint motor program providing stabilization of the shoulder joint as a biomechanical prerequisite for precise finger manipulation. This study examines single motor unit (MU) firing pattern in the right trapezius muscle during fast movements of ipsilateral or contralateral index finger. A modulation of the MU firing rate would support the existence of a general multi joint motor program, while a generally increased and continuous firing rate would support the attention related muscle activation.

Method: Twelve healthy female subjects were seated at a computer work place with elbows and forearms supported. Ten double clicks (DC) were performed with right and left index finger on a computer mouse instrumented with a trigger. Surface electromyographic signals (EMG) was recorded from right and left trapezius muscle. Intramuscular EMG was recorded with a quadripolar wire electrode inserted into the right trapezius. Surface EMG was analyzed as RMS and presented as %MVE. The intramuscular EMG signals were decomposed into individual MU action potential trains using a computer algorithm based on signal shape recognition and manual editing. Instantaneous firing rate (IFR) was calculated as the inverse of each inter-spike interval (ISI). All ISI shorter than 20 ms were defined as doublets. For all MU IFR was spike triggered averaged across the 10 DC to show the modulation during DC as well as for calculation of the cross correlation coefficient (CCC).

Results: All subjects showed surface EMG activity in both right and left trapezius ranging from 1.8 %MVE to 2.5 %MVE. Regarding intramuscular EMG during right hand DC a total of 32 MUs were identified. Four subjects showed no MU activity. Four showed MU activity with low mean firing rate (MFR) with weak or no variations related to the timing of DC. Four subjects showed firing patterns with large modulation in IFR with a clear temporal relation to the DC. During left hand DC 15 MUs were identified in four subjects, for two of the subjects with IFR modulations clearly related to DC. During both ipsi- and contralateral DC, doublets occurred sporadically as well as related to DC

Conclusion: In conclusion, DC with ipsi- and contralateral fast movements of the index finger was found to evoke biomechanically as well as attention related activity pattern in the trapezius muscle. Doublets were for three of the subjects found as an integrated part of MU activation in the trapezius muscle and for one subject temporarily related to DC.

Keywords: computer mouse double clicking, single motor units, trapezius, doublets, contralateral activity

INTRODUCTION

During data entry on the computer low static activity in the upper trapezius muscle has been reported even at an optimal ergonomic work station with full elbow support and no obvious biomechanical need (Kitahara et al., 2000; Thorn et al., 2007). For the uppermost clavicular subpart of the trapezius muscle the activity may be due to the need for postural neck extension and/or gaze stabilization during computer work. In contrast, the acromial subpart that is most prone for myalgia is mostly involved in scapular stabilization and accordingly, presents with a low activity level in the resting upright sitting position. However, in spite of the same modest biomechanical demand during computer work in the same position, it often shows activity above resting level (Sjøgaard et al., 2006). This low level but sustained muscle activity has been associated with development of trapezius myalgia and it has been speculated, whether it is mainly related to the mental demand of attention. (Sjøgaard et al., 2000; Wærsted, 2000; Zennaro et al., 2003). An alternative suggestion is that the trapezius muscle plays a role in a general multi joint motor program providing stabilization of the shoulder joint, as this in many situations may be a biomechanical prerequisite for precise finger manipulations (Alexander and Harrison, 2003). During computer

work the double click (DC) task on the computer mouse is an example of such a specialized finger manipulation demanding a small but fast flexion and extension in the metacarpophalangeal joint of the index finger. Earlier studies have shown that during this dynamic task, modulated firing patterns often involving doublets are evoked in the extensor digitorum communis muscle (Sjøgaard et al., 2001; Søgaard et al., 2001). If the computer work related trapezius activation is mainly caused by attention demand, a regular continuous firing pattern in both ipsi- and contralateral shoulder muscles would be expected during the dynamic DC task. However, if activation is related to biomechanical demands, the firing pattern in the stabilizing trapezius muscle would show task related modulation during the actual finger task.

The aim of the study was to examine single motor unit (MU) firing pattern in the right trapezius muscle during fast movements of the ipsilateral or contralateral index finger. A mechanical task related modulation of the MU firing would support the existence of a general multi joint motor program, while a bilateral generally increased continuous firing rate would support the attention related motor control.

MATERIALS AND METHODS

SUBJECTS AND PROCEDURE

Twelve healthy female subjects volunteered in the study after giving informed consent [Age: 24 (19–38) years, height 1.70 (1.62–1.78) m, and 63 (56–74) kg]. The participants reported no discomfort in the upper body regions within the week prior to testing. Eleven subjects reported to be right handed, while one subject (ID 11) reported to be left handed. All subjects were experienced computer users in their daily work and operated the computer mouse with their right hand. Therefore, in the present study the MU firing pattern was investigated for the right trapezius muscle for all subjects. During the experiment the subjects were seated upright in a height adjustable chair with forearms and elbows fully supported on the surface of a height adjusted table.

The subject performed ten DC interspersed by approximately two s on a computer mouse instrumented with a custom built trigger under the left mouse key. The task was intended to simulate the normal task of double clicking during computer work in order to evoke a function seen on the computer screen and thus was not particularly attention demanding. The subject was instructed to pay attention and follow the visual cue of a color change on the screen throughout the 10 DC sequence, but no particular emphasis on timing or speed was expressed. In a balanced order the subjects performed the DC task with the left as well as the right index finger. During the recordings no auditory or visual feedback of the MU activity was given to the subject. The study was approved by the local ethical committee (KF 01–298/00).

MEASUREMENTS

Surface EMG

Electromyographic signals (EMG) were recorded bilaterally from the upper trapezius muscles using bipolar surface electrodes (Ag–AgCl electrodes, type 72001-K, Medicotest, Denmark). The center of each pair of electrodes was placed 2 cm medial to the midpoint between the seventh cervical vertebrae and the lateral end of acromion. The inter-electrode distance was 20 mm. In **Figure 1** the electrode configuration also including the wire electrode is shown for the right trapezius muscle. The EMG signal was amplified, low-pass filtered (eighth order Butterworth filter, cut-off 400 Hz), and sampled on a computer with a sampling frequency of 1024 Hz. The signals were visually checked and high-pass filtered (cut-off 10 Hz), full-wave rectified, and root mean-square converted within windows of 100 ms duration. The resting EMG signal was recorded during 5 s of instructed rest in the same postural position as during the DC recordings. Visual feedback was provided on the screen to help the subject eliminate visible EMG activity, and the resting EMG amplitude was quadratically subtracted from all other EMG signals. For normalization, the maximal EMG amplitude obtained during three maximal voluntary isometric contractions was used. The contraction was performed bilaterally in the position of 90° shoulder flexion with resistance just proximal to the elbow. The maximal EMG amplitude (MVE) was calculated as the highest mean EMG amplitude obtained with a 1-s window moving in steps of 100 ms.

INTRAMUSCULAR EMG

Intramuscular EMG was recorded from the right trapezius muscle. A quadripolar wire electrode (Silvergold, Type 1, EGG Company, Tokyo, Japan) with a hooked end was inserted 3–4 cm into the muscle at an angle of 30° to the skin surface with a 27 gage cannula, that was subsequently withdrawn leaving the recording site of the wire in the trapezius approximately 2 cm medial to the midpoint between acromion and C7, i.e., in close spatial vicinity of the surface electrode recording site (See **Figure 1**). The wire consisted of four 50 μm urethane coated silvergold wires embedded in an epoxy coating. The distance in the exposed wire endings were 150–200 μm in a rectangular configuration. The four wires were combined to provide three

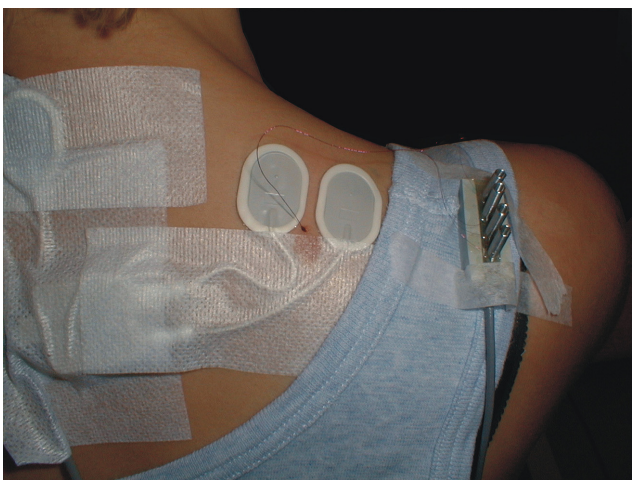

FIGURE 1 | Surface electrode configuration and also including the wire electrode for the right trapezius muscle. Note that the intramuscular quadripolar wire electrode is inserted at a 30° angle and at a distance so the location of the tip of the wire is estimated to be between the surface electrodes and in the recorded muscle volume.

highly selective bipolar recordings of micro MU action potentials (see **Figure 2**, upper part). All signals were analog filtered with a 10–10 kHz bandpass filter and A/D converted and stored on a computer with a sampling rate of 50 kHz for off-line analysis.

Before and after each DC recording it was tested, that the position of the wire electrode was in the vicinity of low threshold trapezius MUs by asking the subject to slightly lift the forearms from the table. The scheduled timing of each DC was given by a color change on the screen in front of the subject, and the actual time was recorded by a custom build computer mouse instrumented with a trigger under the left mouse button.

DATA ANALYSIS

The intramuscular EMG signals were decomposed into individual MU action potential trains (MUAPT) using a computer algorithm based on shape recognition from the three bipolar channels and manual editing. The program is able to handle superimpositions of MUs by composing a comparison signal based on the waveform shape of available active MUs potentials and time shifting these. When a match in shape with the real superimposed signal is available, the combination and timing of MUs in the composed signal is suggested to the operator. (For details see Olsen and Søgaard, 1999; Olsen et al., 2001).

The performance of the program, when operated by the same highly skilled operator as in the present study, was evaluated by decomposition of 18 synthetic signals presenting different degrees of difficulties. The program showed a high accuracy of classification (always above 96%, in 17 out of 18 cases above 99%) and robustness to handling of irregular firing statistics, superimposed action potentials and shape changing of the same MU as well as MU shape similarity between different MU (Farina et al., 2001).

The program is highly interactive and provides excellent visual guidance for manual classification as well as automatically suggested best choice guided by high cross correlation and lowest normalized error power.

The results of the automatic decomposition algorithm were only accepted after detailed manual checking and editing, with calculated parameters as useful guidelines. Results are presented as inter firing interval (IFI) bar plots giving median firing rate for the time period presented (see **Figure 2**, lower part).

Instantaneous firing rate was calculated as the inverse of each IFI. Continuous activity was defined as MUAPT with no IFI longer than three times the median IFI of the train. Mean firing rate (MFR) was calculated as mean of IFR and presented as pulses per second and SD. IFIs shorter than 20 ms were defined as doublets, these were registered but the inter spike interval in the doublet was disregarded in the calculation of MFR, thus counting the doublet as one firing.

A more robust description of the shape of IFR modulation during a DC for each MUAPT was evaluated by spike triggered averaging the IFR across the 10 DCs using the trigger signal for alignment of 1 s pre and 1 s post the time of first click in each DC registered by the computer mouse button. For the spike triggered average all MU with continuous firing rate within the 2 s time interval around DC were included.

Finally, as a measure of the common drive and using the spike triggered averaging of the IFR over the 10 DC, the normalized cross correlation coefficients (CCC) between the averaged IFR profiles for MUs in each recording were calculated (De Luca et al., 1982, 2009). Before cross correlation of the averaged IFR for MU pairs the DC component of the MFR were removed using a digital zero-phase band pass filter from 0.75 to 12 Hz (De Luca et al., 2009). Signals were time shifted from one s back in time to one s forward in time to find the peak CCC and the time lag for the peak CCC.

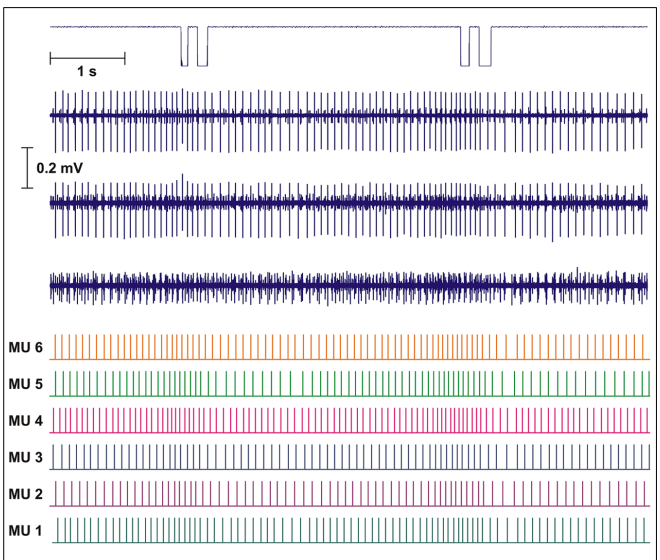

FIGURE 2 | Recording and analysis of two double DC. Upper part shows mouse key trigger signal and the three intramuscular bipolar recordings of the raw electromyographic signals (EMG). The lower part shows the result of the decomposition presented as the inter firing bar plot for each of the identified motor unit (MUs). Based on the distance of the bars the instantaneous firing rate (IFR) for each MU was calculated.

STATISTICS

Differences in surface EMG were tested with a two way repeated measures ANOVA for interaction between left/right trapezius and contralateral/ipsilateral DC. The MU firing pattern associations and temporal relations to DC were determined as "no," "weak," and "strong" subjectively, based on visual examination of individual IFR profiles, rather than assessed quantitatively. CCC among averaged IFR profiles for MUs from recording sites with modulating versus constant firing rates were too few for a proper statistical comparison and are presented only as graphs and descriptive data.

RESULTS

SURFACE EMG

During the 10 DCs surface EMG revealed activity above resting level for all 12 subjects in both right and left shoulder. During right hand DC the activity in right and left trapezius was a mean of 2.4 (1.6) %MVE and 1.8 (1.0) %MVE, respectively. During left hand DC the activity in right and left trapezius was a mean 2.0 (1.1) %MVE and 2.5 (1.1) %MVE, respectively.

The two way ANOVA showed no significant interaction between ipsi/contralateral DC and left/right trapezius ($P = 0{,}134$) or any main effect for ipsi/contralateral DC ($P = 0{,}989$) or left/right Trap ($P = 0{,}726$).

INTRAMUSCULAR EMG

An overview of the results for all subjects showing single MU recordings during right and left hand DC is shown in **Table 1**. The brief low level contraction of the trapezius just before and after the recording showed, that the tip of the wire was in a position, where potentially active MUs were in the vicinity of the tip of the intramuscular wire for all subjects. During the right and left hand DC task eight and four subjects, respectively, showed single MU activity. The three subjects (ID 5, 7, and 11) who did not show single MU activity during either right or left hand DC, showed a similar %MVE recorded from surface EMG as the subjects showing single MU activity.

While only 18 and 10 MU showed continuous firing for the whole recording (**Table 1**), 28 and 14 MUs fulfilled the criteria of continuous firing for the 2 s period surrounding the ipsilateral and contralateral DCs, respectively, and were used for spike triggered averaging IFR and for calculating CCC (**Table 2**). Peak CCC was a mean of 0.76 with a range between subjects from 0.40 to 0.93; while the mean time lag was zero with a range from −39 to 30 ms (See **Table 2**).

IPSILATERAL DC

During the right index finger DC, eight subjects showed MU activity in the right trapezius muscle, and all had one or more continuously active MUs. As a total 32 MUs were identified and 18 MUs showed continuous activity with MFR ranging from 7.4 to 13.4 pps. The number of identified firings in the MUAPT ranged from 169 to 529 discharges.

Four subjects showed continuous activity with only a weak or no association between the small fluctuation in IFR and the timing of DC For an example see **Figure 3**, showing the IFR for the two continuously active MU identified in ID 9. In general MFR for these MUs was low, and no pronounced changes in firing rate were present during the DC. For each subject a general common trend in IFR modulation was seen for all the identified MUs resulting in CCCs among MU averaged IFR for these subjects ranging from 0.40 to 0.81.

For the remaining four subjects a common pattern of IFR with large modulation in firing rate with a clear temporal relation to the DC was present. The lowest level of IFR was found right after the DC with a gradual increase in firing rate for all identified MUs until the next DC. As an example, see **Figure 4A** showing the IFR for the active MUs identified in subject 12 and the temporal relation to the DC shown in the top. The CCC among MU averaged IFR for the subjects showing IFR modulation ranged from 0.64 to 0.96.

In three subjects double discharges were identified. In one of the subjects the modulation in IFR was accentuated by the recruitment of a MU almost only being active with doublets (shown in the bottom of **Figure 4A**). All 16 doublets were coincidental with the peaks in IFR of the concurrently active MU. Another two subjects showed a few occasional doublets, which however, did not seem to be timely related to the DC.

For all eight subjects showing MU activity during DC, the result of the spike triggered averaging of the IFR for all the identified continuously active MUs during the 10 right hand DCs is presented in **Figure 5A**. For four subjects (left side of **Figure 5A**) a more or less pronounced anticipatory increase in IFR can be seen, Subjects 3 showed a special DC related modulation with rather a decrease in IFR, while the remaining three subjects (right side in **Figure 5A**)

Table 1 | Overview of identified MU.

Subject ID	MU (N)	Continuously active MU throughout recording (N)	Range of MFR (pps)	Range of SD in MFR	Range of number of firings (N)
Ipsilateral double click (DC)					
1	5	4	10.4–11.7	1.5–1.8	256–285
3	3	1	11.1	1.7	432
4*	8	2	9.6–12.2	2.2–2.8	278–346
6	2	2	8.6–9.8	0.9–1.1	169–170
8*	5	4	11.6–13.1	2.4–3.2	210–236
9	3	2	7.4–8.1	1.0–1.2	191–207
10	2	2	8.0–8.6	1.0–1.5	344–381
12*	4	1	13.4	2.3	529
Contralateral DC					
2*	1	0	–	–	–
4	2	0	–	–	–
8	5	5	13.3–16.1	1.9–3.0	311–376
12*	7	5	10.1–13.0	1.6–2.3	468–603

The asterisk indicates recordings including double discharges.

Table 2 | Overview of CCC during DC for continuously active MU.

	Continuously active ±1 s around DC (N)	Mean peak CCC (Range)	Time lag (Range) (s)
Ipsilateral DC			
ID 1	4	0.70 (0.64–0.77)	0.004 (–0.009–0.010)
ID 3	3	0.77 (0.73–0.81)	0.006 (0.005–0.007)
ID 4	7	0.78 (0.65–0.89)	0.007 (–0.023–0.042)
ID 6	2	0.73	–0.039
ID 8	5	0.90 (0.86–0.96)	0.001 (–0.006–0.005)
ID 9	2	0.40	0.030
ID 10	2	0.78	0.010
ID 12	3	0.91 (0.84–0.96)	0.008 (–0.005–0.016)
Contralateral DC			
ID 2	1	–	–
ID 4	1	–	–
ID 8	5	0.68 (0.71–0.76)	0.001 (–0.004–0.007)
ID 12	7	0.93 (0.89–0.97)	0.008 (–0.018–0.032)

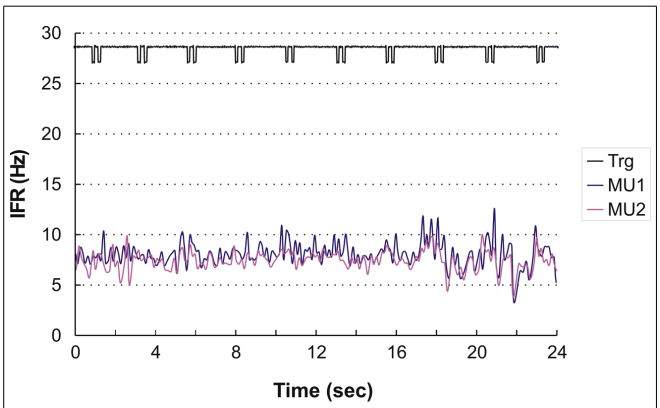

FIGURE 3 | Instantaneous firing rate plot showing the firing pattern of the identified MU in subject 9 during right hand double clicking. The solid line at the top shows the signal from the trigger incorporated in the left mouse key, giving the time of activation of the key.

showed a very modest or no anticipatory increase in IFR in relation to the timing of double clicking. Further, the lack of modulation in IFR for these three subjects also was associated with a low firing rate staying below 10 pps. In **Figure 5B** the cross correlation function of the averaged IFR for all MU pairs in each recording is shown. The shared common synaptic input is clearly visible as a common modulation of the averaged IFR of all identified MUs from each recording site as well as evidenced by the high peak CCC shown below. Mean time shift for peak CCC among subjects was 3 ms and ranged from −39 to 30 ms (See **Table 2**; **Figure 5B**).

CONTRALATERAL DC

For the left index finger DC task contralateral activity was found in the right trapezius muscle for 4 of the 12 subjects but only two subjects showed continuously active MUAPTs. In total 15 MUs were identified and 10 of these showed continuous activity with MFR from 10.1 to 16.1 pps. The number of identified firings in the MUAPT ranged from 311 to 603 (see **Table 1**). Fourteen MUs were continuously active in the 2 s period surrounding the DC and were included in the averaged IFR used for the calculation of CCC presented in **Table 2**. Peak CCC was as a mean 0.90 and ranged from 0.82 to 0.99 with a mean time lag of 5 ms ranging from 18 to 39 ms for the two subjects.

A temporal relation of the IFR and DC was seen for two of the subjects, while the two other showed a weak or no association. Subject 12 who showed peak related doublets for ipsilateral DC also showed nine peak related doublets during the contralateral DC (see **Figure 4B**). In this recording the anticipatory activity can clearly be seen in the end of the recording, where the subject obviously is preparing for the DC number 11 before being stopped by the experimenter.

Interestingly, one of the subjects who presented with complete silence during right index finger movements had one active MU during left index finger DC with a rather distinct firing pattern. The MU activity pattern is presented in **Figure 6** and shows initial repetitive doublet firings according to the criteria of Bawa and Calancie (1983). The doublets showed no relation to the timing of the finger movement, and after 29 doublets firing the MU transitioned from repetitive doublet firing to single spike firing pattern with an abrupt rise in firing rate compared to the firing rate of the doublets disregarding the inter-doublet spike interval. During the remaining recording the MU occasionally reverted to short doublet series, interrupting the single-spike firing.

DISCUSSION

While there was no difference in the overall activity recorded as surface EMG, three different responses on a single MU level were seen in the trapezius muscle during DC: no activity, activity weakly

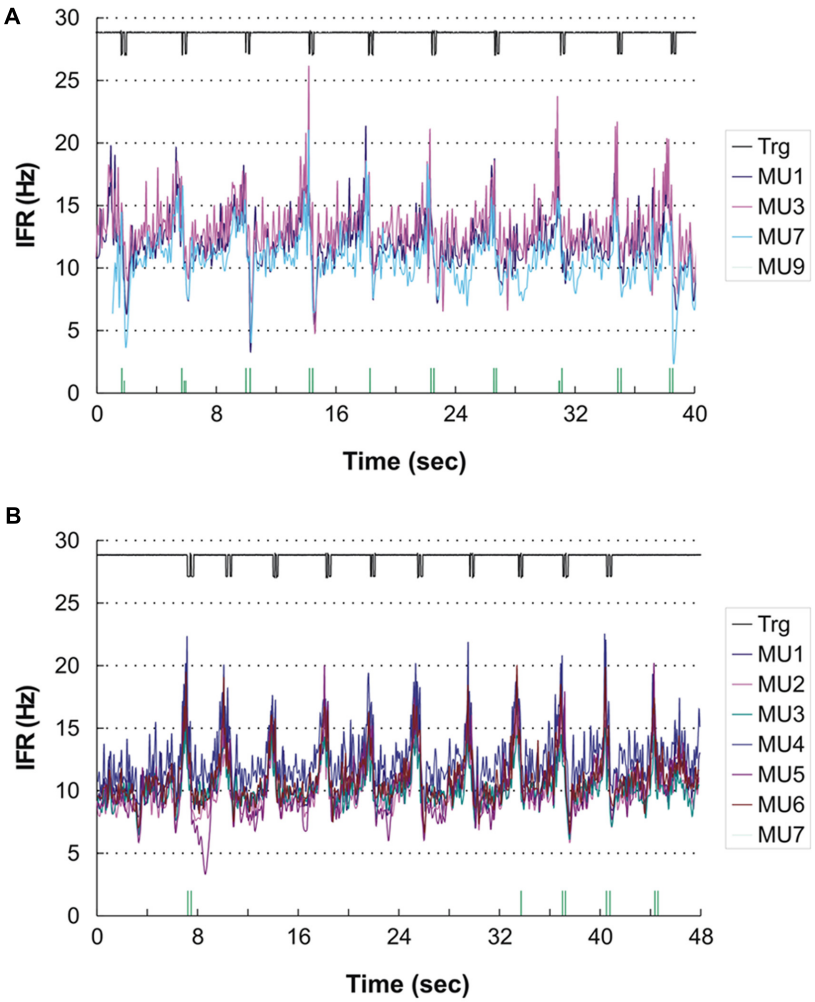

FIGURE 4 | (A,B) Instantaneous firing rate plot showing the firing pattern of the identified MUs in subject 12 during right hand **(A)** and left hand **(B)** double click (DC). As MUs cannot be traced across recordings the MU numbering is arbitrary. The solid line at the top shows the signal from the trigger incorporated in the mouse key, giving the time of activation of the key. The vertical bars in the bottom of **(A)** and **(B)** present the inter firing bar plot for MU 9 and MU 7, respectively, as their intermittent firing pattern is not suited for an IFR presentation. MU doublets are illustrated by high bars.

related and activity strongly related to the mechanical task of fast finger movements. Interestingly, in each recording all the averaged IFR of the identified MUs followed the same pattern with pairs of MUs showing high peak CCC indicating a shared synaptic common drive.

A clear connection between the mechanical performance of the index finger and the MU firing rate modulation in the ipsilateral trapezius was seen in four of the subjects. This supports the idea of the mechanically related multi-joint motor program activating the trapezius muscle anticipatory to the planned movement of the index finger. However, for two of the subjects the same pattern was seen for contralateral index finger movements with no obvious biomechanical reason as has also been reported for the extensor digitorum communis muscle during finger movements (Søgaard et al., 2001). This may be a pre-programmed bilateral overflow phenomenon of an anticipatory motor program aiming at the provision of a stable shoulder girdle in order to perform precise finger manipulations. In the present study we do not have simultaneous intramuscular recordings from both shoulders to reveal whether an increase in activity before each DC is equally pronounced in both shoulders. If this is the case, it may be evoked by a generally increased alertness before each DC performance. Further, in the present study no simultaneous recordings were performed to reveal the temporal relation in activation for the agonistic extensor digitorum communis actually lifting the index finger and trapezius to indicate a multi joint activation program.

A MU firing pattern only weakly related to the actual mechanical task to be performed was seen in four subjects, which may be interpreted as attention related background activity independent of the finger activity. Interestingly, four subjects apparently were able to perform right hand DC without any identified MU activity although low threshold MU were active during the standard contractions before and after the DC task.

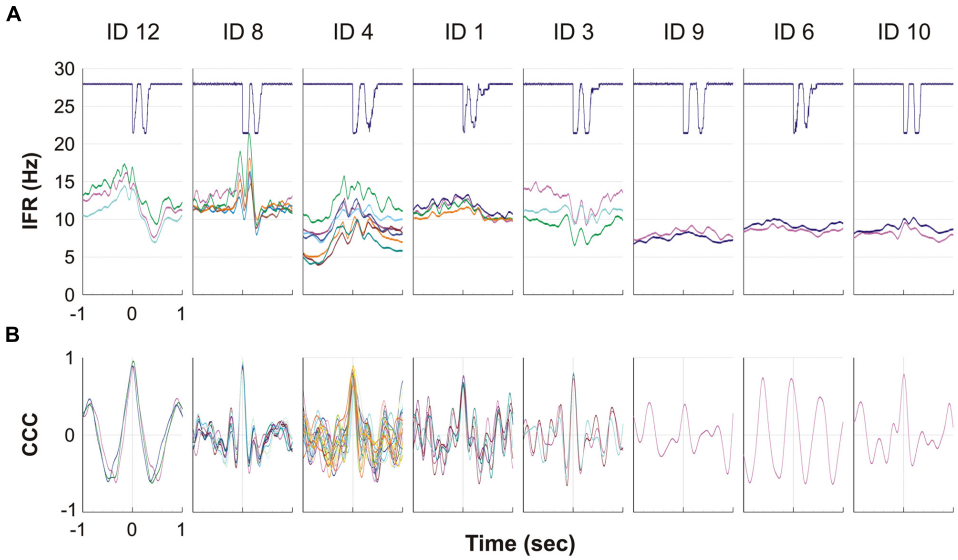

FIGURE 5 | (A,B) The eight graphs, one for each of the eight subjects with MUs with continuous activity, show the averaged firing rate for each MU during the ipsilateral DC. The solid line at the top shows the signal from the trigger, giving the time of activation of the mouse key, which is used to align DC before averaging the firing rate. Each of the lines below represents a MU and shows the IFR averaged for the 10 DC. **(B)** For each of the eight subjects the cross correlation function is given for the averaged IFR of pairs of all MU presented in **(A)**. Please note that the time axis is here giving the 1 s time shift back and forth for the cross correlation function.

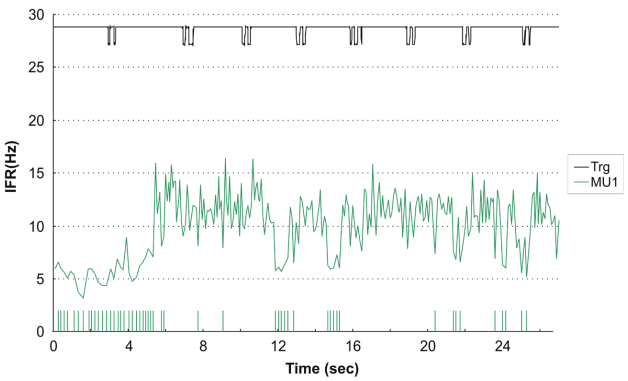

FIGURE 6 | Instantaneous firing rate plot showing the firing pattern of one identified MU in subject 2 during left hand double clicking. The solid line at the top shows the signal from the trigger incorporated in the mouse key, giving the time of activation of the key. After 29 doublets the MU transitioned from repetitive doublet firing to single spike firing pattern. IFR is for the whole MU action potential trains (MUAPT) calculated disregarding the inter-doublet spike interval. The vertical high bars in the bottom indicate the occurrence of doublets. Note, that the doublet firing rate is markly lower than the single spike firing rate and this is evident at all remissions to doublet firings in the recording.

Since the highly selective electrode only records activity within a small muscle volume in the vicinity of the recording area, the wire electrode may just happen to be in a silent subpart of the muscle for this task. A visual inspection as well as the CCC shows a clear dependence between activity patterns in MUs from the same recording site. As each recording site is in a specific subject, results are here given on a subject level, keeping in mind that differences may just represent the difference in activity patterns between trapezius muscle subparts. Indeed, earlier studies on single MU activity in the trapezius muscle have indicated that such a spatial inhomogeneity is likely to exist with a task specific selective activation (Stephenson and Maluf, 2010). Of note is that all subjects had similar level of surface EMG representing the summarized activity of active MUs within the muscle volume below the recording surface electrodes. This strongly indicates that each intramuscular recording may rather be considered as an activity pattern in a specific recording area of the muscle than as a difference between subjects.

Both the attention related MU activity in the trapezius muscle with constant low MFR as well as the activity in MUs with large modulation of IFR in clear relation to the mechanical performance of the index finger could be avoided from a biomechanical point of view as the provided support of the elbow should minimize the need for shoulder joint stabilization. While the MUs with no modulation in firing rate generally are activated at a low firing rate level, the modulating MUs generally are activated at higher MFR with peak levels. Both activity patterns in the perspective of prevention of myalgia represent activation patterns that are not caused by a biomechanical demand but evoked or maintained just by the planning and performance of the movement of the index finger. Such activity may metabolically compromise intracellular homeostasis and eventually impair Ca^{2+} transients resulting in elevated cytosolic $[Ca^{2+}]$ (Westerblad et al., 1991; Gissel, 2000). This process may promote breakdown of the muscle membrane and a leaky cell membrane may cause muscle metabolites to stimulate the nociceptive free nerve endings in the interstitium (for more details see Sjøgaard and Søgaard, 1998).

The present study was not designed to specifically evoke doublets, but doublets were identified for 4 subjects out of 12. One subject showed doublets in the right trapezius muscle related to the fast movements of both the ipsi- and contralateral index finger, in two other subjects MUs occasionally showed doublets unrelated to DC and one subject during contralateral DC showed repetitive doublets. This indicates that doublets are not only linked to demand of high contraction velocity to lift the finger as earlier reported for the finger extensors (Sjøgaard et al., 2001; Søgaard et al., 2001) but also can be evoked by the need of fast stabilization of the shoulder joints and that this may be an integrated part of normal motor control for some subparts of the motor neuron pool.

Stimulation studies have shown, that a doublet can potentiate the twitch force and in good agreement, this activation strategy in voluntary contractions is mainly found when high rate of force development and/or shortening velocity are required (Kossev and Christova, 1998; Thomas et al., 1999; Cheng et al., 2013). In addition to the large initial rate of force development, also a much larger force is maintained during the following excitations, a phenomenon termed catch like properties (Sandercock and Heckman, 1997). This implies that a doublet may impose a higher tension during the subsequent activity than indicated by the actual firing rate of the MU.

Since the earliest description of doublets by Denslow (1948) the origin has been debated and so has the distinction between the occasionally evoked doublets and the repetitive doublets (Bawa et al., 2000; Kudina and Andreeva, 2010). However, as recently stated doublets are rarely recorded during voluntary muscle activity in human subjects (Piotrkiewicz and Kuraszkiewicz, 2014). It is therefore interesting that in the present study simulating free living conditions with no feedback to the subject, a MU presented a firing pattern with repetitive doublets transitioning from stable doublets to stable single spikes firing. Such a firing behavior was earlier described by Bawa and Calancie (1983) and later provoked with MU feedback by Kudina and Andreeva (2010). Repetitive doublets followed by a jump in firing rate could be considered as the bistable firing behavior characterizing a plateau potential. Human plateau potentials have been suggested as an underlying cause of the sustained low level activation of some MU (Kiehn and Eken, 1997; Gorassini et al., 1998). Interestingly, two other studies have reported similar observations of plateau potential like behavior in trapezius MU, one of them actually during almost similar conditions of finger movements (Westad et al., 2004; Stephenson and Maluf, 2010).

One of the strengths of the present study is the use of an averaged IFR profile summarizing detailed information on MUAPTs from 10 repetitions of the studied finger task in the characterization. To our knowledge, this approach to a robust description of the IFR modulation during a certain task has not earlier been conducted in other studies. Further, the task is a functional unrestricted task performed with no feedback on the discharge rate provided for the subject. Therefore, the recorded MU activity for each subject may to a high degree reflect the motor activation pattern used in daily life. A limitation is the use of highly selective wire electrodes providing information on activity in only a small localized part of the upper trapezius muscle that may not be representative for the whole subpart of the Trapezius muscle in each of the subjects. Further, it should be acknowledged that the study is based on a limited number of MUs only allowing an exploratory approach.

CONCLUSION

Several rather distinct MU IFR patterns were identified in the right trapezius muscle during DC with ipsi- and contralateral index finger, in spite of similar low level of surface EMG activity in all subjects. In 75% of the subjects, an attention related IFR activity pattern and/or index finger movement related activity pattern for ipsi- or contralateral DC were seen. In 25% of the subjects doublets were seen, for one subject temporarily related to peak activity in concurrently active MU during both ipsi- and contralateral DC and for the other two subjects as occasional events. The continuous activity of some MUs, the contralateral central drive overflow, and the doublet phenomenon possibly causing an increase in mechanical MU loading may contribute to an explanation of computer work related development of fatigue and myalgia in the trapezius muscle.

AUTHOR CONTRIBUTIONS

All authors substantially contributed to the conception and design of the work and in the interpretation of data. All authors participated in the data acquisition led by Anne K. Blangsted as well as in the analysis led by Henrik B. Olsen. Karen Søgaard and Henrik B. Olsen drafted the work and all authors revised it critically for important intellectual content. All authors finally approved the version to be published.

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Could motor unit control strategies be partially preserved after stroke?

S. Jayne Garland[1]*, Courtney L. Pollock[2] and Tanya D. Ivanova[1]

[1] *Department of Physical Therapy, University of British Columbia, Vancouver, BC, Canada*
[2] *Graduate Program in Rehabilitation Sciences, Department of Physical Therapy, University of British Columbia, Vancouver, BC, Canada*
**Correspondence: jayne.garland@ubc.ca*

Edited by:
Annie Schmied, National Center for Scientific Research, France

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Andrew Joseph Fuglevand, University of Arizona, USA
Inge Zijdewind, University Medical Center, Netherlands

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INTRODUCTION

There is considerable evidence on the impairments that a cerebral stroke will have down-stream of the stroke, i.e., in the spinal motoneuron and the muscle. Motor impairment following stroke has been documented as force production that is slow, weak, and lacking in precision (Garland et al., 2009) and is associated with difficulty in fully activating the muscle (Klein et al., 2013). Furthermore, in functional tasks such as standing balance and gait, there is evidence of deficits in intra-limb coordination of muscles even on the non-paretic side (Marigold and Eng, 2006; Raja et al., 2012). In this opinion paper, we will first briefly review the changes observed at the level of the motor unit (MU) after stroke and second reflect upon whether some changes in the intrinsic properties of motoneurons, typically considered to be maladaptive, might also reflect a positive adaptation that could assist in force production. Lastly, this paper will explore the control of MUs between limbs during standing balance and suggest that, while some impairment may exist, there remains the possibility of a preservation of fundamental motor control strategies after stroke that might be a target for rehabilitation.

MOTOR UNIT/MUSCLE CHARACTERISTICS

At the level of the MU, studies have demonstrated a loss of spinal motoneurons following stroke (McComas et al., 1973; Hara et al., 2004; Lukacs, 2005; Li et al., 2011), particularly those that innervate type II MUs (Lukacs et al., 2008). It has been suggested that chronically paretic muscle is made up of fewer, but larger, MUs due to collateral sprouting of the remaining motoneurons to innervate a greater number of muscle fibers (Lukacs, 2005; Kallenberg and Hermens, 2011; Li et al., 2011) and this process could result in a mismatch of muscle fiber type and motoneuron characteristics (Young and Mayer, 1982; Dattola et al., 1993). Ultimately both of these changes may result in muscle contractions with slower rates of force development and decreased levels of force production (Garland et al., 2009).

In addition to the MU remodeling described above, MU discharge characteristics have been observed to change following stroke. For instance, MU firing rates in the paretic muscle are lower than the non-paretic muscle during ramp isometric contractions (Gemperline et al., 1995; Frontera et al., 1997; Chou et al., 2013; Mottram et al., 2014). It has also been demonstrated that the range of modulation of firing rates is compressed in paretic muscle, such that the increase in firing rate in response to increased force production is limited (Gemperline et al., 1995; Frontera et al., 1997) and the modest increases in firing rate saturate with higher forces (Mottram et al., 2014). Chou et al. (2013) observed that the decreased MU firing rate was associated with a decreased rate and magnitude of force production, suggesting that the limitation in firing rate offered a potential mechanism for the decreased speed of voluntary movement in people post-stroke.

Most of the MU research has been performed in chronic stroke survivors and hence changes would be expected at the level of the muscle when disuse could be a contributing factor. Gray et al. (2012) summarized that chronic stroke results in a decrease in muscle mass, decrease in fiber length, and a smaller pennation angle which would affect the paretic muscle's ability to generate force. A systematic review and meta-analysis performed by English et al. (2010) demonstrated that lean paretic muscle mass was significantly less than that of non-paretic muscle, in both upper and lower extremities, in people at least 6 months post-stroke. However, Klein et al. (2013) and Ramsay et al. (2011) showed that the tibialis anterior (TA) muscle was not atrophied in people after chronic stroke, despite the prevalence of foot-drop in gait (Kesar et al., 2010). Ramsay et al. (2011) also demonstrated that in the plantarflexor complex, the gastrocnemii muscles show significantly greater atrophy than the soleus muscle. Considering the differences in function and muscle morphology (TA and soleus have fewer fast twitch MU than gastrocnemii; Johnson et al., 1973) across the muscles, the impact of MU loss and remodeling following stroke may be muscle- or task-dependent and could explain differences across studies.

MOTONEURON INTRINSIC PROPERTIES

One way to examine the intrinsic properties of the motoneuron in humans is to use the Interval Death Rate (IDR) analysis, which estimates the time-course

of the motoneuron afterhyperpolarization (AHP) based on the discharge pattern of the MUs (Matthews, 1996). The duration of the AHP is important because it influences the discharge rate of the MU (Bakels and Kernell, 1993a,b; MacDonell et al., 2008). MU size and parameters of the AHP have been shown to be inversely related to each other in mammalian models; that is, the greater the amplitude of the AHP and the longer the AHP half decay time, the smaller the MU (Powers and Binder, 2001). The IDR model has been used in humans to show the relationship of the contractile properties of an intrinsic hand muscle to the AHP time-course (Gossen et al., 2003). This "speed-match" between the time course of the motoneuron AHP and the time course of its muscle unit twitch is thought to be functionally-relevant so that the minimal firing rate of the MU is matched to the twitch contraction properties (Gardiner and Kernell, 1990).

We performed the IDR analysis in TA of young healthy subjects and also in people after stroke. While the AHP time constant estimated on the non-paretic side (36.2 ± 6.4 ms, Ivanova et al., 2014) was consistent with that of young healthy individuals (32.9 ± 4.4 ms, MacDonell et al., 2008 and 33.6 ± 4.5 ms, Christie and Kamen, 2010), and older participants (37.3 ± 4.7 ms, Christie and Kamen, 2010), the AHP decay time-constant was significantly prolonged on the paretic side (41.7 ± 8.5 ms, Ivanova et al., 2014). Significant prolongation of the AHP time course after stroke in the upper extremity motoneurons was also reported by Liang et al. (2010) for the biceps brachii in participants with spasticity awaiting botox injection and by Suresh et al. (2014) for the first dorsal interosseous muscle. Our work revealed that the AHP time constants in TA were significantly longer in the low recovery group than in the high recovery group, indicating a relationship between the severity of motor impairment and AHP prolongation (Ivanova et al., 2014), albeit such a relationship was not found in a small hand muscle (Suresh et al., 2014). Nevertheless, Suresh et al. (2014) did suggest that the longer duration of the AHP contributed to the lower MU discharge rate in the paretic muscle after stroke. In the rat, prolongation in the AHP has been observed with chronic spinalization (Bennett et al., 2001) or tetrodotoxin-induced paralysis (Cormery et al., 2000), both being examples of motoneuron plasticity accompanying muscle disuse.

Given the above findings, it is clear that the motoneuron AHP is prolonged in the presence of chronic stroke and this may contribute to the limitations in MU firing rate and motor impairments observed after stroke. It is important to note however, that although there is *on average* a prolongation of AHP after stroke, there is also substantial overlap in AHP time-constants with that found in healthy subjects. What is not clear is whether the AHP prolongation might reflect positive adaptations of the motoneuron to maintain the "speed match." Deficits in velocity of movement are well-documented after stroke (Bohannon, 1987; Davies et al., 1996; Lum et al., 2004). Could the motoneuron be adapting to the remodeling at the level of the muscle? No studies have yet been performed to determine if the time-course of the AHP and the muscle unit remain matched after stroke. These experiments would need to be performed to determine if the adaptations of the motoneuron and firing rate represent a necessary compensation to the muscle changes.

COMMON MODULATION OF MOTOR UNIT DURING STANDING

While the previous section discussed the way in which MUs might control force within a muscle, the motor control of functional tasks like standing require the coordination of MU activity between the limbs. Earlier work from our laboratory explored the role of soleus muscles bilaterally in the postural control of standing by quantifying MU synchronization, common drive and coherence (Mochizuki et al., 2005, 2007). Although soleus MUs within a single muscle had modest levels of synchronization during quiet stance, the incidence of synchronization between the soleus muscles bilaterally was very low (Mochizuki et al., 2005). In contrast, Gibbs et al. (1995) showed evidence of synchronization in 7/10 healthy subjects between bilateral gastrocnemius muscles during a demanding balancing task, possibly revealing a task- or muscle-dependent response. The strength of common modulation of MU discharge was greater in unilateral than bilateral MU pairs, and greater during postural tasks than voluntary isometric tasks (Mochizuki et al., 2007). Altering proprioceptive input by vibrating one leg resulted in a significant reduction in common drive, suggesting that sensory input contributes to the common modulation of MU discharge during postural tasks (Mochizuki et al., 2007). Given the sensorimotor impairments after stroke, one would expect a significant disruption in the common modulation between the paretic and the non-paretic limb.

Whereas the soleus muscle demonstrates tonic EMG activation in standing, the EMG activity of the medial gastrocnemius muscle is more responsive to anterior displacement of the center of pressure (COP) (Di Giulio et al., 2009). We examined the common drive in people after stroke in the medial gastrocnemius muscle during an external perturbation task in standing. Eleven experiments in six participants in the chronic stage after stroke (time post-stroke, 6.8 ± 3.8 years) with mild to moderate levels of motor impairment (Chedoke-McMaster Stroke Assessment foot score, 4.3 ± 1.5/7) and 5 experiments in 5 healthy controls were conducted. A belt placed around the hips was attached to a horizontal cable in front of the subject. Loads were applied by dropping weights of 1% body mass into a basket on the end of the cable every 30 s until a maximum of 5% body mass was in the basket. This resulted in a gradual progression of the anterior COP (**Figure 1**). During the 25–30 s between load drops, MUs that were firing steadily were analyzed. Common drive analysis involved converting MU spike trains to continuous firing rate signals (De Luca et al., 1982). The signals were then high-pass filtered and cross-correlated over a moving window of 3–5 s epochs (Monsour et al., 2012) to produce a common drive coefficient (rho) that was averaged across epochs.

Two-Way ANOVA analysis (with Tukey *post-hocs*) revealed that common drive in MU pairs derived from bilateral muscles (**Figure 1**) was significantly lower in people after stroke (15 MU pairs, rho = 0.44 ± 0.13; mean ± SD) than controls (17 MU pairs, rho = 0.58 ± 0.06, $p < 0.01$), as well as for unilateral MU pairs in the paretic muscles after stroke (10 MU pairs,

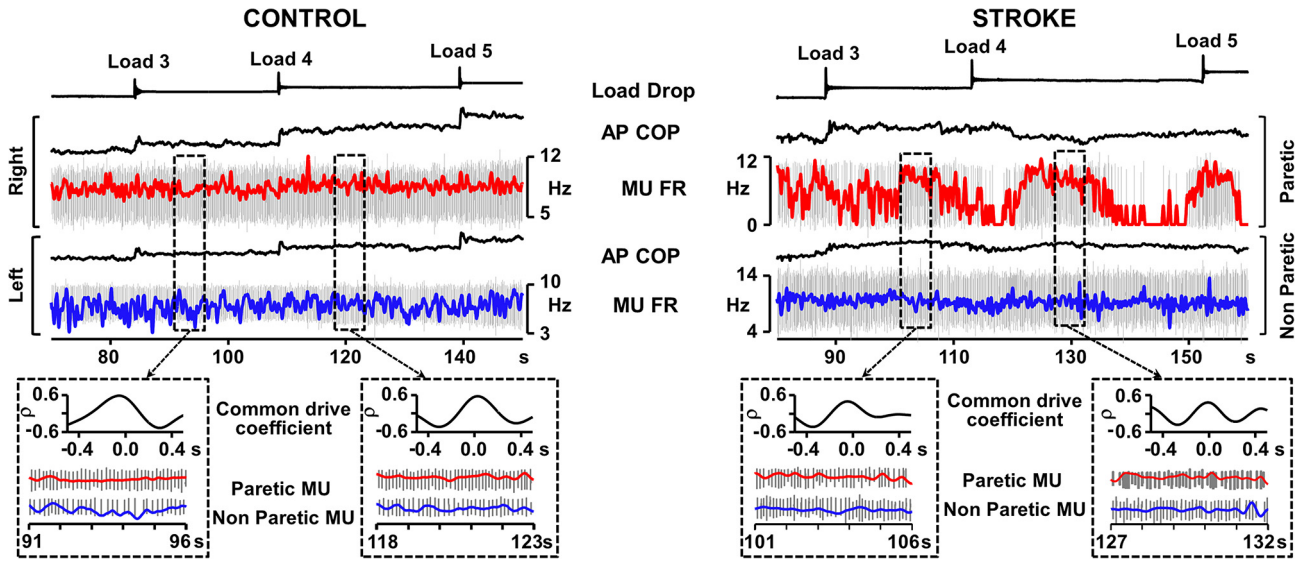

FIGURE 1 | Representative recordings from Control and Stroke participants demonstrating common modulation of firing rate. Traces from top; load drop, anterior-posterior center of pressure (AP COP), and smoothed motor unit (MU) firing rate superimposed on motor unit action potentials (red trace, right leg control, paretic leg), AP COP and MU firing rate (FR) superimposed on MU trains (blue trace, left leg controls, non-paretic leg). Dashed boxes outline examples of 5 s epochs where both motor units in the pair were firing steadily and the common drive coefficient was calculated. Cross-correlograms and expanded motor unit firing rate and action potential traces are presented for each of the outlined epochs. Despite the difficulty in maintaining consistent MU firing rate in the paretic leg, the epochs in which the MU was firing steadily rendered common drive coefficients that were similar to control.

rho = 0.67 ± 0.07) than controls (12 MU pairs, rho = 0.77 ± 0.06, $p < 0.01$). However, the level of common drive *within* the paretic medial gastrocnemius muscle remained relatively high and there was a moderate level of common drive *bilaterally* after stroke indicating that MUs in the paretic muscle still co-modulated with the non-paretic side. Given the range of common drive coefficients, there were MU pairs after stroke that exhibited the same amount of common modulation as that found in healthy persons (**Figure 1**). These findings suggest that MU control strategies such as common drive during postural tasks, while diminished, remain present after stroke.

CONCLUDING REMARKS

There is no doubt that there are changes in the MU discharge characteristics after stroke. But the AHP and common drive data suggest that residual motor control strategies may remain after stroke, albeit diminished, and may reveal a need to consider functional task-dependency in future research to explore MU impairment and adaptation post-stroke. It remains to be seen whether treatments that challenge the neuromuscular system could prevent the muscle remodeling and any compensatory MU control adaptations.

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Single motor unit firing rate after stroke is higher on the less-affected side during stable low-level voluntary contractions

Penelope A. McNulty[1,2]*, Gaven Lin[1] and Catherine G. Doust[1,2]

[1] *Neuroscience Research Australia, Sydney, NSW, Australia*
[2] *School of Medical Sciences, UNSW Australia, Sydney, NSW, Australia*

Edited by:
Parveen N. S. Bawa, Simon Fraser University, Canada

Reviewed by:
Carol Jean Mottram, Rehabilitation Institute of Chicago, USA
Parveen N. S. Bawa, Simon Fraser University, Canada

***Correspondence:**
Penelope A. McNulty, Neuroscience Research Australia, Barker Street, Randwick, Sydney, NSW 2031, Australia
e-mail: p.mcnulty@neura.edu.au

Muscle weakness is the most common outcome after stroke and a leading cause of adult-acquired motor disability. Single motor unit properties provide insight into the mechanisms of post-stroke motor impairment. Motor units on the more-affected side are reported to have lower peak firing rates, reduced discharge variability and a more compressed dynamic range than healthy subjects. The activity of 169 motor units was discriminated from surface electromyography in 28 stroke patients during sustained voluntary contractions 10% of maximal and compared to 110 units recorded in 16 healthy subjects. Motor units were recorded in three series: ankle dorsiflexion, wrist flexion and elbow flexion. Mean firing rates after stroke were significantly lower on the more-affected than the less-affected side ($p < 0.001$) with no differences between dominant and non-dominant sides for healthy subjects. When data were combined, firing rates on the less-affected side were significantly higher than those either on the more-affected side or healthy subjects ($p < 0.001$). Motor unit mean firing rate was higher in the upper-limb than the lower-limb ($p < 0.05$). The coefficient of variation of motor unit discharge rate was lower for motor units after stroke compared to controls for wrist flexion ($p < 0.05$) but not ankle dorsiflexion. However the dynamic range of motor units was compressed only for motor units on the more-affected side during wrist flexion. Our results show that the pathological change in motor unit firing rate occurs on the less-affected side after stroke and not the more-affected side as previously reported, and suggest that motor unit behavior recorded in a single muscle after stroke cannot be generalized to muscles acting on other joints even within the same limb. These data emphasize that the less-affected side does not provide a valid control for physiological studies on the more-affected side after stroke and that both sides should be compared to data from age- and sex-matched healthy subjects.

Keywords: hemiparesis, motor unit firing rate, motor unit firing variability, torque control, stroke

INTRODUCTION

Stroke is the leading cause of adult-acquired motor disability in developed countries (WHO, 2003). The most common outcome after stroke, and the most common cause of motor disability, is hemiparesis or weakness on the side of the body contralateral to the stroke lesion (e.g., Chang et al., 2013). Although the acute lesion is restricted to the brain, secondary adaptive and maladaptive changes may contribute to hemiparesis. There are four principal sites where such degeneration has the capacity to contribute to muscle weakness: (i) cerebral diaschisis (Feeney and Baron, 1986); (ii) reduced corticospinal tract integrity (Fries et al., 1993; Pineiro et al., 2000; Sterr et al., 2010; Stinear et al., 2012); (iii) changes to peripheral motor axon properties (Jankelowitz et al., 2007; Huynh et al., 2013); and (iv) anatomical and physiological changes within the muscle and its constituent single motor units. This study will consider single motor unit discharge behavior.

There are both anatomical and physiological changes within the muscles of the more-affected side after stroke. The anatomical changes may include disuse atrophy (Jørgensen and Jacobsen, 2001; Ryan et al., 2002; Hara et al., 2004; Arasaki et al., 2006; Li et al., 2011); altered muscle phenotype (Jakobsson et al., 1991; De Deyne et al., 2004; Lukács et al., 2008; McKenzie et al., 2009); and reinnervation (Dattola et al., 1993; Hara et al., 2004; Lukács, 2005). Physiological changes include altered motoneuron pool activation so that there is a reduction in the mean motor unit discharge rate and the variability of this discharge (Rosenfalck and Andreassen, 1980; Dietz et al., 1986; Gemperline et al., 1995; Chou et al., 2013); disrupted recruitment threshold (including lower recruitment thresholds, reversed recruitment thresholds so that fast motor units are recruited before slower motor units, and a reduced range over which recruitment occurs), reduced modulation of firing rates, and compression of the dynamic range of motor unit discharge rates (Rosenfalck and Andreassen, 1980;

Gemperline et al., 1995; Hu et al., 2012; Chou et al., 2013). Such changes contribute not only to hemiparesis, but also to reduced control of muscles on the more-affected side after stroke.

Single motor units are the smallest functional division of muscles. They represent the most distal component of the motor pathway and their discharge behavior reflects the intrinsic properties of both the motoneuron and the muscle fibers in addition to the net synaptic drive through this pathway. Recent data from our group recorded during post-stroke therapy demonstrated that the activity of isolated single motor units in severely paretic muscles precedes the development of compound muscle activity (i.e., multiple motor units recruited through voluntary commands), and that this progression is a hallmark of improved movement ability, even many years post-stroke (see McNulty et al., 2013; Thompson-Butel et al., 2013). To understand the process of recovery from isolated single motor unit activity to compound activity during dynamic movements it is simpler to begin with more controlled static tasks so that changes in the properties of single motor units, and the mechanisms controlling this behavior, can be investigated more systematically. The aim of this study was to examine the pattern of motor unit behavior during sustained static contractions.

The changes in motor unit discharge properties noted above have been measured over brief periods, usually from 5–20 s with a range of different tasks and levels of voluntary contraction. Each of these differences may be sufficient to alter the net synaptic drive to the motoneuron pool. For this reason, we extracted the action potentials of single motor units that were either spontaneously active or task-driven during a sustained isometric voluntary contraction at a functionally relevant duration and force intensity during ankle dorsiflexion, wrist flexion and elbow flexion. Motor units were recorded from both the more- and less-affected side after stroke and on both the dominant and non-dominant side in healthy subjects. Motor unit activity during contractions acting on three joints was studied because there are anatomical and functional differences in the control of muscles in the upper and lower limbs, and between proximal and distal muscles of the upper-limb. These differences include different innervation ratios (Buchthal and Schmalbruch, 1980), more numerous monosynaptic corticospinal (Palmer and Ashby, 1992) or bilateral (Colebatch et al., 1990) projections, and differences in mean firing rates (Petajan and Philip, 1969; de Luca, 1985). These differences are superimposed on functional recovery after stroke that is typically greater for the lower-limb than for the upper-limb although the reason for this is not clear. To ensure the results of this study do not simply reflect the differences listed here, data were collected during contractions at three joints. We compared differences in firing rates and the variability of the firing rate between sides and between the upper and lower limb. Data were recorded during elbow flexion from stroke subjects only to examine the effect of hand dominance on the control of motor unit behavior after stroke. Our results suggest that although motor units on the more-affected side have a reduced firing rate compared to the less-affected side as reported previously, the important difference is that the firing rate of motor units on the less-affected side after stroke is higher than both the more-affected side and motor units of healthy subjects.

MATERIALS AND METHODS

SUBJECTS

The activity of 169 single motor units in 28 stroke patients was recorded in the course of three studies of low-level isometric force control: series 1: ankle dorsiflexion; series 2: wrist flexion; and series 3: elbow flexion (**Figure 1**). Activity from 110 single motor units was recorded in 16 healthy subjects who participated in the ankle and wrist experiments. Stroke patients were hemiparetic after a unilateral stroke with muscle weakness in the test limb. Those participating in series 1 could walk >15 m unassisted and none used lower-limb splints, braces or orthoses. Patients in series 2 and 3 had voluntary movement ≥10° at the test joint. Control subjects were neurologically healthy at the time of testing and all participants were cognitively competent (assessed as a Mini-Mental State Examination score ≥24). Participants were excluded if they had uncorrected vision or hearing, unstable blood pressure, or co-morbidities other than stroke that significantly affected sensorimotor function. Five patients and two healthy subjects participated in both the lower-limb and wrist experiments (participant demographics are presented in **Table 1**). All participants gave written, informed consent and these studies were approved by the Human Research Ethics Committees of the University of New South Wales and St Vincent's Hospital, Sydney. Experiments were conducted in accordance with the Declaration of Helsinki.

EXPERIMENTAL PROCEDURE

All data were recorded bilaterally with the exception of ankle dorsiflexion torque which was recorded on the active side only. Single motor unit electromyography (EMG) potentials were recorded using transducers (single motor unit (SMU) electrodes) specifically designed for this purpose with two parallel recording surfaces 1 × 10 mm, fixed 10 mm apart (DE2.3, Delsys, USA). The SMU electrode position was optimized prior to data collection and was not repositioned. Additional surface EMG data were recorded using standard 10 mm Ag/AgCl electrodes positioned in a belly-tendon montage with ∼40 mm interelectrode distance (120 mm for triceps surae), hereafter referred to as EMG electrodes.

Series 1: ankle dorsiflexion

Participants sat with the knee in ∼120° extension and the foot securely strapped to the myograph with the ankle positioned at the mid-point of passive range-of-motion (**Figure 1**). Torque was recorded on the test side only. The SMU electrodes were positioned over tibialis anterior with additional EMG electrodes over tibialis anterior, peroneus longus and triceps surae muscles.

Series 2: wrist flexion

Participants sat with the forearm and hand securely strapped to the myograph in a semi-pronated position with the wrist flexed to 30° (**Figure 1**). The digits were unconstrained. The SMU electrode was positioned over flexor carpi radialis muscle with additional EMG electrodes over flexor and extensor carpi radialis muscles.

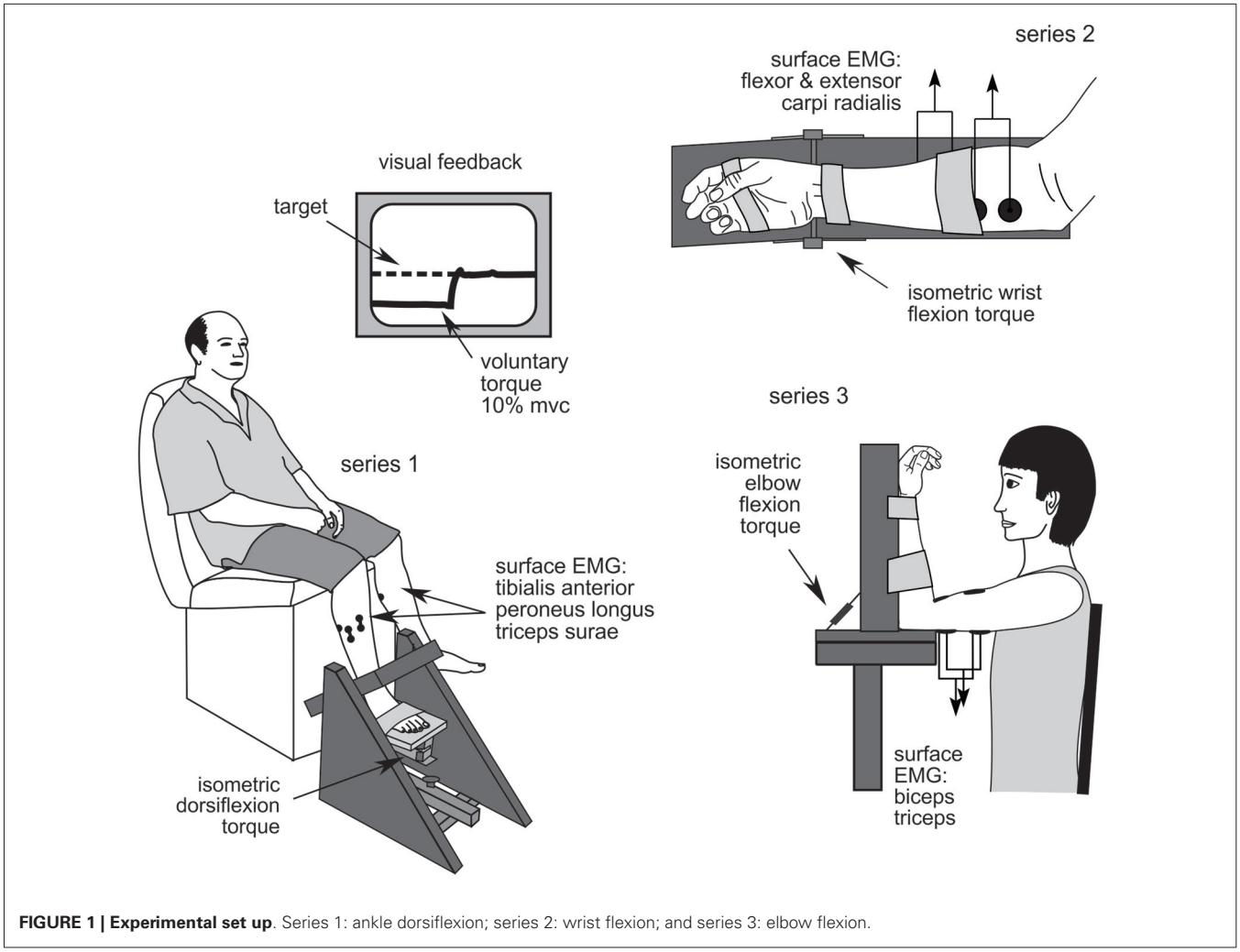

FIGURE 1 | Experimental set up. Series 1: ankle dorsiflexion; series 2: wrist flexion; and series 3: elbow flexion.

Table 1 | Participant demographics and summary of single motor unit recordings.

Series		1 Ankle dorsiflexion	2 Wrist flexion	3 Elbow flexion
Stroke patients	age (years; mean ± SD)	61.5 ± 11.6 (45–75)	65.3 ± 14.6 (42–83)	60.5 ± 16.4 (23–75)
	n (female, male)	10 (2, 8)	7 (2, 5)	11 (3, 8)
	time post-stroke (months)	59.0 ± 17.6 (7–168)	15.0 ± 4.8 (1–38)	31.4 ± 15.8 (3–150)
	walking speed ($m.s^{-1}$)	1.1 ± 0.2 (0.4–2.1)		
	Fugl-Meyer upper-limb motor subscale (score)		42.1 ± 5.2 (14–57)	55.2 ± 2.6 (40–65)
	SMU count (units per patient, mean)	80 (8.0)	54 (8.5)	38 (4.8)
	SMU more-affected, less-affected side	45, 35	31, 23	21, 17
	SMU task driven, spontaneous	25, 55	15, 39	6, 32
	SMU ipsilateral (active side), contralateral to active side	45, 35	29, 25	21, 17
Healthy subjects	age (years; mean ± SD)	60.9 ± 11.6 (45–72)	55.7 ± 14.7 (39–71)	
	n (female, male)	10 (2, 8)	6 (2, 4)	
	SMU count (units per subject, mean)	67 (6.7)	43 (7.2)	
	SMU dominant, non-dominant side	23, 44	8, 35	
	SMU task driven, spontaneous	23, 44	9, 34	
	SMU ipsilateral (active side), contralateral to active side	15, 52	17, 26	

Note that the maximum upper-limb motor Fugl-Meyer Assessment score is 66. There is no control subject matched to the oldest stroke patient for the wrist flexion series (83 years), if this patient is excluded the stroke patients in series 2 were aged 62.3 ± 13.5 years (range 42–74 years). Data are presented as mean ± standard error of the mean (SEM) unless indicated otherwise. Note that motor units in series 1 were recorded from 4 EMG channels, and from 3 EMG channels in series 2 and 3. SMU: single motor unit.

Series 3: elbow flexion

Participants sat with the elbow supported by the myograph so that the shoulder and elbow were flexed to 90° and the forearm supinated and securely restrained (**Figure 1**) or as close as possible to this position for patients with joint stiffness or spasticity. The hand was unconstrained. The SMU electrode was positioned over biceps brachii muscle with EMG electrodes over biceps and triceps brachii muscles.

Single motor unit data were amplified 100–1000 times, band-pass filtered 20–450 Hz (custom amplifier) and digitized at 5 kHz. All other EMG data were amplified 200–1000 times, band-pass filtered 10–1,000 Hz (1902, CED, UK; or IP511, Grass, USA) and sampled at 5 kHz. Torque data were recorded using either a 1 kN (series 1–2) or 2 kN (series 3) load cell (Applied Measurements, Australia) amplified 175–550 times, filtered DC-20 Hz (2044B, Applied Measurements, Australia) and sampled at 2 kHz. All data were digitized and recorded using a 1401 data acquisition card and Spike2 software (CED, UK).

PROTOCOL

Maximum voluntary contraction (MVC) torque was measured on each side as the peak torque recorded during three brief (2–3 s) efforts with strong verbal encouragement and visual feedback. Single motor unit activity was recorded during a voluntary contraction 10% of maximum. The target torque on each side was 10% of the maximum voluntary torque for that side so that a relatively constant proportion of the available motoneuron pool on each side was tested. The target torque was produced unilaterally with the contralateral limb at rest, although torque data were recorded bilaterally during wrist and elbow flexion. The target was displayed with the voluntary torque signal and projected to ensure clear visibility for all participants regardless of eyesight. The display gain was standardized so that the data occupied ∼30% of the screen. Participants were instructed to contract or "pull" until the voluntary torque matched the target and then to maintain this as steadily as possible for 6 min. At the conclusion of each trial participants fully relaxed before performing two brief MVCs to assess fatigue. Gentle verbal prompts were provided as necessary to ensure that voluntary torque matched the target as closely as possible, care was taken to avoid startle responses. Participants were unaware of the single motor unit recordings and were given no feedback regarding these data. After familiarization and practice, the study began on the less-affected side (or dominant side for healthy subjects) with 1–3 repetitions before three trials were recorded on the more-affected side (or non-dominant side for healthy subjects). This enabled participants to become familiar with the protocol on the better performing side. Trials were separated by a rest of 5–10 min to minimize the potential for fatigue. The functional ability of the patient cohorts was tested as 15 m walking speed for series 1 and with the upper-limb motor Fugl-Meyer Assessment for series 2–3.

DATA ANALYSIS

All EMG recordings were inspected for single motor unit action potentials which were discriminated based on spike amplitude and morphology using the template matching algorithms of Spike2 software (CED, UK) during either task-driven or spontaneous activity. The activity of single motor units was analyzed from periods of stable firing so that the initial increase in firing rate following recruitment was excluded, as was any slowing prior to derecruitment. The mode of activation was determined for each single motor unit, discriminating between activity that was task-driven or spontaneously active. The former was identified by recruitment that coincided with an experimental event, and the latter when no such trigger could be identified (**Figure 2**). The mean firing rate was calculated over the duration of each unit's stable discharge. Histograms were constructed for the mean interspike interval of motor unit discharge rates with a bin width of 10 Hz to enable comparison with previous reports. The dynamic range was defined as the frequency between the lowest and highest mean motor unit firing rate for a given side and series.

There were no significant differences in the firing rate or coefficient of variation of the mean firing rate for each unit between motor units on the dominant and non-dominant sides of healthy subjects when all series were combined and within each series. For this reason, and to account for the unbalanced sample between sides for the healthy subjects, these data were combined and hereafter referred to as control data. Differences in single motor unit discharge properties were investigated using a general linear model with *post hoc* Holm-Sidak pairwise comparisons. In separate analyses the dependent variable was either motor unit firing rate or the coefficient of variation of the firing rate, with factors of *series* (ankle dorsiflexion, wrist flexion) and *side* (stroke more-affected, stroke less-affected, control data). The same analyses were used to compare the firing rate and coefficient of variation of units that were task-driven or spontaneously active. The relationship between the functional ability of stroke patients, the time post-stroke and the number of motor units recorded was investigated using Pearson correlations when all series were combined and for each series. Series 3 data were not included in these statistical analyses due to the absence of control data, but were subsequently analyzed to compare the more-affected side to the less-affected side using Mann Whitney rank sum tests with Bonferroni corrections for multiple comparisons. Unless indicated otherwise, data are presented as mean ± standard error of the mean (SEM). Differences were considered significant when $p < 0.05$.

RESULTS

The number of motor units recorded in each series and each side for both stroke patients and healthy subjects are summarized in **Table 1**. In each experiment either three or four EMG channels were recorded on each side. In 16 instances two motor units were discriminated from the same EMG channel and in two instances three motor units were discriminated using template matching. For the remaining recordings, only one motor unit was discriminated per recording. For stroke patients there was no relationship between the level of functional ability, time post-stroke and the number of motor units recorded either when all data were combined or within each series. Single motor unit recordings were obtained for all

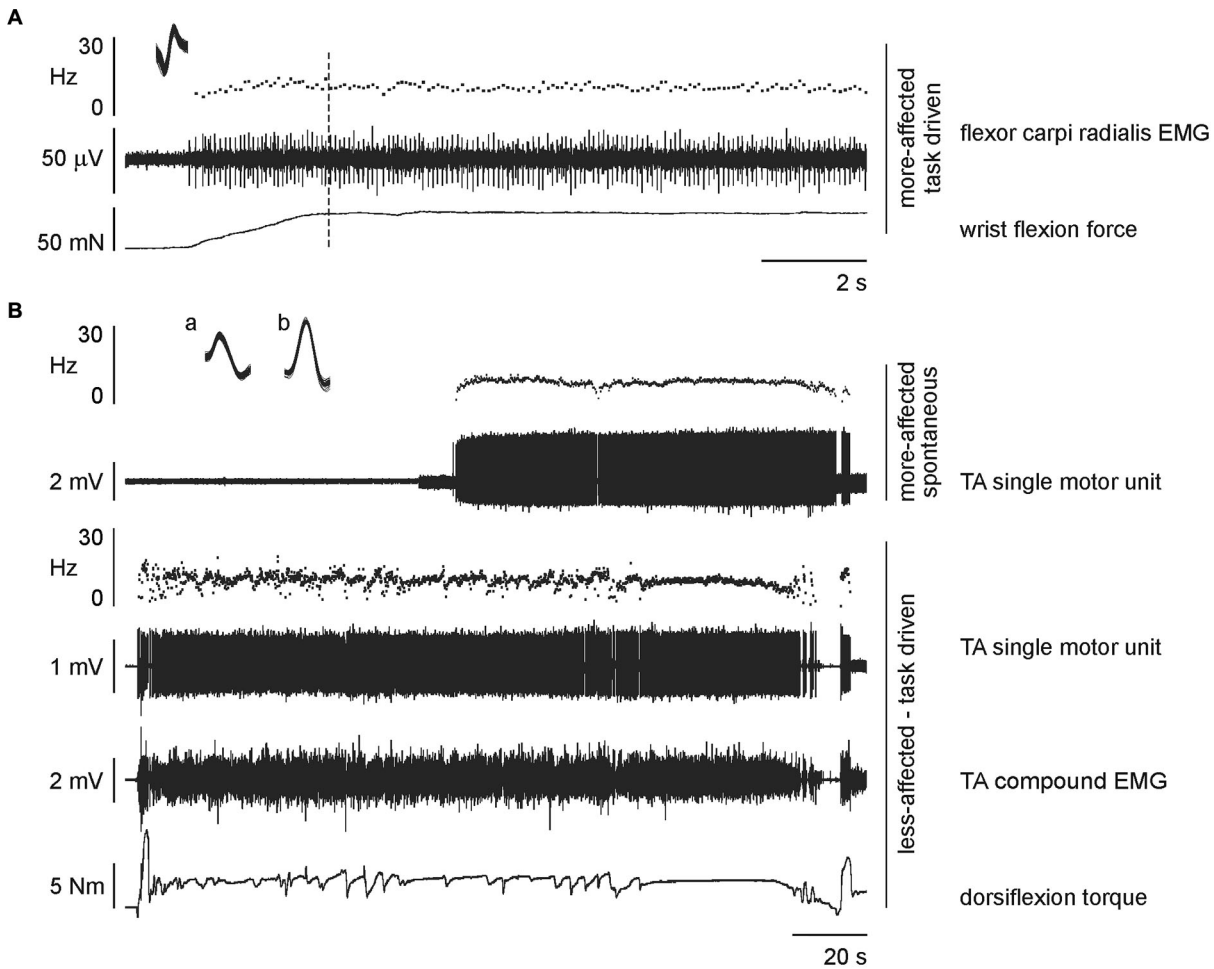

FIGURE 2 | Raw data from single stroke patients. (A) expanded time scale recording to show a task-driven motor unit recorded on the more-affected side during wrist flexion by a 74 year old male, 11 months post-stroke. The unit was recruited at the beginning of the task (and continued to fire throughout the task) but data were analyzed from the dotted line, i.e., after the acceleration in discharge rate associated with increasing force (discharge rate 9.44 ± 0.06 Hz, mean and standard error of the mean (SEM)). Inset: superimposed action potentials demonstrating a unitary recording. **(B)** concurrently recorded units during dorsiflexion of the less-affected tibialis anterior by a 72 year old male, 39 months post-stroke. The single motor unit on the active less-affected side is task-driven with activation relating to the total torque output (discharge rate 9.55 ± 0.05 Hz, mean and SEM; superimposed spikes marked a). The single motor unit on the passive more-affected side is spontaneously active and unrelated to the task on the contralateral leg (discharge rate 8.85 ± 0.03 Hz, mean and SEM; superimposed spikes marked b).

healthy subjects in each series and for all stroke patients in the ankle dorsiflexion series, 6 of the 7 patients during wrist flexion, and 8 of 11 patients during elbow flexion. An example of raw data showing simultaneously recorded task-driven and spontaneously-active single motor unit activity is shown in **Figure 2**. There were no differences in the mean firing rate or coefficient of variation for motor units that were task-driven or spontaneously active, either when all series were combined or within each series. Therefore the mode of motor unit activation was not considered further. The pattern of motor unit firing during each series and on each side is presented in histograms in **Figure 3**. The post-trial MVCs were not different to those performed at the beginning of the study suggesting that physiological fatigue did not influence motor unit discharge properties.

FIRING RATE DURING ANKLE DORSIFLEXION AND WRIST FLEXION

There was a significant difference between the firing rates for single motor units during wrist flexion and ankle dorsiflexion with firing rates higher for wrist flexion 11.85 ± 0.26 Hz than for ankle dorsiflexion 8.61 ± 0.21 Hz ($F_{(1,238)} = 93.298$, $p < 0.001$) (**Figures 3**, **4A**). Mean firing rates were highest on the less-affected side and lowest on the more-affected side of stroke patients with a significant effect of side ($F_{(2,238)} = 9.977$, $p < 0.001$). *Post hoc* pairwise comparisons revealed differences between the more- and less-affected side of stroke patients and between the less-affected side of stroke patients and control data (both $p < 0.001$), but not between the more-affected side of stroke patients and control data. Firing rates were higher on the less-affected side in each comparison. There was no interaction between side and series. The shift to higher firing rates (shorter interspike intervals) on the

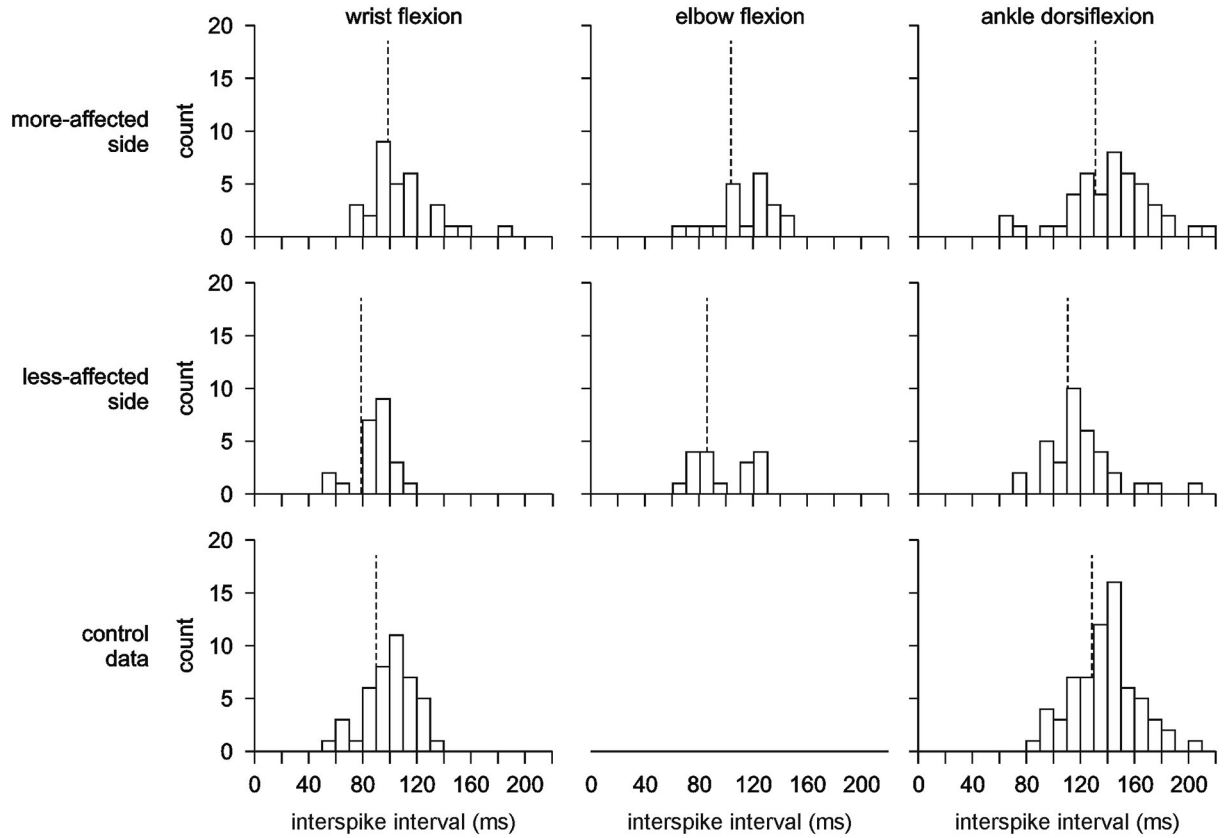

FIGURE 3 | Interspike interval histograms showing the mean discharge pattern for all motor units recorded. There is a clear shift to the left (higher motor unit firing rates) for motor units recorded on the less-affected side compared to the more-affected side and control data. The vertical dash line indicates the mean for each histogram. Note that no control data was recorded for elbow flexion.

less-affected side after stroke is clearly evident in the histograms in **Figure 3**.

FIRING RATE VARIABILITY (COEFFICIENT OF VARIATION) DURING ANKLE DORSIFLEXION AND WRIST FLEXION

An interaction was found between *side* and *series* for the coefficient of variation of single motor unit discharge ($F_{(2,238)}$ = 7.604, $p < 0.001$) (**Figure 4B**). There was a conditional effect of *series* ($F_{(1,238)}$ = 37.862, $p < 0.001$) with firing rate variability higher for combined wrist flexion data than for ankle dorsiflexion data. *Post hoc* pairwise comparisons for wrist flexion show that variability was lowest on the more-affected side compared to either the less-affected side ($p = 0.02$) or control data ($p < 0.001$), and that there were no differences between the less-affected side and control data. There were no differences in firing rate variability between sides during ankle dorsiflexion. For the conditional effect of *side* (both ankle dorsiflexion and wrist flexion series combined) ($F_{(2,238)}$ = 3.234, $p = 0.041$), *post hoc* comparisons confirmed that the firing rate variability was lower on the more-affected side compared to control data ($p = 0.01$) but that there were no differences between the more- and less-affected sides, or the less-affected side and controls.

MOTOR UNIT BEHAVIOR DURING ELBOW FLEXION

The firing rate of motor units during elbow flexion was higher on the less-affected side than the more-affected side ($p = 0.021$, **Figures 3**, **4A**) as was the coefficient of variation of the firing rate ($p = 0.024$). The values for both measures fell between that of motor units recorded during ankle dorsiflexion and wrist flexion. The incidence of 4.8 motor units per subject recorded during elbow flexion was lower than that during ankle dorsiflexion, wrist flexion, or for controls (**Table 1**). The mean firing rate of motor units recorded during elbow flexion was not different to that recorded during wrist flexion or ankle dorsiflexion (which were different, see above).

DYNAMIC RANGE OF MEAN MOTOR UNIT FIRING RATES FOR THE THREE SERIES

The dynamic range (the difference between the highest and lowest mean motor unit firing rate) during wrist flexion was lower on the more-affected side than the less-affected side and control data but the amplitude of the range was compressed only slightly, being 11.1, 13.7, 13.3 Hz, respectively (**Figure 4C**). For ankle dorsiflexion the dynamic range was not compressed on the more-affected side, being larger than both the less-affected side and control data. The magnitude of the range was 14.6 Hz for the

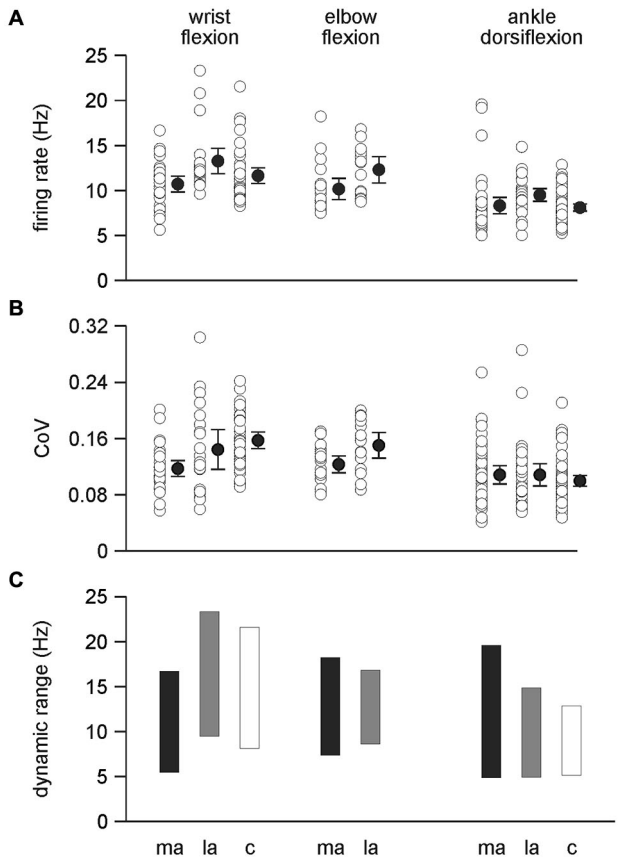

FIGURE 4 | Single motor unit discharge behavior. (A) Firing rates were significantly higher during wrist flexion than ankle dorsiflexion ($p < 0$ .001) and also higher on the less-affected side compared to both the more-affected side and control data ($p < 0.001$). There was no difference between the more-affected side and control data for wrist flexion and ankle dorsiflexion. Firing rates were higher on the less-affected side compared to the more-affected side during elbow flexion ($p = 0.021$). **(B)** The co-efficient of variation of motor unit discharge rates was higher for wrist flexion data than ankle dorsiflexion data ($p < 0.001$). During wrist flexion the co-efficient of variation was lowest on the more-affected side compared to the less-affected side ($p = 0.02$) and control data ($p < 0.001$). There was no difference between the less-affected side and control data during wrist flexion, or between sides during ankle dorsiflexion. The co-efficient of variation during elbow flexion was lower on the more-affected side compared to the less-affected side ($p = 0.024$). **A** and **B**: data are presented for each motor unit (open symbols); mean (filled symbols) and 95% confidence intervals. **(C)** the dynamic range of mean motor unit firing rates. The dynamic range was compressed on the more-affected side only for motor units recorded during wrist flexion. ma: more-affected side; la: less-affected side; c: control data (healthy subjects).

more-affected side, 9.8 Hz for the less-affected side, and 7.6 Hz for control data. The magnitude of the dynamic range for elbow flexion was larger on the more-affected side than on the less-affected side, being 10.7 and 8.1 Hz, respectively.

DISCUSSION

We investigated the discharge properties of single motor units after stroke using a consistent protocol of stable low-level isometric contractions at three joints in comparison to age- and sex-matched healthy subjects. Participants were unaware of the single motor unit recordings and were instructed to hold a voluntary contraction as steadily as possible at a functionally relevant intensity 10% of maximum for a functionally appropriate duration of 6 min. The most important finding of this study was that although motor units on the more-affected side discharged at lower firing rates than those on the less-affected side as reported previously, the significant difference was that motor units on the less-affected side had a higher firing rate than those on either the less-affected side or for control data, so that the firing rate on the more-affected side was no different to control data. Firing rate variability was depressed after stroke but this was only significant for motor units on the more-affected side during wrist flexion. Similarly, the dynamic range of stable motor unit firing was compressed only on the more-affected side during wrist flexion. In contrast the dynamic range was largest on the more-affected side during ankle dorsiflexion and elbow flexion. These data suggest that motor unit discharge properties from muscles acting across a single joint after stroke cannot be generalized to muscles acting across other joints, even within the same limb. More importantly, the less-affected side does not provide a valid control for motor unit activity on the more-affected side, control data must also be recorded in age- and sex-matched healthy subjects.

WHY IS SINGLE MOTOR UNIT FIRING RATE HIGHER ON THE LESS-AFFECTED SIDE AFTER STROKE?

The most parsimonious explanation for changes in motor unit properties after stroke is of reduced corticofugal output from the lesioned hemisphere (e.g., Cicinelli et al., 1997; Murase et al., 2004) or a reduction in the connectivity of this pathway (Stinear et al., 2007). This accords with the reduced variability of motor unit discharge in wrist muscles but does not explain increased firing rate on the less-affected side. Higher firing rates on the less-affected side may result from compensation for learned non-use of the more-affected side (Taub et al., 2006). However this explanation is unlikely because the mean MVC on the less-affected side, although higher than the more-affected side, was less than for control data. The influence of ipsilateral projections from the contralesional hemisphere is also unlikely to influence motor unit properties after stroke. In macaque monkeys this pathway was found to make little contribution to the recovery of motor function after experimental stroke (Zaaimi et al., 2012). We hypothesize that the higher firing rates on the less-affected side are due to increased excitability of the contralesional hemisphere as a consequence of reduced transcallosal inhibition from the lesioned hemisphere (Murase et al., 2004; Duque et al., 2005; Takeuchi et al., 2008; Nowak et al., 2009; Stinear et al., 2014). The resulting asymmetry in interhemispheric inhibition with cortical excitability decreased ipsilesionally and increased contralesionally, compounds the already diminished neuronal drive of the lesioned motor cortex (Murase et al., 2004), further reducing motor output and limiting functional movement and the potential for motor recovery (Duque et al., 2005; Bolognini et al., 2009; Calautti et al., 2010) on the more-affected side. It is clear however, that these changes cannot be attributed to changes in the peripheral motor axons. Changes in the biophysical properties of motor axons after stroke are subtle and because they are mostly associated with

activity-dependent hyperpolarization (Jankelowitz et al., 2007), should only become apparent with fatigue, which was not evident in the current study.

DIFFERENCES IN FIRING RATE VARIABILITY

The natural variabilty of motor unit discharge has been estimated as ≤40% (Gandevia et al., 1990) which may reflect a functional balance between potentiation and fatigue during sustained activity (McNulty and Macefield, 2005). In this study the variability of motor unit firing rates was not different between spontaneously-active and task-driven motor units and was lower on the more-affected side but this was only significant during wrist flexion. The origin of motor unit activity might explain some of the differences in discharge variability. One source of spontaneous motor unit activity is persistent inward currents which produced extremely low discharge variability when manifest in humans after spinal cord injury as spasm (Gorassini et al., 2004). Mottram et al. (2009, 2010) investigated whether this monoaminergic modulation of motor unit behavior might explain the origin of spontaneous motor unit discharges in spastic muscles after stroke and whether less variable discharges resulted (2010). They suggested that the discharge variability, which was ∼3 times higher after stroke than after spinal cord injury, was not explained by persistent inward currents. The level of variability in the current study was similar but ∼3% lower to that reported by Mottram et al. (2010) which in addition to our finding of no difference between task-driven and spontaneously active motor unit firing properties supports this suggestion. Mottram et al. (2010) proposed that motor unit discharge variability arises from tonic depolarizing synaptic drive with either a descending or segmental origin. Spontaneous activity was shown to be unrelated to spasticity, strength or force variability (Chang et al., 2013). Regardless of the mechanism, the reduced co-efficient of variation during wrist flexion presents a loss of fine motor control. This damped dynamic response may not provide the capacity for subtle responses to exogenous perturbations during functional tasks, but whether this is due to reduced neural drive or hyperexcitability at the monosynaptic Ia afferent-α-motoneuron synapse (as suggested by hyperreflexia) requires further study.

THE PATTERN OF ALTERED MOTOR UNIT BEHAVIOR

Despite different functional roles, the pattern of changes was consistent across the different series of this study although not all were statistically significant. The differences were greater in the upper limb and this may reflect the observation that the upper extremity recovers less after stroke than the lower limb and this has a larger impact on functional disability (Kalra et al., 1993; Feys et al., 1998). The changes in motor unit behavior in both the upper and lower limbs may result from altered physiology such as decreased central drive (e.g., Nielsen et al., 2008; Klein et al., 2013) but also from altered anatomy. Within muscles there may be a reduction in the number of functioning motor units (Hara et al., 2000, 2004; Arasaki et al., 2006; Lukács et al., 2008); and muscle fiber atrophy (Scelsi et al., 1984; Slager et al., 1985) associated with muscle disuse (Ramsay et al., 2011). Perhaps the least understood change in muscles after stroke, and one that will affect the behavior of individual motor units, is the pattern of denervation and reinnervation that is thought to underpin changes in muscle phenotype (for review see Hafer-Macko et al., 2008). The results of muscle biopsy after stroke are variable with both hypertrophy and increased numbers of type I muscle fibers demonstrated on the more-affected side (e.g., Edström, 1970; Dietz et al., 1986; Dattola et al., 1993; Lukács et al., 2008). Conversely, a shift from type I to type IIx muscle fibers has also been demonstrated (e.g., Frontera et al., 1997; De Deyne et al., 2004; McKenzie et al., 2009). Changes in motor unit twitch contraction times on the more-affected side (McComas et al., 1973; Young and Mayer, 1982; Dattola et al., 1993; Frontera et al., 1997) and motor unit twitch force (McComas et al., 1973; Young and Mayer, 1982; Lukács, 2005; Lukács et al., 2009) may reflect transsynaptic degeneration (McComas et al., 1973; Lukács, 2005; Lukács et al., 2009), collateral sprouting and re-innervation (Dattola et al., 1993; Kallenberg and Hermens, 2011) or a combination of these processes. The data from this study suggest that the pattern, if not the magnitude, of these changes is consistent across the more-affected side.

METHODOLOGICAL CONSIDERATIONS

Motor units on the more-affected side of stroke patients are reported to have lower peak firing rates, reduced discharge variability and a compressed firing range (Rosenfalck and Andreassen, 1980; Dietz et al., 1986; Jakobsson et al., 1992; Gemperline et al., 1995; Frontera et al., 1997; Suresh et al., 2008, 2012; Hu et al., 2012; Chou et al., 2013). Our results suggest the pattern is more complex and this may be due to methodological differences. We know of no other study to investigate the firing rate of single motor units on both sides of stroke patients in comparison to healthy subjects, except during fatigue (Hu et al., 2006). The more-affected side is most commonly compared to the less-affected side (Dietz et al., 1986; Gemperline et al., 1995; Frontera et al., 1997; Suresh et al., 2008, 2012; Hu et al., 2012; Chou et al., 2013), but occasionally the comparison is with data recorded in healthy subjects (Rosenfalck and Andreassen, 1980; Jakobsson et al., 1992). It is now beyond dispute that the side ipsilateral to the lesion is not unaffected or non-paretic, hence we use the term less-affected for this side (see Colebatch and Gandevia, 1989; Horstman et al., 2008). Our data suggest that it is only when motor unit activity on the more-affected side is compared to that of both the less-affected side and control data that the pattern of single motor unit behavior on the more-affected side after stroke can be fully understood. The motor unit discharge rates during wrist flexion were no higher than those during ankle dorsiflexion than would be expected for muscles of the upper and lower limbs (Petajan and Philip, 1969; de Luca, 1985).

The motor unit activity in this study was recorded during stable, low-level isometric voluntary contractions. The 10% MVC target was chosen to reflect the level of muscle activation typically required during everyday tasks (Thomas et al., 2005; Tikkanen et al., 2013). The target was set in proportion to the maximal output of the active side, rather than as the same absolute torque for both sides (Gemperline et al., 1995; Suresh et al., 2011). In this manner a relatively constant proportion of the available motoneurone pool was activated during all trials even when the MVC torque was asymmetric. Stable motor unit activity has

previously been studied for between 2 s (e.g., Hu et al., 2012) and a maximum of 20 s (e.g., Rosenfalck and Andreassen, 1980) generally as the hold component of a trapezoidal (e.g., Chou et al., 2013) or triangular (e.g., Gemperline et al., 1995) ramp. Again, the longer duration was chosen to reflect activities of daily living such as carrying items like shopping or a dinner tray. Patients reported that the stable hold required less concentration than contractions involving ramps and changing force (unpublished data) and the consistent amplitude of the MVC at the conclusion of each trial demonstrates that physiological fatigue did not affect motor unit behavior. Finally, the motor units in this study were all recorded using surface electrodes, rather than intramuscular needle or fine wire electrodes (Rosenfalck and Andreassen, 1980; Dietz et al., 1986; Jakobsson et al., 1992; Gemperline et al., 1995; Frontera et al., 1997; Chou et al., 2013). More recent studies have decomposed single motor unit action potentials from surface array electrodes with multiple recording surfaces (Suresh et al., 2008, 2012; Hu et al., 2012). We used surface electrodes optimized for single motor unit recordings and this minimized the need for spike sorting or signal decomposition. However, the use of surface electrodes produces a bias towards more superficial motor units which in turn contain a higher proportion of fast type motor units (Čebašek et al., 1996; Kernell, 1998; Knight and Kamen, 2005) and this bias may in part, explain some of the differences found in this study.

A large proportion of motor units recorded in this study were active on the contralateral "resting" side. Although we could not simultaneously record torque contralaterally during ankle dorsiflexion contractions, torque was recorded bilaterally during wrist and elbow flexion contractions to ensure the posture of the resting limb was the same on both sides and that there were no changes in passive tension. There was no discernible torque recorded that could be associated with the activity of motor units on the contralateral side. There are two possible explanations for the absence of recorded torque. First, although the force transducers used in this study have a linear response they are not designed to record the torque produced by single motor units. Second, the first recruited motor units of a voluntary contraction (McNulty and Cresswell, 2004) or a single active motor unit in an otherwise quiescent muscle (McNulty and Macefield, 2005) may not generate measureable force as their action is thought to increase internal muscle tension and stiffness so that the slack in the muscle-tendon unit is reduced, allowing any subsequent increase in tension to be transduced. The activation of contralateral motor units could reflect bilateral projections to skeletal muscles (Colebatch et al., 1990; Ridderikhoff et al., 2005) or contralateral irradiation (Zijdewind and Kernell, 2001) but presumably only for task-driven motor units. The majority of contralateral motor units were spontaneously active and as such their discharge is unlikely to be related to the descending motor command. The origin of this activity remains uncertain.

LIMITATIONS

The SMU electrodes used in this study were designed for an amplifier using a low-pass filter of 450 Hz, half that commonly used for surface EMG recordings. However this did not affect either the identification or the discrimination of single motor unit potentials in this study for several reasons. First, the power of the EMG signal lies below 100 Hz; second, because the majority of units were spontaneously active, their action potentials were not superimposed on compound EMG; and finally, there was no difference in the ability to discriminate units recorded using these electrodes and those recorded using standard surface electrodes and a 1,000 Hz low-pass filter.

In this study the behavior of motor units recorded during elbow flexion fell between that recorded during ankle dorsiflexion and wrist flexion. These data were recorded in stroke subjects only (there being no effect of side in healthy subjects at the wrist) to exclude the potential confounding effect of handedness, particularly if the more-affected hand had been dominant pre-stroke. The effect of handedness should be more pronounced in distal compared to proximal muscles of the upper-limb. The absence of control data during elbow flexion is a limitation of the study. Evidence for the effect of hand dominance or lesion laterality on recovery after stroke is not definitive. A dominant more-affected hand resulted in less impairment than a non-dominant more-affected hand, although this difference was only apparent in functional assessments and not in the performance of activities of daily living (Harris and Eng, 2006). A strong hand preference in monkeys, regardless of side, was associated with less recovery than more bilateral hand use and this was independent of lesion volume (Darling et al., 2013). Handedness influenced the extent of less-affected hand use but not that of the more-affected hand in stroke survivors (Rinehart et al., 2009), and there was no effect of handedness for Constraint-induced Movement Therapy in which the less-affected hand is restrained (Langan and van Donkelaar, 2008). That motor unit recordings from biceps brachii followed the pattern of the wrist flexion suggests that our results during wrist flexion are not affected by handedness. The difference in the properties of motor units of muscles acting on the wrist and elbow can be explained by a lower innervation ratio (Buchthal and Schmalbruch, 1980), more numerous monosynaptic corticospinal projections (Palmer and Ashby, 1992), and fewer bilateral projections in distal muscles such as those controlling the wrist compared to more proximal muscles flexing the elbow (Colebatch et al., 1990).

CONCLUSIONS

This study examined the discharge properties of single motor units during contractions acting on joints in the upper and lower limbs of both sides after stroke and in healthy age- and sex-matched controls. The pattern of changes, but not the magnitude, was consistent across the more-affected side after stroke. Although motor units on the more-affected side had lower firing rates compared to the less-affected side as reported previously, this difference does not reveal the true pattern of altered motor unit behavior after stroke. The most significant finding of this study was that motor unit firing rates on the more-affected side were not different to control data. Rather the pathological change in motor unit firing rate occurred on the less-affected side, where mean rates were significantly higher than either the more-affected side or control data. Motor unit discharge variability was lower, but this was only significant for wrist muscles on the more-affected side. We hypothesize both changes reflect the asymmetric

interhemispheric inhibition known to develop after stroke. Most importantly, this study demonstrates that in order to understand physiological changes after stroke it is necessary to compare data from the more-affected side, the less-affected side *and* healthy age- and sex-matched subjects.

AUTHOR CONTRIBUTIONS

Penelope A. McNulty Conceived and designed the experiments, supervised data collection and analysis, wrote and revised the manuscript, approved the final version.

Gaven G. Lin Performed data acquisition and analysis, contributed to manuscript drafting and revision, approved the final version.

Catherine G. Doust Performed data collection and analysis, contributed to manuscript drafting and revision, approved the final version.

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Motor unit firing pattern, synchrony and coherence in a deafferented patient

Annie Schmied[1]*, Robert Forget[2] and Jean-Pierre Vedel[1]

[1] *National Center for Scientific Research (Centre National de la Recherche Scientifique), Plasticité et Pathophysiologie du Mouvement, Institut de Neuroscience de la Timone, University Aix Marseilles, Marseille, France*

[2] *Faculté de Médecine, Ecole de Réadaptation, Centre de Recherche Interdisciplinaire en Réadaptation du Montréal Métropolitain, Institut de Réadaptation Gingras-Lindsay de Montréal, Université de Montréal, Montréal, QC, Canada*

Edited by:
Rachael D. Seidler, University of Michigan, USA

Reviewed by:
David M. Halliday, York University, UK
Kemal Sitki Turker, Koc University School of Medicine, Turkey

****Correspondence:***
Annie Schmied, Plasticité et Pathophysiologie des Réseaux Moteurs Rythmiques (P3M), Institut de Neurosciences de la Timone, Campus Santé Timone, 27 Boulevard Jean Moulin, 13385 Marseilles Cedex 5, France
e-mail: schmied.annie@univ-amu.fr

The firing of spinal motoneurons (MNs) is controlled continuously by inputs from muscle, joint and skin receptors. Besides altering MN synaptic drive, the removal of these inputs is liable to alter the synaptic noise and, thus, the variability of their tonic activity. Sensory afferents, which are a major source of common and/or synchronized inputs shared by several MNs, may also contribute to the coupling in the time and frequency domains (synchrony and coherence, respectively) observed when cross-correlation and coherence analyses are applied to the discharges of MN pairs. Surprisingly, no consistent changes in firing frequency, nor in synchrony and coherence were reported to affect the activity of 3 pairs of motor units (MUs) tested in a case of sensory polyradiculoneuropathy (SPRNP), leading to an irreversible loss of large diameter sensory afferents (Farmer et al., 1993). Such a limited sample, however, precludes a definite conclusion about the actual impact that a chronic loss of muscle and cutaneous afferents may have on the firing properties of human MUs. To address this issue, the firing pattern of 92 MU pairs was analyzed at low contraction force in a case of SPRNP leading similarly to a permanent loss of proprioceptive inputs. Compared with 8 control subjects, MNs in this patient tended to discharge with slightly shorter inter-spike intervals but with greater variability. Synchronous firing tended to occur more frequently with a tighter coupling in the patient. There was no consistent change in coherence in the 15–30 Hz frequency range attributed to the MN corticospinal drive, but a greater coherence was observed below 5 Hz and between 30 and 60 Hz in the patient. The possible origins of the greater irregularity in MN tonic discharges, the tighter coupling of the synchronous firing and the changes in coherence observed in the absence of proprioceptive inputs are discussed.

Keywords: motor unit, firing rate, firing variability, synchronization, coherence, proprioception

INTRODUCTION

Whether engaged in postural control or movement, the activity of spinal and supraspinal motor neurons is under the continuous control of sensory feedback provided by muscle, joint and skin receptors. In humans, stimulation of muscular and cutaneous large diameter afferents produces short-latency excitatory and/or inhibitory responses of MNs (Buller et al., 1980; Garnett and Stephens, 1980; Chalmers and Bawa, 1997; Marchand-Pauvert et al., 2000). In monkeys and humans, the effectiveness of the coupling between MNs and peripheral afferents is demonstrated by the consistent post-spike changes in EMG activity obtained through spike-triggered averaging (Flament et al., 1992; Kakuda et al., 1998; McNulty et al., 1999; McNulty and Macefield, 2001; Fallon et al., 2005; Baker et al., 2006).

Very little is known concerning the alterations that may affect MN firing pattern when cutaneous and proprioceptive feedback is lacking. During transient removal of peripheral feedback from their target muscle, MNs have been found to discharge at lower frequencies than in normal conditions (Fukushima et al., 1976; Gandevia et al., 1990; Macefield et al., 1993), in keeping with a net facilitatory contribution of peripheral afferents to the MN synaptic drive. A decrease in the net excitatory drive of proprioceptive origin may, however, be compensated for by an increase in the MN synaptic drive of cortical origin, which may eventually result in higher firing rates (Garland and Miles, 1997a). Besides suppressing part of the MN net excitatory synaptic drive, the removal of afferent feedback is liable to alter synaptic noise and, hence, the variability in MN tonic activity (Calvin and Stevens, 1968; Matthews, 1996). During transient removal of peripheral feedback from their target hand muscle, human motor axons recorded during maximal contraction were found to discharge more regularly than MUs tested under normal conditions (Gandevia et al., 1990). At submaximal contraction levels, however, a marked increase in discharge variability was reported to occur (Fukushima et al., 1976; Gandevia et al., 1990; Garland and Miles, 1997a).

As a major source of common and/or synchronized inputs shared by MNs, sensory afferents may also contribute to the

coupling of the discharges of MN pairs in the time and frequency domains (synchrony and coherence, respectively). The temporal coupling observed within a few ms (short-term synchronization) or tens of ms (broad-peak synchronization) by cross-correlating MN discharges (Sears and Stagg, 1976; Kirkwood et al., 1982; Datta and Stephens, 1990; Schmied et al., 1993), and the common frequency content assessed through coherence analyses (Rosenberg et al., 1989; Farmer et al., 1993; Halliday, 2000) are interpreted as reflecting the activity of common inputs and/or inputs synchronized at the pre-motoneuronal level. Short-term or broad-peak synchronization can arise when inputs fire either stochastically or rhythmically, whereas coherence within specific frequency bands is a result of rhythmically firing inputs (Rosenberg et al., 1989; Kirkwood and Sears, 1991; Baker et al., 1999; Halliday, 2000). During steady contraction, coherence between the discharges of single MUs is often prominent between 15 and 35 Hz in arm and leg muscles (Davey et al., 1993; Farmer et al., 1993; Mills and Schubert, 1995; Salenius et al., 1997; Kim et al., 2001; Kilner et al., 2002; Semmler et al., 2004). This frequency range is similar to the beta-range oscillatory coupling observed between the electroencephalographic, or magnetoencephalographic activity of the sensorimotor cortex, and the electromyographic (EMG) activity (corticomuscular coherence) in humans (Conway et al., 1995; Brown et al., 1999; Mima and Hallett, 1999; Brown, 2000; Marsden et al., 2000; Grosse et al., 2002; Salenius and Hari, 2003). Oscillatory coupling in the beta-range can similarly be found between the EMG activities of coactivated muscles (intermuscular coherence) during static isometric contraction (Kilner et al., 1999; Grosse et al., 2002).

Clinical and experimental evidence converges in support of a central origin for the synchronous activity and coherence observed between voluntarily activated MUs, with a major contribution of corticospinal pathways (Adams et al., 1989; Powers et al., 1989; Davey et al., 1990; Datta et al., 1991; Farmer et al., 1993; Schmied et al., 1993, 1999, 2000). Indeed, with the exception of one study showing that ischemic deafferentation could reduce single MU coherence between 6 and 10 Hz (Christakos et al., 2006), transient alteration of peripheral feedback was not found to change the amount of synchrony or coherence observed between tonically firing MNs in decerebrate cats (Prather et al., 2002) or in humans (Garland and Miles, 1997b).

Data reported above were obtained during transient alteration of somesthetic inputs. Documentation concerning the adaptation of MU firing patterns and EMG activity after an irreversible loss of proprioceptive feedback is limited. In a study performed in a patient (IW) with a quasi-total loss of large diameter sensory afferents, 3 MU pairs were reported to fire with no consistent changes in frequency, synchrony or coherence compared to normal subjects (Farmer et al., 1993). Such a limited sample precluded, however, a detailed assessment of the changes which might have affected the firing pattern and the oscillatory and/or non-oscillatory synchronous activity of MUs in this patient compared to healthy subjects. In another patient (GL) affected similarly by a quasi-total loss of large diameter sensory afferents, no major change was reported to affect corticomuscular coherence in the beta-range (Patino et al., 2008), whereas, in the same patient, inter-muscular coherence in the beta-range was found to be lacking during steady contraction of finger muscles (Kilner et al., 2004). Taken together, these data suggest that during isometric contraction, group I and II sensory afferents are necessary for the synchronization of the EMG activity of synergistic MN pools (Kilner et al., 2004), but not for the coupling of motor cortex and MN activity in the beta frequency range (Patino et al., 2008), nor for the synchrony and beta range coherence observed between MU discharges within a MN pool (Farmer et al., 1993). Although IW and GL both showed a quasi-total loss of large diameter afferent fibers due to SPRNP, there were, nonetheless, some differences in the extent of deafferentation, which went from the feet up to the neck in the first case, and up to the nose in the second (Cole and Paillard, 1995), and the persistence of movement-evoked potentials in self-paced movement in the first, but not in the second case (Cole et al., 1995; Kristeva et al., 2006).

The present study aimed at investigating the influence of muscle and cutaneous afferents on the frequency and variability of single MU tonic activity, and on the coupling of the discharges of MU pairs in the time and frequency domains in the patient GL in whom corticomuscular and intermuscular coherence had previously been investigated (Kilner et al., 2004; Patino et al., 2008). To this aim, inter-spike interval, cross-correlation and coherence analyses were applied to the activity of 92 MU pairs in the wrist extensor muscles tested during low-force handgrip. Data were compared with those obtained for 171 MU pairs tested under the same conditions in 8 healthy subjects. Part of the data has been preliminarily published elsewhere (Schmied et al., 1995).

MATERIALS AND METHODS

The patient GL (female) suffering from a major sensory polyradiculoneuropathy was tested at the ages of 47 and 61, in 2 sets of recordings (a, b) including 5 and 2 sessions, respectively. Data were compared with those obtained in a single session with 8 healthy female subjects of similar ages (42–63) with no signs of neurologic impairments. Experiments were conducted with the approval of the Ethics Committee of the local Medical University (CCPPRB-Marseilles I, approval No 92/74), and the informed consent of the patient and control subjects to the experimental procedure.

CLINICAL DESCRIPTION

The patient GL followed at the Centre Hospitalier de l'Université de Montréal, Pavillon Hôtel Dieu (Canada), had been suffering from a permanent and specific loss of the large peripheral myelinated sensory fibers for 15 and 29 years at the time of the first and second testing, respectively. At age 28, she first developed Guillain-Barré syndrome with motor and sensory symptoms from which she completely recovered. Then, at 32, she had another episode of polyradiculoneuropathy that affected strictly her peripheral sensory nervous system, but with no recovery. History and disease characteristics were extensively described in Forget (1986). In short, this resulted in a loss of light and crude touch, vibration perception, kinaesthesia, and position sense in her four limbs, trunk, neck and face below the nose. All tendon reflexes were absent. She can feel strong pressure, as well as pain and temperature. No sensory nerve action potentials were

observed from antidromic or orthodromic stimulation of the superficial radial, median and ulnar nerves in either hand, or from the left and right sural and superficial peroneal nerves. No sensory evoked potentials could be detected in the cortical sensorimotor areas. These observations have been confirmed and proven stable for the past 30 years. A sural nerve biopsy revealed a complete loss of A-β myelinated fibers larger than 9 μm (Cooke et al., 1985; Forget and Lamarre, 1995). The motor pathways were not affected and motor nerve conduction velocities and needle EMG investigation of the muscles of the arm were normal without any clinical evidence of weakness. Although confined to a wheelchair, the patient is able to do most of her daily manual work at home and, after years of training, has completely recovered fine movements such as handwriting, but only under visual guidance.

EXPERIMENTAL PARADIGM

The patient and control subjects were all right-handed. Experiments were performed in an adjustable armchair with the right forearm held in a cushioned groove. The distal end of the forearm was immobilized in a U-shaped device maintaining the hand in a semi-prone position, with the wrist flexed at 10°. In the rest position, subjects had their fingers passively flexed around a fixed cylinder (diameter: 4 cm; length: 10 cm) placed vertically against the palm of the hand. During the recordings, subjects closed their hand around the manipulandum and maintained the position and pressure of their fingers around it as steady as possible for the tonic discharges of 2 low-threshold MUs to be recorded for 2–3 min. The MU recordings were monitored on oscilloscopes and computer screens. On-line discrimination was performed by means of dual window discriminator units (Bak electronics) to provide the subjects with visual and auditory feedback for the 2 MU discharges.

Wrist extensor and flexor EMG activity was recorded using pairs of non-polarizable single-use surface electrodes (Ag-AgCl, 16 mm^2, Alpine Biomed) placed 2 cm apart. Single MU discharges were simultaneously recorded in the extensor carpi radialis muscles by means of two tungsten microelectrodes (impedance 12 M?, tested at 1 kHz, Frederick Haer and Co., USA) inserted transcutaneously (1–2 cm apart), and moved in tiny steps until a stable recording was obtained. EMG and MU activities were amplified and filtered (band-pass at 30 Hz-1 kHz and 300–3000 Hz, respectively). EMG and MU signals were digitized (sampling rates of 5 and 30 KHz, respectively) and stored on a computer using an acquisition device (1401-plus) driven by Spike 2–5 software (Cambridge Electronic Design, Cambridge, UK).

Root mean square (RMS) values for wrist extensor and flexor EMG activity were computed across each of the recording periods. At the end of the experiment, microelectrodes were removed and subjects were asked to produce 3 bouts of maximal isometric contraction of the wrist extensor and flexor muscles, under strong verbal encouragements. The highest level of EMG activity assessed in these bouts was subsequently used to normalize EMG activity in percentages of maximal voluntary muscle contraction (% MVC). Single MU action potentials were re-discriminated offline and analyzed using Spike 2–5 software. The firing behavior of each MU was plotted on an instantaneous frequency curve, as illustrated in **Figures 1A,B** (MU1, MU2, bottom traces). The presence of abnormally low or high instantaneous frequency values was carefully monitored to ensure that no spike had been missed or erroneously included in the discrimination process.

MU firing patterns were characterized on the basis of the inter-spike interval (ISI) mean duration (ISI_{mean}), excluding those longer than 300 ms (about 3–4 times the mean), resulting from pauses in MU tonic activity. The discharge variability was evaluated on the basis of the ISI standard deviation (ISI_{SD}) and ISI coefficient of variation (ISI_{CV}) across each recording ($ISI_{CV} = 100 * ISI_{SD}/ISI_{mean}$). The firing pattern of a given MU pair was thereafter described in terms of ISI_{mean} and ISI_{CV} geometric means ($ISIgeo = (ISI_{mean1} * ISI_{mean2})^{-2}$; $CVgeo = (ISI_{CV\,1} * ISI_{CV\,2})^{-2}$).

ANALYSIS OF MU SYNCHRONOUS ACTIVITY

Synchronous activity in MU pairs was analyzed by cross-correlating the 2 spike trains, as shown in **Figures 1C,D**. The cross-correlograms yielded the distribution of impulses produced by one MU in 1 ms bins, 100 ms before and after the trigger impulses produced by the second MU, chosen as that with the lower firing rate. Synchronous impulses formed a central peak in the cross-correlograms. The peak duration (black bar around 0, **Figures 1C,D**) was delimited by means of the cumulative sum (CUSUM) method (Ellaway, 1978) in reference to a baseline extending from −100 to −20 ms in the cross-correlogram. In the absence of clear-cut changes in the CUSUM bin count around time 0, the strength of synchronization was arbitrarily calculated over a 20-ms window centered on 0. The synchronization strength was evaluated in terms of synchronous impulse probability (SIP) and synchronous impulse frequency (SIF). The SIP is given by the peak count above the baseline mean divided by the number of trigger spikes (i.e., imp./trig.), whereas the SIF is given by the peak count above the baseline mean divided by the duration of the recording (i.e., imp./s). The statistical significance of changes in the peak region as compared to the baseline was evaluated at $p < 0.05$ on the basis of the Z score value ($z = 1.96$) of the peak count (Garnett et al., 1976).

ANALYSIS OF MU COHERENT ACTIVITY

Coherence analysis provides an estimate of the frequency content and the strength of the coupling between 2 spike trains (Rosenberg et al., 1989). Coherence estimates (C) were calculated using the freeware toolbox (NeuroSpec 2.0, GNU GPL) developed in MatLab (MatWorks, Natick, MA, USA) by D. M. Halliday (University of York, York, UK). For each MU pair, analysis was performed on a number L of non-overlapping spike train segments with the following parameters:

- samp_rate = 1000 (spike train sampling rate in Hz).
- sec_tot = recording duration in s.
- seg_pwr = 10 (frequency resolution = 0.98 Hz).
- $T = 1.024$ s (segment length).
- L = sec_tot/T.
- opt_str = w4 (full cosine taper applied to each segment, 50% tapering at each end).
- To take into account the possible influence of the firing rates and the recording duration on synchrony and/or coherence

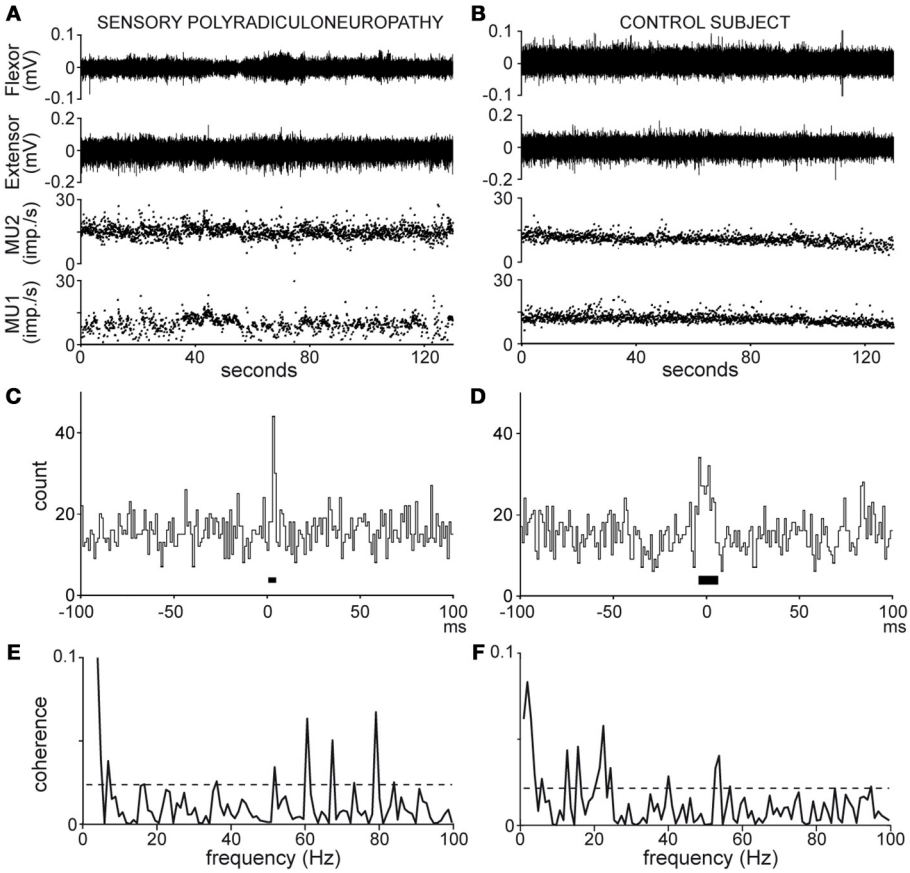

FIGURE 1 | Recordings (A,B) and analyses (C–F) of MU pair discharges in the patient (A,C,E) and healthy subject n°8 (B,D,F); (A,B), wrist flexor and extensor EMG activity (top traces), MU 1 and 2 instantaneous frequency (bottom traces); (C,D), cross-correlograms [synchronization peak width (black bar) 4 and 12 ms, synchronous impulse probability 0.04 and 0.08]; (E,F), coherence spectra (dotted line, significance limit).

(Bokil et al., 2007; Maris et al., 2007; Schmied and Descarreaux, 2010; Negro and Farina, 2012), 2 subsets of 45 MU pairs were selected on the basis of the similarity in their range of firing rates and analyzed over a fixed duration of 120 s.

- Coherence spectra were computed from 0 to 100 Hz as shown in **Figures 1E,F**. In each spectrum, a 95% confidence level (dotted lines, **Figures 1E,F**) was calculated [$C_{cl} = 1 - (0.05)^{1/L-1}$] under the assumption that the 2 spike trains were independent (Rosenberg et al., 1989). Any coherence value reaching this level was considered to reflect significant coupling between the 2 spike trains at that frequency. The rate of occurrence of significant coherence at a given frequency (expressed as a percentage of MU pairs tested) was obtained by counting the number of pairs showing significant coherence in that bin.
- Global estimates of coherence strength were obtained for both the patient and control subjects, and subsequently compared through pooling (Amjad et al., 1997), The pooling procedure checked the homogeneity of the coherence estimates among all MU pairs within each group with an extended form of the test used to detect significant differences between two single coherence estimates (Rosenberg et al., 1989), and, provided a pooled estimate for each group. The extended difference of coherence test (Amjad et al., 1997) was used again to detect any difference between the two pooled coherence estimates which may occur beyond those detected within each pool. In the case of the subsets of 45 MUs analyzed over 120 s, significant differences in coherence were detected by subtracting the inverse arc hyperbolic tangent ($\tanh^{-1}$) of the patient subset coherence estimate from the control one (Rosenberg et al., 1989).

Coherence strength was also evaluated in terms of Z scores obtained using the Fisher transformation of the coherence inverse arc hyperbolic tangent at each frequency ($Z = \tanh^{-1}(\sqrt{C}) * 2L^{-2}$). An estimate of population coherence was obtained by averaging the coherence Z scores in each bin across all MU pairs tested. Coherence significance and strength were further examined within eight frequency bands (band I, 0–5 Hz; band II, 5–10 Hz; band III, 10–15 Hz; band IV, 15–30 Hz; band V, 30–45 Hz; band VI, 45–60 Hz; band VII, 60–75 Hz; band VIII, 75–90 Hz) for each MU pair. Each band includes the lower frequency limit and excluded the upper one. The rate of occurrence of significant coherence observed for N MU pairs in a given band of n bins was obtained by dividing the sum (S) of occurrences of significant coherence observed for all pairs throughout the successive bins by the product of the number of pairs and bins [S/(n*N)]. For each pair, an estimate of the coherence strength in each band (band

Z score) was obtained by averaging the Z scores of the band's n bins. An estimate of the population coherence in each band was obtained by averaging the band Z scores of the N MU pairs.

STATISTICS

Given that most of the variables assessed in the patient and the control group did not follow a normal distribution, the Wilcoxon rank sum and Kolmogoroff Smirnoff tests were used to determine statistically significant differences between the groups. The rates of occurrence of significant coherence per bin and per band observed in the patient and control subjects were compared using Fisher's exact test on an n*m contingency table. Statistical analyses were conducted with scripts from the MatLab statistical toolbox and central file exchange (MatWorks, Natick, MA, USA). In all tests, the level of significance was set at $P = 0.05$. Unless explicitly stated, pooled data are expressed in terms of median and inter-quartile deviation (IQD) values.

RESULTS

It is noteworthy that, to compensate for the loss of proprioceptive feedback, the patient had to develop a motor strategy heavily dependent upon visual feedback, as previously reported in similar conditions (Rothwell et al., 1982; Sanes et al., 1985). She had to continuously focus her attention on her hand position, as well as on the MU discharges displayed on the oscilloscope screen. Even if her ability to maintain a tonic discharge of the MUs was slightly better during the second testing, constant reliance on visual feedback remained necessary.

A total of 92 and 171 MU pairs were tested in the patient and the control subjects, respectively, at similar levels of EMG activity in the wrist extensor [median (IQD) = 8.9 (5.8) vs. (8.4) (4.4)% MVC] and flexor [6.1 (2.9) vs. 6.3 (3.9)% MVC]. There was no significant difference between groups in the recording durations [121 (83) vs. 132 (59) s, $P = 0.2$] or in the number of spikes used as triggers in the cross-correlation analyses [1229 (902) vs. 1332 (766), $P = 0.6$]. The 2 subsets of 45 MU pairs selected on the basis of their similarity in firing rates included 20 and 25 pairs in the patient recording sets a and b, and 6, 6, 3, 6, 4, 6, 6, and 8 pairs in the 8 control subjects.

SINGLE MU FIRING PATTERN

In the patient, MUs tended to fire at slightly faster rates, and greater variability than in control subjects (**Table 1**). The geometric means of ISI_{mean} [77.2 (17.4) ms and 87.8 (13.8) ms, respectively] and ISI_{CV} [25.7 (10.8)% and 18 (5.7)%, respectively] differed significantly between the patient and the control group ($P < 0.0001$). It is well established that MUs with faster firing rates (i.e., shorter ISI_{mean}) tend to discharge more regularly (i.e., shorter ISI_{SD}) than those with slower firing rates (Person and Kudina, 1972; Kukulka and Clamann, 1981; Matthews, 1996). This was confirmed in both groups. As shown in **Figure 2**, an exponential relationship in the form of $ISI_{SD} = a^{*}ISI_{mean}^{b}$ was

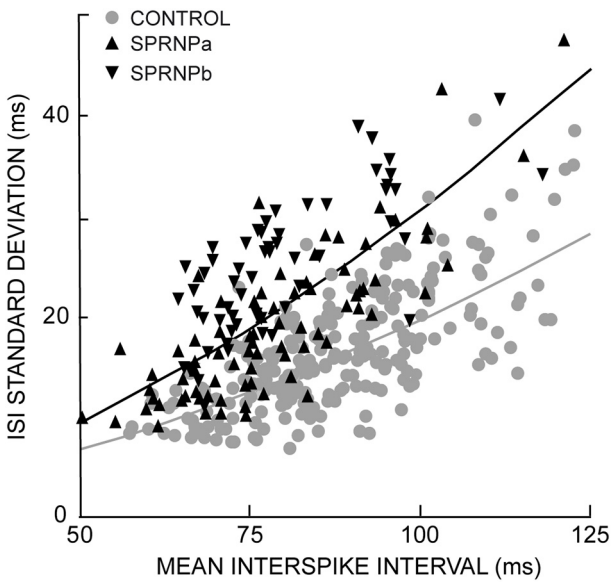

FIGURE 2 | Exponential relationship between each MU standard deviation and mean ISIs; healthy subjects gray curve and dots; SPRNP patient black curve and triangles (tip up and down, set a and b, respectively).

Table 1 | Characteristics of MU firing pattern.

Subject	Age	*N* pairs	Length (s)	ISIgeo (ms)	CVgeo (%)	N trig	Synchro (%)	W (ms)	SIP (imp./trig)	SIF (imp./s)
SPRNPa	47	55	101 (81)	76 (18.2)	22.1 (6.4)	1154 (993)	100	9 (5)	0.06 (0.03)	0.7 (0.3)
SPRNPb	61	37	142 (67)	78.3 (15.5)	31.6 (5.6)	1407 (825)	100	10 (3.5)	0.05 (0.02)	0.4 (0.3)
C1	42	19	170 (123)	82.9 (11)	12.4 (2.5)	2002 (1099)	74	9 (11)	0.03 (0.01)	0.3 (0.2)
C2	48	38	130 (118)	92.1 (17.8)	17.5 (3.9)	1206 (1047)	87	11.5 (5)	0.04 (0.01)	0.4 (0.1)
C3	50	9	125 (21)	92.4 (9.1)	23.1 (3.3)	1234 (390)	89	11 (7)	0.06 (0.04)	0.5 (0.4)
C4	51	18	121 (53)	78.9 (10.7)	18.7 (4.3)	1272 (757)	67	11 (10)	0.04 (0.02)	0.4 (0.2)
C5	52	19	141 (76)	96.4 (17.2)	16.7 (4.2)	1217 (891)	84	13 (6)	0.04 (0.02)	0.3 (0.2)
C6	53	26	131 (53)	88.2 (10.2)	20 (5)	1272 (701)	100	14 (8.5)	0.05 (0.03)	0.5 (0.4)
C7	57	10	128 (26)	84.9 (13.2)	20 (3)	1328 (310)	80	10 (7.5)	0.03 (0.01)	0.3 (0.2)
C8	63	32	142 (44)	83.1 (11.4)	19.1 (5.9)	1443 (585)	94	14.5 (5.5)	0.07 (0.03)	0.8 (0.3)

Recording's duration (length), geometric mean of inter-spike intervals (ISIgeo) and of its coefficient of variation (CVgeo), number of triggers (N trig), rate of significant synchrony (%), peak width (W) and strength of synchronous impulse probability (SIP) and of synchronous impulse frequency (SIF) of MU pairs in session a and b of patient GL (SPRNPa, SPRNPb), and in 8 control subjects (C1–C8).

found in both the patient ($a = 0.2$, $b = 1.8$, goodness of fit $P < 0.0001$) and the control subjects ($a = 0.2$, $b = 1.5$, goodness of fit $P < 0.0001$). However, in both of the patient's testing sessions (SPRNPa and SPRNPb), ISI_{SD} values were consistently greater than in the control group across the whole range of ISIs (**Figure 2**, black triangles and gray dots, respectively). The greater firing variability of the patient's MUs as compared to control subjects was confirmed by the values of ISI_{SD} [21 (12) ms vs. 12 (5) ms, $P < 0.0001$] observed with the subsets of 45 pairs (**Table 3**) discharging with similar ISIs [80 (17) ms vs. 81 (14) ms, $P = 0.8$].

SYNCHRONY BETWEEN MU DISCHARGES

The rate of occurrence of significant synchronization, its time course (peak width) and its strength (SIP and SIF) are summarized in **Table 1** for the 2 sets of recordings performed with the patient (SPRNPa and SPRNPb) and for the 8 sessions performed with the control subjects (C1–C8). In the patient, all 92 MU pairs tested showed a significant synchronization peak whereas in the control subjects, 23 of the 171 pairs tested showed no significant synchrony. The contingency table analysis yielded a significant difference in the likelihood ratio of synchronization occurrence ($P = 0.0001$). The distribution (cumulative density function) of the significant peak width and SIP values (**Figures 3A,B**) also differed significantly (Kolmogoroff Smirnoff test, $P < 0.0001$ and $P = 0.002$, respectively). Upon pooling the data, the duration of significant peaks was consistently narrower in the patient than in the control group [10 (4) vs. 12 (6) ms), $P < 0.0001$]. Moreover, synchronization peaks shorter than 10 ms were observed more frequently in the patient than in the control group (48 vs. 25%, $P < 0.0001$). Synchronous firing probability and frequency indices were both found to be greater in the patient than in control subjects [SIP: 0.058 (0.027) vs. 0.046 (0.028) imp./trig., $P = 0.0004$, and SIF: 0.64 (0.38) vs. 0.45 (0.35) imp./s, $P = 0.0005$].

Comparing the subsets of 45 pairs with similar firing rates tested over 120 s confirmed that significant synchrony occurred more frequently (100 vs. 64 %) with shorter peaks [9 (4) ms vs. 13 (5) ms, $P < 0.0001$] in the patient than in the control subsets (**Table 3**). The SIP and SIF indices, however, did not differ significantly between patient and control subsets [SIP: 0.05 (0.02) vs. 0.05 (0.03) imp./trig., $P = 0.8$, and SIF: 0. 52 (0.33) vs. 0.55 (0.30) imp./s, $P = 0.7$]. There was a tendency for peaks with more bins to contain more spikes, as illustrated in **Figure 3C**. In both the patient and the control subsets, peak SIP values and widths were positively correlated ($r^2 = 0.59$, $P < 0.0001$, $r^2 = 0.25$, $P = 0.0004$, respectively) with a similar slope (0.003, $P = 0.8$) but significantly different intercepts (0.025 vs. 0.013, $P = 0.001$). Similarly, with the whole population of MUs tested, the intercept value was significantly higher in the patient than in control subjects (not illustrated), suggesting a greater effectiveness of the synchronization process.

COHERENCE BETWEEN MU DISCHARGES

Significant coherence values were observed more frequently in the patient than in the control subjects. This is illustrated in **Figure 4** using the pooled coherence estimates computed for the whole populations of MUs tested in the patient and control group, and for the 2 subsets of 45 pairs with similar firing rates (**Figures 4A,B**, respectively). Applying Fisher's exact test to each bin of the spectrum with the whole MU populations revealed a significantly higher rate of significant coherence in the patient as compared to the control subjects below 10 Hz and between 27 and 79 Hz (black dots, **Figure 4A**), whereas the reverse was observed at 22 Hz (gray dot, **Figure 4A**). In the case of the subsets of 45 pairs analyzed over 120 s, the rate of occurrence of significant coherence was significantly higher in the patient than in the control group between 30 and 39 Hz (black dots, **Figure 4B**), whereas the reverse was observed at 21 Hz (gray dot, **Figure 4B**). The percentages of significant coherence and Z score values observed in each of the 8 frequency bands are summarized in **Table 2** for each recording set performed with the patient (SPRNPa and SPRNPb) and for the 8 sessions with the control subjects (C1–C8). The percentages observed with the whole population of MUs tested in the patient were significantly higher than those observed for the control subjects in bands II, III, V, VI, VII, and VIII, whereas the reverse was

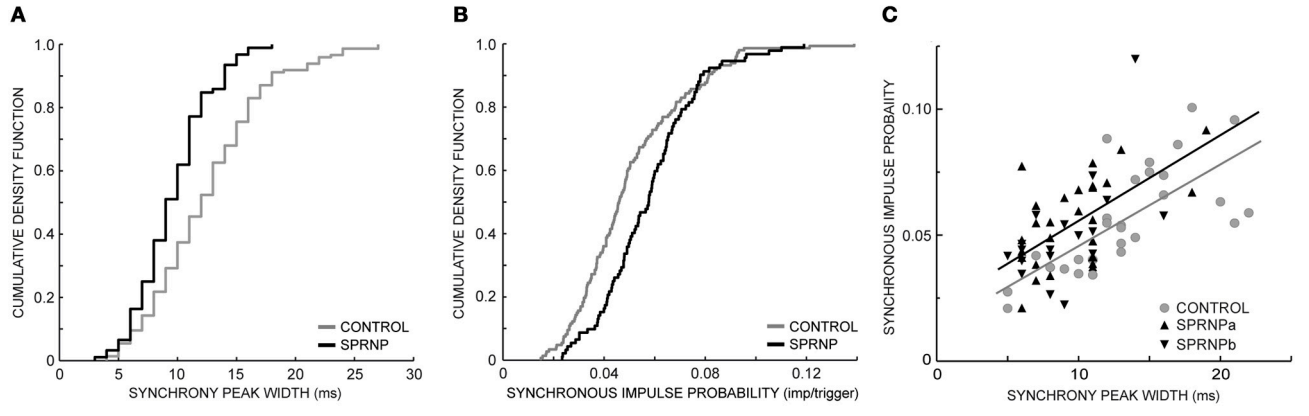

FIGURE 3 | Synchronization peak width (A) and synchronous impulse probability (B) cumulative density function (SPRNP patient and healthy subjects, black and gray staircase lines, respectively); (C) linear relationship between synchronization peak width and amplitude in two sets of 45 MUs (healthy subjects gray line and dots, SPRNP patient black line and triangles, set a tip up, and set b tip down).

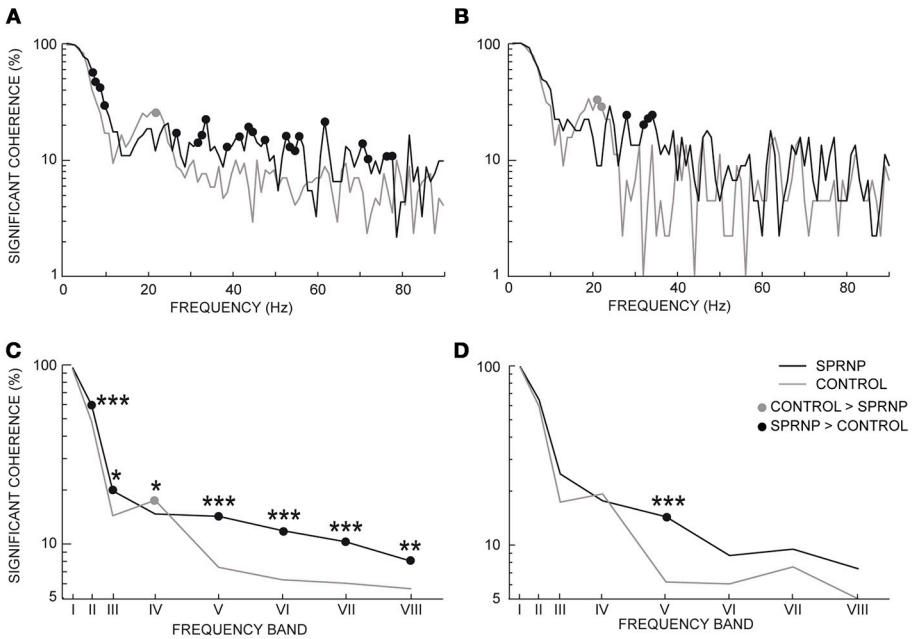

FIGURE 4 | Percentage of significant coherence values per 1 Hz-bin from all MU pairs (A), and 45 pairs firing at similar frequencies for 2 min (B); percentage of significant coherence values within 8 frequency bands for all Ms pairs (C), and the 45 pairs subsets (D); (C,D) abscissa median values of frequency bands I to VIII. (A–D), black and gray lines, SPRNP patient and healthy subjects, respectively; black and gray dots, significantly higher percentages in the patient and healthy subjects, respectively. $^{*}p < 0.05$, $^{**}P < 0.01$, $^{***}p < 0.001$.

Table 2 | MU pairs coherence values.

Subject	*N* pairs	Band I	(%, Z)	Band II	(%, Z)	Band III	(%, Z)	Band IV	(%, Z)	Band V	(%, Z)	Band VI	(%, Z)	Band VII	(%, Z)	Band VIII	(%, Z)
SPNPa	55	94	6.2 (5)	53	2.7 (1.6)	22	1.7 (0.6)	16	1.7 (0.6)	13	1.5 (0.4)	14	1.5 (0.4)	11	1.4 (0.3)	8	1.3 (0.2)
SPNPb	37	100	9.7 (2.8)	70	3 (1.8)	17	1.4 (0.6)	12	1.5 (0.3)	16	1.6 (0.4)	9	1.5 (0.4)	10	1.3 (0.3)	8	1.3 (0.2)
C1	19	100	7.5 (3.9)	48	2.4 (1.4)	16	1.4 (0.7)	7	1.3 (0.2)	10	1.4 (0.2)	6	1.3 (0.3)	9	1.3 (0.4)	6	1.2 (0.1)
C2	38	93	7.9 (4.2)	45	2.4 (0.8)	15	1.5 (0.7)	9	1.3 (0.4)	9	1.3 (0.3)	7	1.3 (0.3)	6	1.2 (0.3)	5	1.2 (0.2)
C3	9	97	5.9 (1.9)	49	2.2 (1.7)	20	1.3 (0.8)	7	1.4 (0.3)	12	1.4 (0.4)	7	1.3 (0.3)	10	1.3 (0.3)	4	1.3 (0.2)
C4	18	86	5.2 (3.3)	38	2.1 (0.9)	8	1.4 (0.6)	8	1.3 (0.3)	4	1.3 (0.4)	7	1.3 (0.3)	6	1.3 (0.2)	5	1.2 (0.2)
C5	19	97	7.1 (3.4)	49	2.8 (1.3)	11	1.6 (0.3)	11	1.3 (0.4)	6	1.2 (0.5)	7	1.3 (0.3)	2	1.2 (0.2)	5	1.2 (0.2)
C6	26	96	7.2 (4.2)	36	2.3 (1.3)	12	1.2 (0.6)	24	1.8 (0.5)	8	1.3 (0.4)	6	1.3 (0.3)	6	1.3 (0.3)	6	1.3 (0.2)
C7	10	100	8.9 (3.7)	54	2.9 (1)	16	1.4 (1.1)	9	1.4 (0.3)	4	1.1(0.2)	3	1.3 (0.2)	7	1.3 (0.3)	6	1.3 (0.2)
C8	32	93	5.9 (2.3)	64	3 (1.1)	19	1.6 (0.8)	44	2.4 (0.7)	6	1.3 (0.2)	6	1.3 (0.3)	5	1.3 (0.3)	7	1.3 (0.2)

Rate of significant coherence (%), and coherence Z score (Z) in session a and b of patient GL (SPRNPa, SPRNPb) and in 8 control subjects (C1–C8) at each frequency band (I, 0–5 Hz; II, 5–10 Hz; III, 10–15 Hz; IV, 15–30 Hz; V, 30–45 Hz; VI, 45–60 Hz; VII, 60–75 Hz; VIII, 75–90 Hz).

found for band IV (**Figure 4C**). In the case of the subsets of 45 pairs (**Table 4**), the significantly higher rate of occurrence of significant coherence observed in the patient was restricted to band V (**Figure 4D**).

Coherence estimates also tended to be stronger in the patient than in the control subjects, as seen in the whole populations of MUs tested in the patient and control subjects, and the 2 subsets of 45 pairs (**Figures 5A,B**, respectively). The Chi^2 test applied to the patient pooled coherence estimate (Amjad et al., 1997) revealed significant differences between MU pairs between 1 and 9 Hz, and at 12 Hz (not illustrated), whereas in the control pool, significant differences between MU pairs occurred from 1 to 7 Hz and from 20 to 22 Hz (not illustrated). Heterogeneity in the control coherence estimate around 20 Hz is consistent with the fact that the coherence values for subjects C6 and C8 were much higher in band IV than for any of the other control subjects (**Table 2**). The extended Chi^2 test applied to the mixed coherence estimate obtained by pooling together the patient and control MU recordings revealed significant differences within a region of the spectrum extending from 27 to 79 Hz (black dots, **Figure 5A**), i.e., well beyond the frequency regions (1–12 Hz and 20–22 Hz) where significant differences were detected within either the patient or

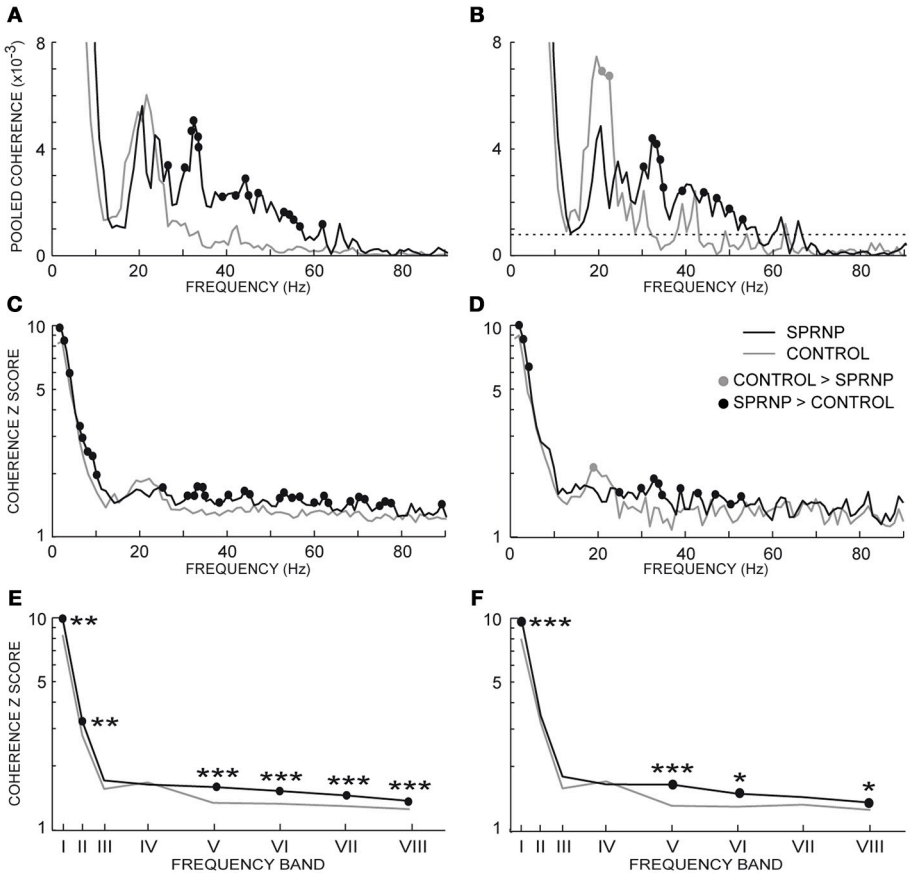

FIGURE 5 | Pooled coherence estimate spectra (1 Hz bin) for all MU pairs (A), and the subsets of 45 pairs (B, dotted line: coherence significance limit); (averaged coherence Z scores per 1 Hz-bin for all MU pairs (C), and the 45 pairs subsets (D); averaged coherence Z scores within 8 frequency bands for all MU pairs (E), and the 45 pairs subsets (F); (E,F) abscissa median values of the frequency bands I to VIII. (A–F), black and gray lines, SPRNP patient and healthy subjects, respectively; black and gray dots, significantly higher percentages in the patient and healthy subjects, respectively. $^{*}p < 0.05$, $^{**}P < 0.01$, $^{***}p < 0.001$.

the control pool. For the subsets of 45 pairs analyzed over 120 s, upon computing the difference between the $\tanh^{-1}$ transformation of each pooled coherence estimate (Rosenberg et al., 1989), coherence was found to be significantly stronger in the patient than in the control subjects below 5 Hz (out of scale, **Figure 5B**) and from 30 to 53 Hz (black dots, **Figure 5B**), whereas the reverse was found between 21 and 22 Hz (gray dots, **Figure 5B**).

Similarly, the Z scores obtained in each 1 Hz bin with all MU pairs were significantly greater in the patient than in the control subjects below 10 Hz and from 30 to 80 Hz (black dots, **Figure 5C**). Conversely, a non-significant trend toward greater Z scores in the control group than in the patient was observed from 18 to 22 Hz. The comparison between the 2 subsets of 45 pairs discharging at similar rates confirmed the presence of greater Z scores in the patient subset below 10 Hz and from 30 to 75 Hz (black dots **Figure 5D**), and the occurrence of significantly greater Z scores around 20 Hz in the control subset (gray circle **Figure 5D**).

The Z score means observed in the 8 frequency bands tested are shown in **Table 2** for each testing in the patient and the 8 control subjects. Comparing the Z scores obtained for each band with all MUs confirmed the occurrence of stronger coherence in bands I, II, V, VI, VII, and VIII (**Figure 5E**) in the patient than in healthy subjects. Upon comparing the 2 subsets of 45 pairs discharging at similar rates (**Table 4**), coherence was also found to be stronger in the patient than in the control subsets in bands I, V, VI, and VIII (**Figure 5F**).

RELATIONSHIPS BETWEEN THE MU FIRING PATTERN, SYNCHRONY AND COHERENCE INDICES

In order to determine whether there was a link between the changes in firing rate and variability, synchronous activity, and coherence found to affect MU discharges in the absence of sensory feedback, the relationships known to exist between some of these parameters in healthy subjects were examined in the patient. The expected positive correlation between the geometric means of ISI_{CV} and ISI_{mean} (Matthews, 1996) was observed in both the patient ($\rho = 0.4$, $P < 0.0001$) and the control group ($\rho = 0.2$, $P = 0.03$). Heterogeneity between subjects may again account for the weaker correlation observed in the control group. As reported previously in healthy subjects (Schmied and Descarreaux, 2010), there was no consistent covariation between

the synchronous impulse probability (SIP) and the ISI geometric mean in both the patient ($\rho = -0.05$, $P = 0.6$) and the control subjects ($\rho = -0.03$, $P = 0.7$), whereas the synchronous impulse frequency (SIF) covaried negatively with the ISI geometric mean ($\rho = -0.25$, $P = 0.001$; $\rho = -0.4$, $P < 0.0001$, respectively) in both. Again, inter-subject heterogeneity may explain the weaker correlation in the control group. A difference appeared in the covariation commonly reported between the synchronous activity and the variability of the MU discharges (e.g., Nordstrom et al., 1992). As expected, in the control group, both the SIP and SIF values were found to increase with the geometric mean of ISI_{CV} ($\rho = 0.4$, $P < 0.0001$; $\rho = 0.3$, $P < 0.0001$, respectively). However, in the patient, the covariation was non-significant or reverted ($\rho = -0.04$, $P = 0.6$; $\rho = -0.4$, $P = 0.0001$, respectively).

The lack of sensory feedback did not seem to affect the relationship between MU firing pattern and coherence strength. As previously reported in healthy subjects (Christou et al., 2007; Schmied and Descarreaux, 2011), greater coherence values in band II (6–10 Hz) were associated with shorter ISIs (i.e., faster firing rates) in both the control and the patient MU populations ($\rho = -0.3$, $P = 0.0001$; $\rho = -0.3$, $P = 0.002$, respectively). Interestingly, in both populations, the coherence strength in band I (1–5 Hz) was found to increase with the ISI geometric mean ($\rho = 0.2$, $P = 0.01$; $\rho = 0.6$, $P < 0.0001$, respectively).

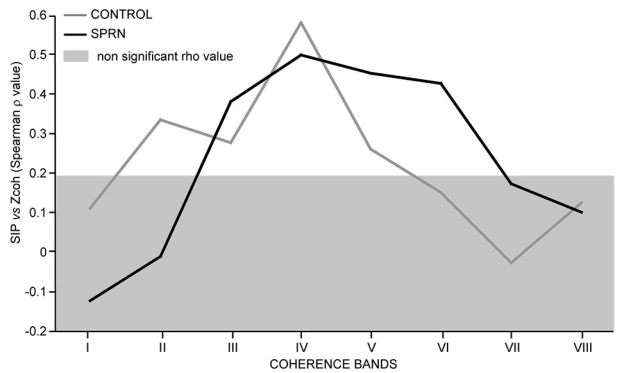

FIGURE 6 | Strength of covariation between the synchronous impulse probability (SIP) and the averaged coherence Z score (Spearman rho value, ordinate) as a function of the frequency range (bands I to VIII, abscissa); Rho values inside the light gray area are not significant; black and gray lines, SPRNP patient and healthy subjects, respectively.

Figure 6 shows the strength of the relationships observed between the synchronous activity (SIP) and the coherence Z scores across the 8 frequency bands tested in the control and patient MU populations. In keeping with previous observations (Farmer et al., 1993; Lowery et al., 2007), the highest correlation between MU synchrony (SIP as well as SIF) and coherence was found in the beta-band IV in the control subjects. This was also seen in the patient. Again confirming previous reports (e.g., Semmler et al., 1997), the lack of correlation between the high level of coherence below 5 Hz (band I) and the amount of synchronous activity (SIP, as well as SIF) in the control group, was also observed in the patient. In the control population, a much weaker, but significant covariation was also observed in bands II, III and V. A very distinct pattern was observed in the patient, however, where the correlation was absent in band II, but particularly strong in the gamma-bands V and VI (**Figure 6**).

COMPARISONS BETWEEN THE TWO TESTING SESSIONS WITH THE PATIENT

Given that the testing sessions were performed at the age of 47 (set a) and 61 (set b) in the patient, it was, necessary to check whether the differences between the patient and the control subjects were present to a similar extent at each testing (cf. **Tables 1–4**).

The ISI geometric means assessed in sets a and b did not differ significantly, and both were significantly higher than for control subject MUs. The geometric means of ISI_{CV} were significantly greater in set b than in set a, but both were significantly higher than for control MUs.

Significant synchronization peaks were observed for all MU pairs in both recording sets. Such a high incidence of synchronization was observed only once among the 8 control subjects. The synchronization peak durations did not differ significantly between the two sessions, and were, in both cases, significantly shorter than those of the control MU pairs. Synchronization indices SIP and SIF were stronger in the first testing than in the second, but were in both cases significantly larger than those in the control group.

MU coherence spectra were remarkably similar in both recording sets, apart from the occurrence of particularly high coherence values below 5 Hz in set b. In both sets, coherence values in bands II, V, VI, and VII were significantly higher than in the control population. Moreover, the Z score values observed in bands V and VII in both of the patient recording sets were consistently greater than those observed in each of the 8 control subjects (**Table 2**). Both sets of recordings showed the same lack of consistent differences from the control MUs in the beta-band IV.

Table 3 | Characteristics of MU subsets with similar firing range.

Groups	*N*	ISI_{geo}	CV_{geo}	Synchro (%)	W (ms)	SIP (imp./trig)	SIF (imp./s)
SPRNPa	27	86.1 (14.8)	26.0 (9.7)	100	10 (4)	0.055 (0.03)	0.53 (0.4)
SPRNPb	18	74.3 (8.0)	29.7 (5.7)	100	8.5 (5)	0.046 (0.02)	0.52 (0.2)
CONT	45	80.2 (12.4)	15.6 (4.4)	93	12 (6)	0.040 (0.03)	0.44 (0.3)

Firing pattern (ISIgeo, CVgeo), rate of significant synchrony (%), peak width and amplitude (W, SIP, SIF) in subsets of 45 MU pairs in patient GL (SPRNPa, SPRNPb) and in control subjects (CONT).

Table 4 | Coherence values of MU pairs with similar firing range.

Groups	*N*	Band I (Z)	Band II (Z)	Band III (Z)	Band IV (Z)	Band V (Z)	Band VI (Z)	Band VII (Z)	Band VIII (Z)
SPRNPa	27	6.6 (2.8)	2.4 (1.1)	1.5 (0.8)	1.6 (0.4)	1.5 (0.4)	1.5 (0.3)	1.3 (0.2)	1.4 (0.3)
SPRNPb	18	8.0 (1.8).	3.3 (0.8)	1.6 (0.3)	1.4 (0.5)	1.5 (0.3)	1.4 (0.2)	1.3 (0.3)	1.3 (0.2)
CONT	45	4.7 (1.5)	2.3 (0.8)	1.4 (0.5)	1.5 (0.7)	1.4 (0.3)	1.3 (0.2)	1.3 (0.2)	1.3 (0.3)

Coherence Z score (Z) in subsets of 45 MU pairs in patient GL (SPRNPa, SPRNPb) and in control subjects (CONT) at each frequency band (I, 0–5 Hz; II, 5–10 Hz; III, 10–15 Hz; IV, 15–30 Hz; V, 30-45 Hz; VI, 45–60 Hz; VII, 60–75 Hz; VIII, 75-90 Hz).

DISCUSSION

The importance of proprioceptive feedback in helping human subjects voluntarily activate single MUs, as well as the necessary role of visual and auditory feedback in the absence of muscle afferent feedback, were highlighted early on (Wagman et al., 1965; Rothwell et al., 1982; Gandevia et al., 1990). Indeed, the training required to produce and maintain steady activity in 2 MUs for 1–3 min was much longer in the patient than in control subjects. She had to visually control her arm, wrist and hand position, look at the MU spikes on the oscilloscope screen, and listen to the audio feedback, all simultaneously and continuously. The task demanded much more attention from her than from the control subjects. It is worth noting, however, that, despite the high attention load, she never complained of any form of fatigue.

In the previous case study performed with the deafferented patient IW, the limited sample of 3 MU pairs precluded a detailed assessment of the changes which might have affected the firing pattern and oscillatory and/or non-oscillatory synchronous activity of MNs, and no consistent difference was reported as compared to healthy subjects (Farmer et al., 1993). In the present study with the patient GL, the firing pattern, synchronization and coherence characteristics of single MUs were extensively documented with 55 and 37 pairs tested 15 and 29 years, respectively, after the irreversible loss of the large peripheral sensory afferents. Notwithstanding the probable existence of age-related differences in the patient's physiological state between the two recording sets, the second testing revealed the same increase in firing rate and variability, stronger short-term synchronization and greater coherence from 30 to 60 Hz as the first testing, compared to the control subjects. It is, therefore, tempting to relate the firing specificities of the MUs tested in the SPRNP patient to a putative reorganization of the MN afferent network, without excluding the possibility of adaptive changes of MN intrinsic properties facing the massive loss of synaptic inputs of sensory origin.

CHANGES IN FIRING RATE

The removal of peripheral afferent feedback is liable to suppress part of the MN net excitatory synaptic drive. A marked reduction in firing rates has been observed in single motor axons and single MUs tested at maximal or submaximal contraction levels during acute deafferentation of hand or leg muscles (Fukushima et al., 1976; Gandevia et al., 1990; Macefield et al., 1993). It has been suggested that the proprioceptive feedback may contribute to up to 30% of the MN net excitatory drive (Macefield et al., 1993). Contrasting with this view a recent meta-review in which the firing rate of MNs from different muscles was analyzed in relation to the number of muscle spindles suggested that, during muscle contraction, the initial excitatory contribution of the peripheral afferent feedback may be followed by a depressing effect on the MN firing frequency (De Luca and Kline, 2012). In keeping with such a depressing effect, a trend for higher firing rates was consistently observed in the wrist extensor muscles of the chronically deafferented patient GL in both testing sessions as compared to the 8 control subjects (**Table 1**). A similar trend for faster firing was reported for MUs tested in healthy subjects during a postural manipulation expected to reduce muscle spindle inputs in a non-invasive way (Garland and Miles, 1997a). With the same experimental paradigm, the responsiveness of single MUs to transcranial magnetic stimulation of the motor cortex was found to be enhanced (Garland and Miles, 1997a). It was therefore suggested that an increase in the corticospinal drive could compensate for the loss of proprioceptive assistance (Garland and Miles, 1997a), in keeping with the greater excitability of the motor cortex consistently observed when sensory feedback is removed transiently (Brasil-Neto et al., 1993; McNulty et al., 2002) or after amputation (Ziemann et al., 1998). In the same way, the faster firing rates observed here in the deafferented patient may reflect a compensatory increase in corticospinal and/or subcortical MN drive allowing submaximal contractions to be sustained in the absence of proprioceptive assistance.

CHANGES IN FIRING VARIABILITY

Divergent results have been reported concerning the way proprioceptive inputs may influence MN firing variability. Motor axons tested during maximum contraction were found to discharge more regularly without peripheral feedback than MUs tested under normal conditions (Gandevia et al., 1990). By contrast, MUs tested at submaximal contraction levels, were found to discharge more irregularly during pharmacological or postural manipulations expected to reduce muscle spindle input (Fukushima et al., 1976; Garland and Miles, 1997a). In the same way, the discharges of MUs tested in the deafferented patient were characterized by a much greater variability than those tested in healthy subjects. The greater irregularity of the patient's MU discharges was observed consistently in both sets recorded almost 15 years apart. Moreover, the differences between the patient and the control subjects persisted in the subsets of MUs tested over 2 min at similar firing frequencies (**Table 2**). The use of visual feedback has been reported to markedly enhance MU firing variability in healthy subjects older than 65 as compared to subjects below 31 (Welsh et al., 2007). In the present study, the control subjects and the patient were tested at similar ages ranging from 42 to 63. Nevertheless, the much stronger dependence of the patient on

visual feedback could at least partly account for greater MU firing variability.

At the cellular level, the regularity of the discharge of a MN depends on synaptic noise and membrane properties (Calvin and Stevens, 1968; Person and Kudina, 1972; Matthews, 1996; Taylor and Enoka, 2004). Synaptic noise is liable to be altered by the suppression of the huge number of synaptic potentials normally generated by cutaneous, muscular and tendinous receptors. No prediction can be made, however, as to the extent and the sign of the putative synaptic noise changes, which may depend on the respective contribution of inhibitory and excitatory inputs still present and/or newly recruited to compensate for the loss of proprioceptive input.

Besides the likeliness of changes in the structure and pattern of synaptic noise, the intrinsic properties of the deafferented MNs may also be modified (Gonzalez-Forero et al., 2002). In a study on the effects of chronic deafferentation on cat alpha MNs, there was, however, no clear-cut evidence for changes affecting the membrane electrical properties (Gustafsson et al., 1982). In humans, a change in the after hyperpolarization duration has been reported to affect MNs in patients with amyotrophic lateral sclerosis, a neurodegenerative disease affecting both the MNs and their corticospinal afferents (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011). Further investigation is required to determine if MN intrinsic properties are affected or not in the present case of deafferentation. It is noteworthy, however, that the positive correlation between the geometric means of ISI_{CV} and ISI_{mean} (Matthews, 1996) was maintained in the patient, suggesting that there was no major alteration of MN membrane properties.

CHANGES IN SYNCHRONOUS ACTIVITY

The present data confirms the early observation by Stephens and colleagues in patient IW (Farmer et al., 1993) that the loss of cutaneous and proprioceptive feedback does not prevent synchronization of single MU discharges. The large sample of MUs tested here in patient GL reveals, however, the existence of changes which can be informative regarding the contribution of sensory inputs to synchronization processes in healthy subjects, as well as regarding the new synchronization patterns in the deafferented patient.

Significant peaks were observed for all MUs in both recording sets with the patient. This occurred only once in the 8 healthy subjects tested (**Table 1**). The most conspicuous change was the shorter duration of the synchronization peaks in the patient as compared to the control group. This was observed in both testing sets and confirmed in the subsets of MUs tested at similar firing frequencies over 120 s. The fact that peaks broader than 12 ms were less frequent in the patient suggests that proprioceptive inputs may contribute to the broad peak synchronization processes thought to involve presynaptic synchronization of MN inputs, with the contribution of segmental networks and spinal interneurons (Kirkwood et al., 1982, 1984; Powers et al., 1989; Datta et al., 1991; Schmied et al., 1994). According to data obtained in MN slice preparations (Turker and Powers, 2002), the loss of common inhibitory inputs of proprioceptive origin may also contribute to the shortening of the synchronization peaks observed in the patient.

Whether expressed in terms of SIP or SIF, synchronization tended to be stronger in the patient than in the control population, although the difference did not reach significance in the second testing. It must be kept in mind, however, that the amplitude and width of the synchronization peaks may covary (Schmied et al., 1994). As a matter of fact, in the subsets of MUs tested with similar firing rates, similar SIP and SIF values were obtained for the broad peaks with moderate bin counts observed in the control subjects and the narrow peaks with large bin counts observed in patient (**Figure 3C**). The higher intercept of the regression line between the peak amplitude and its duration observed in the patient reflected a trend toward greater SIP values, and, hence a greater effectiveness of the synchronization processes.

The trend toward stronger synchrony in the patient could not be accounted for by any differences in firing rate (Schmied and Descarreaux, 2010), excluded *de facto* in the subset comparisons. In addition to the strength and pattern of the synchronizing inputs, membrane properties may also influence MN synchronization (Taylor and Enoka, 2004). A putative link between membrane calcium channels, and the synchrony and the variability of the MN discharges (Taylor and Enoka, 2004) may account for the positive correlation observed between the synchrony and the variability of the MU discharges in the control group, in keeping with previous reports (Nordstrom et al., 1992; Schmied et al., 1994). In the deafferented patient, however, the increase in synchronous activity could not be attributed to the concurrent changes in the firing variability given the lack of correlation between these parameters.

The presence of large and narrow synchronization peaks in the patient suggests an enhancement of the corticospinal inputs thought to contribute to the short-term synchronization process (Datta et al., 1991). The stronger short-term synchrony observed in the patient might be related to the constant visual attention required from her to keep the same MUs discharging as steadily as possible for at least 1 min, in keeping with a previous report (Schmied et al., 2000).

Common oscillatory as well as non-oscillatory inputs are liable to generate synchronization peaks in cross-correlograms (Baker et al., 2001). Some insight into the nature of the common oscillatory inputs which may contribute to the stronger short-term synchronization observed in the deafferented patient can be gained by examining the relationship between the strength of the synchrony activity and the level of coherence in a given frequency band. In both the patient and the control populations, the strongest index of covariation was observed in a similar way in the band IV. The MN oscillatory coupling present in this frequency band (equivalent to the beta-band at the cortical level) is taken to reflect the frequency content of the corticospinal inputs which innervate MNs monosynaptically (Farmer et al., 1993, but see Mills and Schubert, 1995). Given that the synchronous activity and coherence Z score in band IV covaried similarly in the patient and the control population, it can be inferred that the loss of peripheral afferent feedback did not markedly alter the component of MU short-synchronization generated by cortical inputs firing in the beta frequency range. As a matter of fact, the major change observed in the deafferented patient was the

presence of a particularly strong index of covariation between both synchrony indices (SIP as well as SIF) and the coherence Z scores in bands V and VI, contrasting with the lack of consistent covariation in the control population in this region of the spectrum (equivalent to the gamma range at the cortical level). It seems therefore that at least part of the enhanced short-synchrony observed in the patient might be explained by a greater activity of common MN inputs firing within the gamma-frequency range.

CHANGES IN COHERENCE

The whole population of MUs tested in the deafferented patient showed a consistent increase in coherence below 10 Hz and above 30 Hz as compared to the control population. This was confirmed by testing 2 subsets of MUs firing at similar frequencies over 120 s in order to minimize the dependence of coherence on the duration of the spike trains (Bokil et al., 2007) and firing rates (Christou et al., 2007; Negro and Farina, 2012). Under these conditions, the most consistent changes in coherence were found in band I which includes the slow co-modulation or common drive affecting the concurrent firing of MUs (De Luca et al., 1982; Myers et al., 2004), and in bands V and VI reminiscent of the low and high gamma ranges of cortical oscillatory activity.

Common drive

MN coherence in the range of 1–5 Hz is correlated with the common drive index assessed by cross correlating their instantaneous firing rates (Myers et al., 2004). This low-frequency coupling represents the moment-to-moment fluctuations in the synaptic drive which controls a given set of neurons engaged in a common task. It has been observed ubiquitously in humans in MU discharges within a single muscle or in homologous bilateral muscles involved in voluntary and postural motor activity (De Luca et al., 1982; Marsden et al., 1999; Mochizuki et al., 2006), as well as in the discharges of MNs of anesthetized cats driven by cutaneous or muscle receptor afferents (Prather et al., 2002). The existence of a common drive in a muscle devoid of spindles suggests that the presence of proprioceptive afferents is not necessary for this type of coupling to occur (Kamen and De Luca, 1992). This is confirmed by the strong coherence observed within this frequency range in the deafferented patient. The enhancement of MU firing rate co-modulation in the patient is in good agreement with the stronger common drive index observed during transient suppression of proprioceptive feedback (Garland and Miles, 1997a). The enhanced low-frequency coupling between MU discharges observed in deafferented muscles is in keeping with a recent hypothesis according to which proprioceptive feedback may down-regulate the common drive (De Luca et al., 2009). Another hypothesis may also be put forth based on the motor strategy used by the deafferented patient. With respect to this, it is noteworthy that the coherence below 5 Hz was found to covary positively with the variability of the MU discharges. The major contribution of visual feedback in the deafferented patient might explain conjointly the increase in firing variability (Welsh et al., 2007) and the increase in coherence below 5 Hz (McAuley et al., 1999).

Beta-range coherence

Confirming data obtained in a previous study with another deafferented patient IW (Farmer et al., 1993), the range of MU coherence in the 15–30 Hz band did not differ substantially between the deafferented patient GL and the control subjects. The MUs of patient GL as well as of 6 of the control subjects did not show any conspicuous coherence peaks, in contrast to the 2 other healthy subjects. The rather moderate values of coherence observed in the beta-range frequency are in keeping with previous reports in the wrist extensor muscles (Kakuda et al., 1999; Mattei et al., 2003). The 15–30 Hz MU coherence, which is particularly prominent in finger muscles as compared to other muscles (Kim et al., 2001), is thought to reflect the frequency content of MN corticospinal inputs (Farmer et al., 1993; Moritz et al., 2005). A tight correlation between the synchrony indices and the beta-range coherence was similarly observed in the control subjects and the patient, suggesting that the lack of sensory feedback did not alter the prominent contribution of beta-range oscillatory inputs to the MU synchronous activity. In addition to the descending corticospinal drive, sensory ascending pathways have recently been shown to contribute to the beta-range oscillatory coupling of the motor cortex and MN pools with a variable degree of prominence between subjects (Riddle and Baker, 2005; Witham et al., 2011). The lack of conspicuous changes in beta-range coherence observed here at the single MU level in the deafferented patient GL fits well with the persistence of corticomuscular coherence in the beta-range described in the same patient (Patino et al., 2008). This suggests that the contribution of ascending pathways connected to the large diameter sensory afferents is not needed for this type of coupling to occur.

By contrast, the beta-range intermuscular coherence which is thought to reflect at least in part MN corticospinal inputs (Norton and Gorassini, 2006; Fisher et al., 2012) was lacking in the same patient GL (Kilner et al., 2004). This suggests a major contribution of large diameter sensory inputs shared by synergistic MN pools to this type of coupling, in keeping with data obtained in monkeys (Baker et al., 2006). It can therefore be inferred that the common inputs which generate intermuscular coherence in the beta-range frequencies differ at least partly from those which generate MU coherent activity within a muscle, and from those which generate corticomuscular coherence in the same frequency range, in keeping with previous observations (Boonstra et al., 2009; Nishimura et al., 2009; Muthukumaraswamy, 2011).

Gamma-range coherence

In healthy subjects, coherence between single MU discharges tested during steady isometric contractions was non-significant or very low in the gamma range as compared to the beta range frequencies, in keeping with previous studies (Davey et al., 1993; Farmer et al., 1993; Kakuda et al., 1999; Marsden et al., 1999; Kim et al., 2001; Kilner et al., 2002; Semmler et al., 2002; Mattei et al., 2003). By contrast, in the deafferented patient GL, significant coherence values were consistently observed from 30 to 60 Hz, with a greater rate of occurrence and stronger values than in healthy subjects. The occurrence of such changes was

not documented in the previous study based on 3 pairs of finger muscle MUs tested in the deafferented patient IW (Farmer et al., 1993). In the deafferented patient GL, the most conspicuous enhancement of coupling between MU firings was observed in the low gamma range frequencies (30–45 Hz).

Without excluding the possible contribution of the subcortical oscillatory network which may be enhanced in the gamma-range to compensate for the loss of MN drive (Nishimura et al., 2009), one may reasonably assume that at least part of the coherent activity of single MUs originates from motor cortical areas. Corticomuscular coherence, as well as oscillatory activity in the sensorimotor cortex, has been shown to be specifically enhanced in the low-gamma range in relation to the visuomotor context and/or the degree of attention, readiness, and motor preparation in humans (Aoki et al., 1999; Schoffelen et al., 2005, 2011). An enhancement of the oscillatory corticospinal drive in the gamma-frequency range might be expected to occur in a prominent way in the deafferented patient as a result of the greater concentration and visual attention she had to develop to maintain the steady activity of pairs of MUs in the absence of peripheral feedback. This could account for the greater coupling between MU discharges observed here in the gamma frequency range.

In this context, it seems puzzling that in the same deafferented patient GL, corticomuscular coherence in the gamma-range assessed in isometric conditions did not differ from that of healthy subjects (Patino et al., 2008). The two studies differ, however, with regard to the task and muscles involved, i.e., self-adjusted hand clenching in the case of our MU coherence assessment (proactive task) vs. finger flexion in response to an external force, in the case of the corticomuscular coherence assessment (reactive task). A stronger dependence on proprioceptive feedback might be expected when the subject has to counteract an external force rather than produce the amount of force required to keep two MUs firing. Methodological differences may also contribute to the apparent discrepancy between the present single MU study and the previous corticomuscular study (Patino et al., 2008) performed in the same deafferented patient GL. Corticomuscular coherence in the beta as well as in the gamma range is known to depend on the subject's training state (Witham et al., 2011; Mendez-Balbuena et al., 2012). In the corticomuscular coherence study, a single testing session was performed with each of the 6 healthy subjects, in the same way as with the patient (Patino et al., 2008). By contrast, in the present study, MU recordings were obtained in a single session with each of the 8 healthy subjects tested, whereas 2 sets of 5 and 2 recording sessions, respectively, were performed with the patient. The longer training period and the greater effort required from the patient to keep the MU tonic discharges steady, as compared to healthy subjects, may have contributed to the stronger coupling of the MU firings in the gamma frequency ranges. It is worth noting, that, in Patino and colleagues' study, only 2 of the 6 healthy subjects tested showed significant corticomuscular coherence between 30 and 45 Hz in static conditions, while a significant peak around 35 Hz was present in the patient GL (Patino et al., 2008). This is quite consistent with the high level of MU gamma-range coherent activity presently observed in the same patient.

CONCLUSION

The irreversible loss of the large diameter sensory axons which continuously convey cutaneous, muscular and tendinous feedback from the whole body is liable to impact motor control in at least two ways. Firstly, the massive loss of the peripheral afferents which control the firing of mono- and/or poly-synaptic MNs at the segmental level and/or via cortical and subcortical motor pathways must be compensated for. Secondly, new motor strategies relying on continuous visual feedback and constant attention toward the on-going motor task must be developed to replace the missing proprioceptive assistance. These two aspects must be taken into account when explaining the changes observed here in the firing patterns of single MUs, and the coupling of their discharges in the time and frequency domains. Indeed, a compensatory enhancement of corticospinal and/or descending pathways may account for the faster firing rates, greater variability, stronger short-term synchronization, and stronger coherence in the low gamma frequency range of the MUs tested in the deafferented patient. The disappearance of broad peak synchronization in the patient suggests that peripheral afferents may contribute to this type of coupling. The persistence of coherent MU activity in the beta range frequency suggests, however, that peripheral feedback is not necessary for this type of coupling to occur in wrist extensor MNs. Furthermore, the constant attention as well as the permanent reliance on visual feedback developed by the patient to cope with the loss of proprioceptive and cutaneous assistance may at least partially account for the greater firing variability, the stronger oscillatory coupling observed below 5 Hz, in the common drive frequency range, and between 30 and 60 Hz, in the gamma-frequency range.

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Motor unit firing rates during spasms in thenar muscles of spinal cord injured subjects

Inge Zijdewind[1], Rob Bakels[1] and Christine K. Thomas[2]*

[1] Department Neuroscience, Medical Physiology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[2] The Miami Project to Cure Paralysis, Departments of Neurological Surgery, Physiology and Biophysics, University of Miami Miller School of Medicine, Miami, FL, USA

Edited by:
Maria Piotrkiewicz, Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Poland

Reviewed by:
C. J. Heckman, Northwestern University, USA
Monica Ann Gorassini, University of Alberta, Canada

***Correspondence:**
Christine K. Thomas, The Miami Project to Cure Paralysis, Departments of Neurological Surgery, Physiology and Biophysics, University of Miami Miller School of Medicine, LPLC, 1095 NW 14 Tce, R48, Miami, FL 33136, USA
e-mail: cthomas3@med.miami.edu

Involuntary contractions of paralyzed muscles (spasms) commonly disrupt daily activities and rehabilitation after human spinal cord injury (SCI). Our aim was to examine the recruitment, firing rate modulation, and derecruitment of motor units that underlie spasms of thenar muscles after cervical SCI. Intramuscular electromyographic activity (EMG), surface EMG, and force were recorded during thenar muscle spasms that occurred spontaneously or that were triggered by movement of a shoulder or leg. Most spasms were submaximal (mean: 39%, SD: 33 of the force evoked by median nerve stimulation at 50 Hz) with strong relationships between EMG and force (R^2 > 0.69). Unit recruitment occurred over a wide force range (0.2–103% of 50 Hz force). Significant unit rate modulation occurred during spasms (frequency at 25% maximal force: 8.8 Hz, 3.3 SD; at maximal force: 16.1 Hz, 4.1 SD). Mean recruitment frequency (7.1 Hz, 3.2 SD) was significantly higher than derecruitment frequency (5.4 Hz, 2.4 SD). Coactive unit pairs that fired for more than 4 s showed high (R^2 > 0.7, n = 4) or low (R^2:0.3–0.7, n = 12) rate-rate correlations, and derecruitment reversals (21 pairs, 29%). Later recruited units had higher or lower maximal firing rates than lower threshold units. These discrepant data show that coactive motoneurons are drive both by common inputs and by synaptic inputs from different sources during muscle spasms. Further, thenar motoneurons can still fire at high rates in response to various peripheral inputs after SCI, supporting the idea that low maximal voluntary firing rates and forces in thenar muscles result from reduced descending drive.

Keywords: motor unit recruitment, motor unit derecruitment, motor unit firing rate modulation, afferent input, motoneuron, persistent inward current

INTRODUCTION

Force is graded during voluntary contractions by the recruitment of motor units and by changes in motor unit firing rate (Monster and Chan, 1977). Since motor unit forces average 13% (6% SD) of maximal when their axons are stimulated at recruitment frequencies (5 Hz, Person and Kudina, 1972; Thomas et al., 1991), there is a large range over which force can be graded by changes in firing rate. After cervical spinal cord injury (SCI), recruitment becomes more important for force production in thenar muscles. Not only do thenar motor units generate nearly one third of their maximal force at recruitment frequencies (mean twitch/tetanic force ratio: 0.36, 0.11 SD; Håger-Ross et al., 2006), the firing rates achieved by motor units during maximal voluntary contractions are also low (mean 9.2 Hz, 3.1 SD; Zijdewind and Thomas, 2003). In uninjured subjects, maximal thenar motor unit firing rates are about three times higher, averaging 34.1 Hz (10.2 SD; Thomas, 1997). Two findings suggest that these low maximal motor unit firing rates after SCI result from reduced descending drive rather than the inability of motoneurons to respond to various inputs. First, the firing rate of one thenar motor unit was higher during a spasm (involuntary muscle contraction) than during a maximal voluntary contraction (Zijdewind and Thomas, 2003). Second, maximal motor unit firing rates could also be increased by combining a maximal voluntary contraction with an evoked muscle spasm (Zijdewind et al., 2012).

Here, our aim was to record the firing behavior of different thenar motor units during muscle spasms. If the activity of two units was correlated during a muscle spasm, the motoneurons had likely responded to an external, synaptic drive. However, if the firing behavior of unit pairs was not correlated, synaptic inputs may have activated different parts of the motoneuron pool or intrinsic currents may have affected the firing of one motoneuron more than the other. Strong increases in motor unit firing rates during spasms would also support the idea that deficits in descending drive to the thenar motor pool limit maximal voluntary motor unit firing rates and force after cervical SCI.

METHODS

SUBJECTS

Data were recorded from seven individuals with chronic (>1 year) cervical SCI (1 woman, 6 men; aged 20–53 years) due to a fall (n = 1) or a motor vehicle (n = 2), bicycle (n = 1) or diving accident (n = 3) that occurred from 1–26 years ago. Injury level was at C4 (n = 1), C5 (n = 2), C6 (n = 2) or C7 (n = 2) and was classified as A (n = 5) or B (n = 2) using the American Spinal Injury Association impairment scale. The thenar muscles of the hand more likely to contract involuntarily were studied once in each subject (n = 5 left; n = 2, right). Two subjects had some voluntary control of their thenar muscles. The University of Miami Institutional Review Board approved all of the experiments and each subject gave written informed consent to participate.

SETUP FOR THENAR MOTOR UNIT AND MUSCLE MEASUREMENTS

Each subject sat in their wheelchair with the test arm supported in a vacuum cast. The hand lay in modeling clay with the palm up, and was held in place by a metal plate and Velcro straps (Thomas, 1997). Electromyographic activity (EMG) was recorded from the distal and proximal muscle surfaces using wire electrodes taped across the muscle. The distal electrode lay across the interphalangeal joint, the proximal electrode was placed at the base of the thenar eminence, and a common electrode lay across the middle of the muscle bellies (Westling et al., 1990). Potentials from single motor units were recorded intramuscularly using a custom-made tungsten microelectrode. A transducer was aligned with the thumb to measure abduction and flexion forces at right angles. Resultant force was calculated from these forces.

PROTOCOL

Spasms were triggered by shoulder movements, by lifting and dropping one leg on the foot plate of the wheelchair, or they occurred spontaneously. Between spasms (≥1 min), the intramuscular electrode was moved in order to sample different motor units. Maximal thenar muscle force was evoked by stimulating the median nerve just proximal to the wrist for 1 s at 50 Hz using supramaximal pulses (20–50% higher than the intensity that evoked a maximal compound muscle action potential). The force generated during spasms was normalized to the respective 50 Hz force for a given muscle to enable comparison of data across spasms and subjects.

DATA COLLECTION AND ANALYSIS

Intramuscular EMG, surface EMG, and force were sampled online at 12,800 Hz, 3,200 Hz, and 400 Hz, respectively, using a SC/Zoom system (Umeå University, Sweden). Potentials belonging to the same motor unit were identified by amplitude, duration and shape, and verified by overlaying all of the marked potentials for a given spasm. The time between the potentials of a single motor unit was converted to instantaneous frequency by using the reciprocal of the interspike intervals (ISI). For each spasm, firing frequency was determined: (1) when the motor unit was recruited (first ISI shorter than 500 ms; Fuglevand et al., 2006); (2) at 25%, 50%, 75% and 100% maximal force during the increase in spasm force (an average over three consecutive ISIs was calculated for each measure); and (3) unit derecruitment (last ISI less than 1000 ms) during the decrease in spasm force.

When pairs of units were coactive during spasms, estimates were made of the amplitude of persistent inward currents boosting the effective synaptic drive by calculating the change in firing frequency of the first recruited unit (control unit, CU) when the second unit (test unit, TU) was recruited vs. derecruited (ΔF = frequency CU at recruitment of TU- frequency CU at derecruitment of TU; Kiehn and Eken, 1997; Bennett et al., 2001; Gorassini et al., 2004). In cat motoneurons, it has been suggested that the persistent calcium current activates with a time constant of about 50–100 ms (Lee and Heckman, 1998; Venugopal et al., 2011). In order to allow full activation of persistent inward currents in the CU, we selected control and TUs that were recruited at least 1000 ms apart. To measure whether a unit pair was influenced by common drive, the mean firing frequency of each unit was calculated over consecutive 500 ms epochs. Data from simultaneously active units were plotted against each other and the correlation coefficient of the linear-regression analysis was calculated. For the paired unit analysis only unit pairs with an overlap of eight 500 ms-epochs (4 s) and a correlation coefficient (R^2) above 0.7 were used.

Surface EMG was rectified, integrated every 250 ms, and plotted against the resultant force for the rising phase of the spasm and separately for the decline. The association between EMG and force values was determined using linear regression analysis.

STATISTICS

Mean (SD) data are given. Relationships between surface EMG and force, recruitment and derecruitment frequency, maximal frequency and maximal force, and maximal frequency of the first and second recruited units used for paired analysis were all analyzed using least squares linear regression. Differences in rate of force development and decline during the upward and downward phase of a spasm were tested with a multilevel analysis (mixed model analysis, IBM SPSS 22) with force rate as the first level and the muscle status (residual voluntary control or not) as the second level variable. In the analysis the intercept of the relation between the rate of force change and muscle status was first modeled as a fixed variable. Including a random intercept improved our model significantly, but including a random slope made no further improvement (−2 log likelihood did not change significantly).

Motor unit firing rates were compared at recruitment, derecruitment and at different force levels. Differences in motor unit firing rates were analyzed using a multi-level analysis so all data were included in the analysis (not all units could be followed or were not active across all force levels). The data were modeled in a hierarchical multi-level model with unit firing rate as the lowest level variable. To identify that the data came from a specific motor unit and subject, higher level group-identifiers were included. We first assessed possible differences in firing rates between subjects with or without residual voluntary force (muscle status). No significant differences were observed in recruitment, maximal

or derecruitment frequencies. Therefore frequency data were pooled for all subjects. Differences in firing rate were examined at specified force levels. In the analysis the intercept of the relation between firing frequency and force level was first modeled as a fixed variable. Including a random intercept improved our model significantly. Including a random slope, however, did not improve our model any further (−2 log likelihood did not change significantly). Therefore, only the statistical analysis including the random intercept was presented in the Results.

RESULTS

Most thenar muscle spasms occurred spontaneously or spasms were evoked by movement of a shoulder or leg. The mean increase in spasm force lasted 4.17 s (SD, 1.91) while the force decrease lasted 6.50 s (SD, 5.69 s, $n = 37$ spasms). Two other spasms lasted up to 24 s because they had double peaks. Possibly two spasms had occurred in close succession. The rate of change for EMG and force was similar during the upward phase of a spasm (1.89 N/s, SD 3.02) and during the downward phase (1.59 N/s, SD 2.95, $F = 6.635$, $P = 0.58$, **Figure 1**). In general, mean EMG and force were strongly associated during the spasms (**Figures 2A,B**; up: 32/39 spasms, $R^2 > 0.69$; down: 32/39 spasms, $R^2 > 0.52$). Some motor units were not derecruited following spasms but rather continued to fire for several minutes at low firing rates (**Figure 1B**).

MOTOR UNIT FIRING RATE INCREASES DURING SPASMS

The maximal force obtained during spasms averaged 7.5 N (7.3 SD) or 39.5 % (33.2 SD) of the 50 Hz force. We could track 72 motor units throughout or during part of a spasm (**Figures 1**, **3**). The recruitment force of these units varied between 0.04–23.8 N (mean 2.2 N, 3.0 SD) or 0.2–103.1% of 50 Hz force. Thus, both low and high threshold units were analyzed.

Motor unit firing rate at recruitment averaged 7.1 Hz (3.2 SD, $n = 66$ units) and increased to a mean maximal firing frequency of 11.6 Hz (4.4 SD, $n = 64$ units, $P < 0.001$; **Figure 5**) even though the force was often submaximal (<50 Hz force). At derecruitment, motor unit firing rate (5.4 Hz, 2.4 SD, $n = 66$) was significantly lower than both the recruitment and maximal firing rates (both $P < 0.001$). Of these units, 41% (25/61) had higher firing rates at derecruitment than at recruitment in at least one spasm (see e.g., Unit 1 in **Figure 3B**).

Motor units that could be followed during stronger spasms (≥25% maximal force) showed clear rate modulation (**Figure 6**). The average firing rate of units that were active at 25% of maximal (50 Hz) force was 8.8 Hz (3.3 SD, $n = 27$), at 50% was 10.0 Hz (2.9 SD, $n = 24$), at 75% was 12.2 Hz (2.8 SD, $n = 16$) and at 100% was 16.1 Hz (4.1 SD, $n = 10$; **Figure 6**).

Motor units did not necessarily behave similarly in repeat spasms. **Figure 2C** shows one unit behaving similarly during repeat spasms (Unit 1) while the firing behavior of the other unit differed (Unit 2). Other units were coactive during the same spasm and could be followed during multiple spasms (**Figure 3**). All three units co-modulated their firing rate during the force increase of the first spasm (dotted lines), whereas each unit behaved differently during the second (dashed lines) and third spasms (solid lines; **Figure 4**).

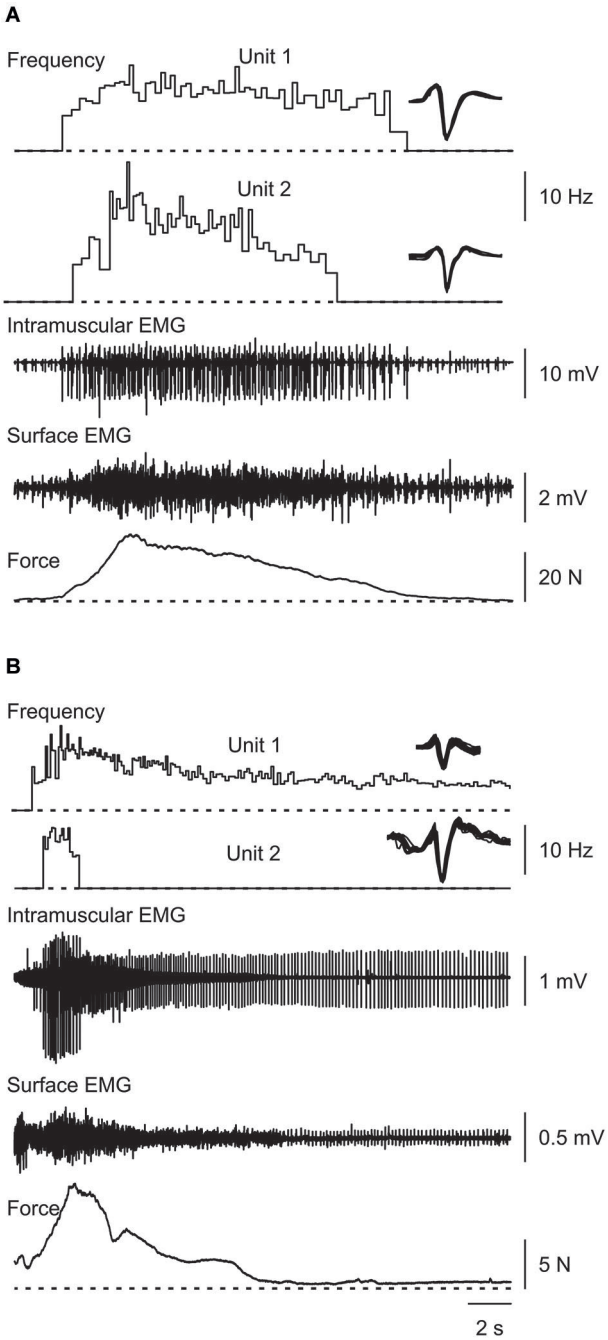

FIGURE 1 | Typical muscle spasms showing two motor units that are recruited, increase their firing rate during the force rise, decrease firing rate as the force declines, and are derecruited (A,B) or continue to fire after the contraction (B). Overlays of the marked potentials show accurate identification of each motor unit (right).

MAXIMAL FIRING RATES OF LATER RECRUITED UNITS WERE EITHER HIGHER OR LOWER THAN EARLIER RECRUITED UNITS

During voluntary contractions, coactive units can display similar rate modulation (common drive; e.g., De Luca and Hostage, 2010) or low threshold units can attain lower maximal firing rates than later recruited units (e.g., Moritz et al., 2005; Oya et al.,

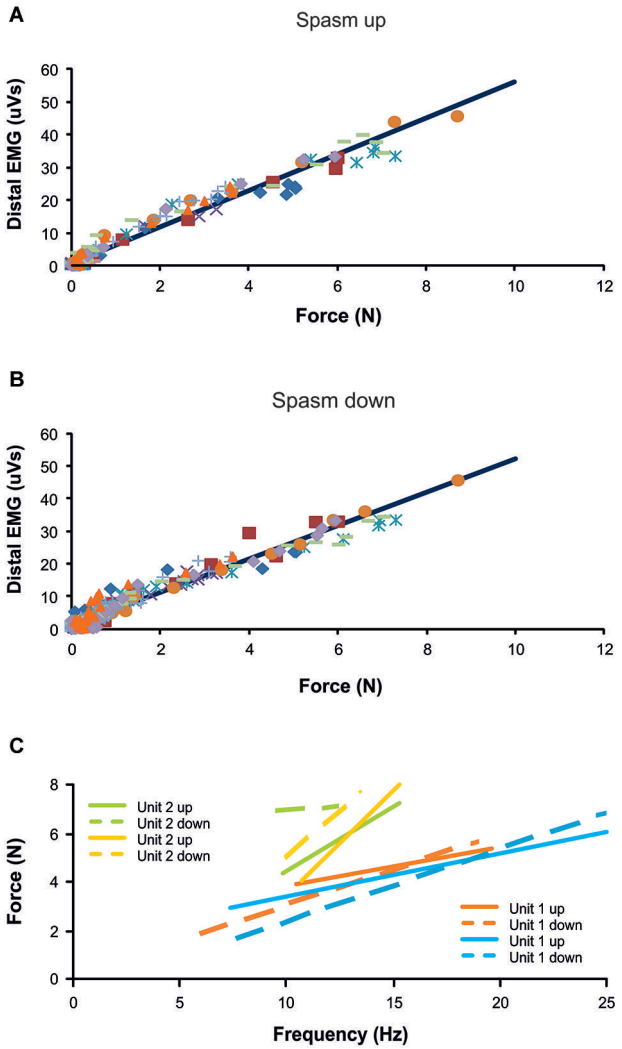

FIGURE 2 | Distal EMG-force relationship during the force rise (A; R^2 = 0.97) and fall (B; R^2 = 0.95) of 10 spasms (different symbols) recorded from one subject. (C) Frequency-force relationship for 2 units, each followed during the force increase and decrease of two different spasms (n = 4 spasms total). Data are from the same subject as in **(A)** and **(B)**.

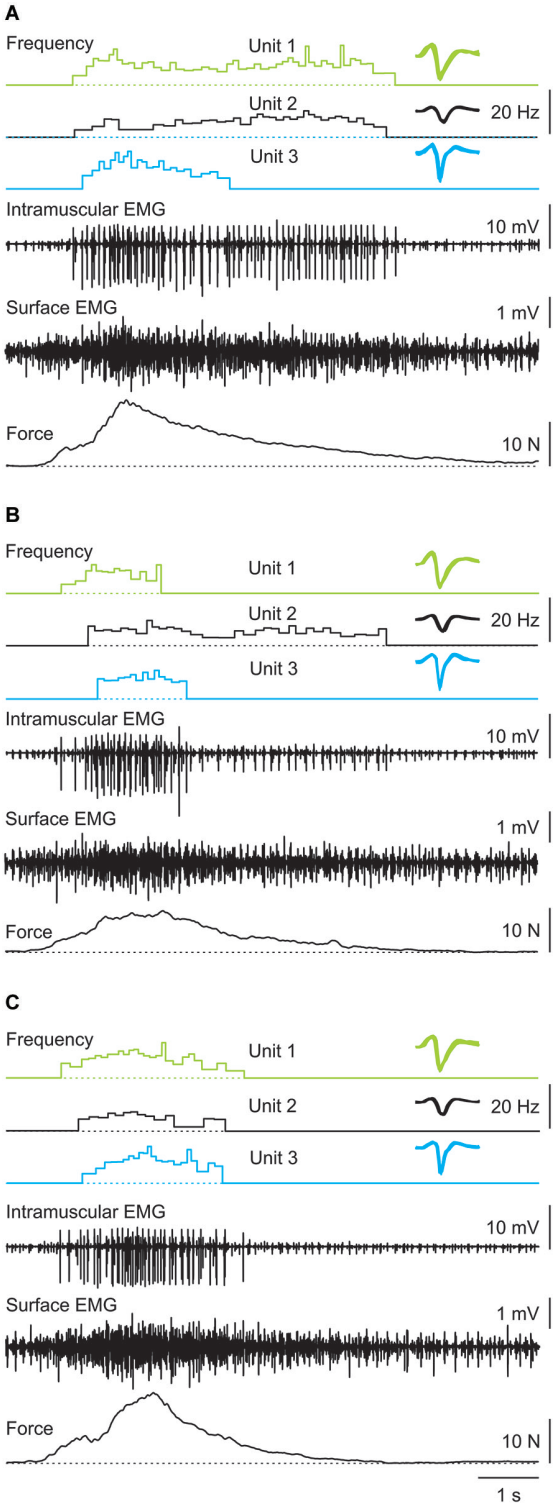

FIGURE 3 | Three consecutive muscle spasms from one subject in which three coactive units behaved differently despite similar rates of force increase and decrease during each spasm. Recruitment order was always Unit 1, 2, then 3. Unit derecruitment occurred in the reverse order in two spasms **(A,C)** but was disordered (Unit 1, 3, 2) in the other spasm **(B)**. Overlays of the marked potentials show accurate identification of each motor unit (right).

2009). When more than one unit could be tracked throughout a spasm (e.g., **Figures 3**, **7**), there was no systematic difference between the maximal firing rates of the later and earlier recruited units. That is, the maximal firing rate of the later recruited units could be either higher or lower than that recorded for earlier recruited units (**Figure 7**).

PAIRED UNIT ANALYSIS

During spasms, 23/92 unit pairs (25%) had a moderate rate-rate correlation ($R^2 > 0.3$), but only 8 pairs showed a high rate-rate correlation ($R^2 > 0.7$). Only 24 of the 92 pairs fired simultaneously for more than 4 s. Of the 24 pairs, 12 pairs (50%) showed a moderate rate-rate correlation, but only 4 pairs showed a high correlation ($R^2 > 0.7$; during voluntary contractions in control

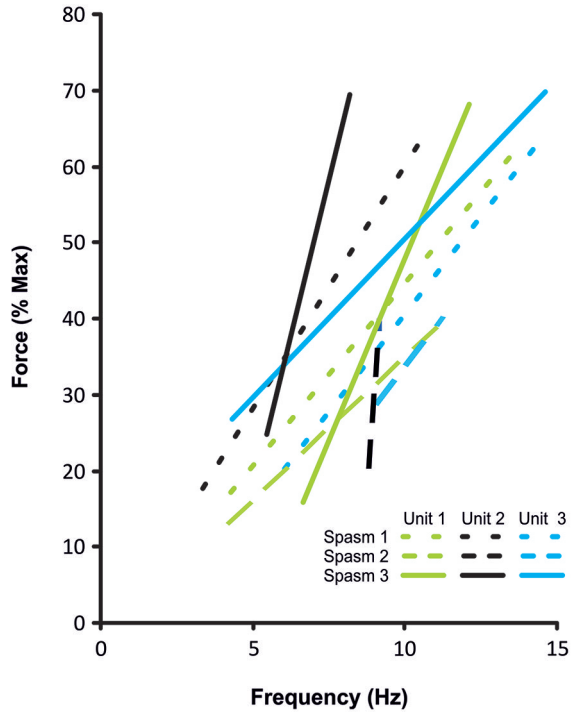

FIGURE 4 | Frequency-force relationship for the 3 coactive units during the force increase of spasm 1, 2 and 3 (dotted, dashed, solid lines, respectively) shown in Figure 3.

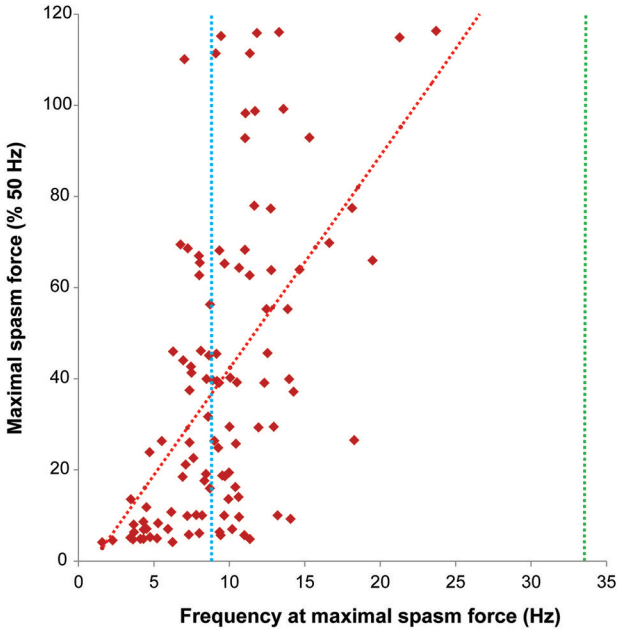

FIGURE 5 | Firing rate (Hz) at maximal spasm force vs. maximal spasm force (% 50 Hz force). The red interrupted line shows the least squares regression line (R^2 = 0.30). The dotted lines indicate the average firing rates of motor units during maximal voluntary contractions of the thenar muscles in subjects after spinal cord injury (SCI) (9.2 Hz, blue; Zijdewind and Thomas, 2003) and in able-bodied subjects (34.1 Hz, green; Thomas, 1997).

subjects mean R^2 = 0.78, Mottram et al., 2009). Each of these 4 units pairs has similar recruitment forces so none of these pairs fulfilled our criterion a recruitment being separated by at least 1000 ms. Thus, none of our unit pairs (coactivated *involuntarily* during spasms) were suitable for the conventional paired unit analysis to estimate the amplitude of the persistent inward current (Gorassini et al., 2004).

Of the 72 unit pairs for which we could determine derecruitment, 21 pairs (29%) showed reversals of derecruitment. That is, the unit that was recruited first was also derecruited first (**Figure 3B**). Eleven unit pairs were followed through multiple spasms (range: 2–4). During a total of 26 repeat contractions, there were two recruitment reversals (7.7%) and 10 derecruitment reversals (38.5%).

DISCUSSION

The main finding of the present study was distinct rate modulation of motor units during thenar muscle spasms in individuals with SCI. These data confirm in a larger group of motor units that thenar motoneurons impacted by SCI are still capable of firing at higher frequencies in response to various peripheral inputs, as are motoneurons innervating limb muscles (Thomas and Ross, 1997; Gorassini et al., 2004). Although most thenar spasms were submaximal in terms of unit firing rate and force, this rate modulation is an important contributor to spasm strength. Paralyzed thenar motor units generate 46% and 72% of their maximal force at 7.1 Hz and 16.1 Hz, the average recruitment and maximal firing rates during spasms, respectively (Håger-Ross et al., 2006). Further, the low or high correlations in rate-rate plots, and the lower or higher maximal firing rates of earlier vs. later recruited units suggest that motor units activated during muscle spasms are not always driven solely by common inputs.

HIGHER MOTOR UNIT FIRING RATES DURING THENAR SPASMS THAN VOLUNTARY CONTRACTIONS

The mechanisms underlying muscle spasms are still not completely understood. After SCI it is thought that constitutively active neuromodulatory receptors (Murray et al., 2010) and long duration excitatory post synaptic potentials (Norton et al., 2008) act to increase the amplitude and the likelihood of activation of persistent inward currents in motoneurons (Button et al., 2008). These changes and reduced inhibition after SCI make motoneurons easier to activate from peripheral inputs (Gorassini et al., 2004; Norton et al., 2008), and thus evoke long-lasting firing (Zijdewind and Thomas, 2003, 2012). Overall, the peripheral inputs to motoneurons (amount, strength, source) and their effects on motoneurons seem to increase after SCI whereas the amount, strength, and effects of descending input can be dramatically reduced (Lemon, 2008; Oudega and Perez, 2012; Thomas et al., 2014). The mean maximal motor unit firing rate was low during spasms (16.1 Hz) compared to the firing rate recorded during maximal voluntary contractions performed by able-bodied subjects (34.1 Hz; Thomas, 1997) or the stimulation frequency needed to evoke maximal force in thenar motor units paralyzed by SCI (30–50 Hz; Håger-Ross et al.,

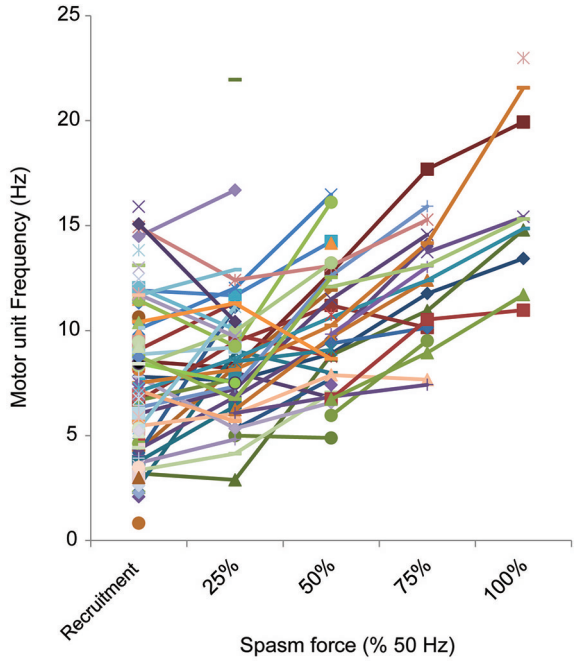

FIGURE 6 | Motor unit firing rate at different force levels (% 50 Hz force). The firing rate of motor units (reciprocal of the mean of 3 interspike intervals) at each 25% force step was plotted, starting at recruitment. Not all spasms attained the force evoked by 50 Hz stimulation or units could not be followed up to maximal force.

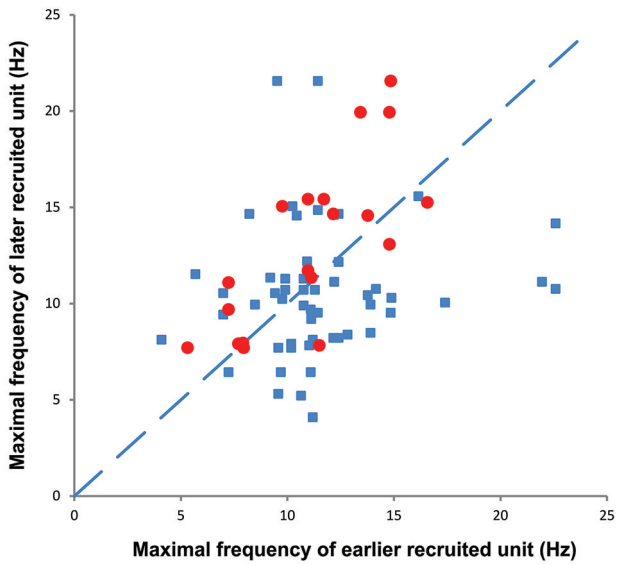

FIGURE 7 | The maximal firing frequency of all motor unit pairs obtained during spasms (blue). Later recruited units had lower, similar, or higher maximal firing rates than earlier recruited units. The red symbols refer to motor unit pairs that display a moderate ($R^2 > 0.3$) to high rate-rate correlation during spasms.

2006). Nevertheless, the thenar motor unit firing rate increased significantly more during spasms than previously seen during maximal voluntary contractions of thenar muscles performed by SCI subjects (Zijdewind and Thomas, 2003). In the latter paper, the units were recruited at 5.7 Hz (2.5 SD) and reached 9.2 Hz (3.1 SD) at maximal voluntary force. When units were recruited at low force levels and monitored up to maximal force they showed little or no rate gradation. The maximal increase in unit firing rate from 25% to 100% maximal voluntary force was only 4.2 Hz and some units actually showed a decrease in rate (maximal decrease 2.5 Hz; Zijdewind and Thomas, 2003; **Figure 2**). Taken together, these results indicate that motor units are not driven maximally during thenar voluntary contractions following SCI.

The combination of voluntary input and weak spasms indeed resulted in higher unit firing rates than did voluntary input alone (13.4 vs. 11.7 Hz; Zijdewind et al., 2012), stressing the importance of peripheral input for increasing firing rates of thenar motoneurons after SCI. In contrast, triceps brachii motor units often fired at high rates during maximal voluntary contractions after SCI (range: 6–54 Hz, for review see, Thomas et al., 2002; Johanson et al., 2013), differences that may relate to stronger monosynaptic corticospinal input to motoneurons that supply hand vs. limb muscles.

PAIRED MOTOR UNIT BEHAVIOR

The short duration and relatively fast rate of force change during naturally occurring spasms makes it difficult to use the paired unit analysis to estimate persistent inward current amplitudes (Kiehn and Eken, 1997; Bennett et al., 2001; Gorassini et al., 2004; however, cf. Revill and Fuglevand, 2011). First, most of our unit pairs were activated within a short time window (<500 ms), which suggests that these units were activated by the same trigger. Second, most of our unit pairs were coactive for short periods. Third, only a few unit pairs showed a high rate-rate correlation which suggests that after recruitment these units received different inputs or modulated their firing on the basis of intrinsic motoneuron properties. The present data illustrate how difficult it is to use the paired unit method when *both units are activated during a spasm*. In a previous paper (Gorassini et al., 2004) these paired unit methods were useful because contraction duration and recruitment separations were controlled. The CU was steadily activated voluntarily before the spasm and compared to a TU that was only activated during the spasm.

Nevertheless, these persistent currents probably underlie the prolonged motor unit firing that occurs after some spasms (**Figure 1B**) and the lower mean frequency of individual motor units at derecruitment (5.4 Hz, SD 2.4) vs. recruitment (7.1 Hz, SD 3.2). In contrast, recruitment and derecruitment frequencies did not differ significantly when SCI subjects activated thenar units voluntarily (recruitment: 5.6 Hz, SD 2.7; derecruitment: 5.2, SD 2.0; Zijdewind and Thomas, 2003). Whether this observation reflects larger persistent inward currents during spasms than during voluntary activation is unclear.

Motor unit derecruitment reversals (first recruited unit also stops firing first) were observed in 29% of the motor unit pairs during spasms, which is comparable to the percentage found in units left under voluntary control after injury (25%; Zijdewind and Thomas, 2012) but lower than the reversal percentage for

unit pairs that were spontaneously active (56%; Zijdewind and Thomas, 2012). The derecruitment reversals, and the higher motor unit firing frequencies at derecruitment vs. recruitment probably indicate differences in input across motoneurons. It is known that inhibition effectively closes ion channels involved in persistent currents (Hyngstrom et al., 2008). Thus, an uneven distribution of inhibition over the motoneuron pool could result in inhibition and/or derecruitment of some motoneurons but not others.

MAXIMAL FIRING RATES OF LATER RECRUITED UNITS ARE HIGHER OR LOWER THAN THOSE OF EARLIER RECRUITED UNITS

In able-bodied subjects, motor units often show an association between recruitment threshold and mean motor unit firing rates but these relationships can differ. In some studies, motor units recruited at higher forces fire at a lower rate than units activated earlier at weaker forces; i.e., an onion skin profile (e.g., De Luca and Hostage, 2010). This theory of common drive relies upon variations in motoneuron properties (e.g., input resistance) to account for recruitment of motoneurons in the same pool and assumes that these neurons receive shared synaptic drive resulting in comparable firing rate modulation. However, this explanation does not take into account other variations in motoneuron properties (e.g., differences in input-output relations; i.e., gain, amplitude and kinetics of persistent currents, Lee and Heckman, 1998), the importance of which is suggested by data that show earlier recruited units reach lower maximal firing rates than later recruited units (e.g., Moritz et al., 2005; Oya et al., 2009).

During spasms there was no systematic relationship between the maximal firing rate of earlier and later recruited units (**Figure 7**), even for unit pairs in which the rate-rate correlation was significant. Further, some units that showed significant co-variation in firing rate still had lower correlation coefficients than reported for units from control subjects during voluntary contractions (R^2 = 0.78; Mottram et al., 2009). This behavior suggests that during a spasm some motoneurons share common input that results in similar firing rate modulation. However, at the same time, simultaneously active motoneurons in the same pool probably also have different input-output relations (Kernell and Hultborn, 1990) or receive different amounts and/or sources of synaptic input to give uncorrelated data. Not only may this result in different unit firing rates and durations, but also in derecruitment reversals. In **Figure 3**, Unit 1 was recruited first in all three spasms but was derecruited last in two spasms and first in the other despite similar rates of force development and decline across spasms.

FUNCTIONAL IMPLICATIONS

The present data show the importance of various afferent inputs for motor unit rate modulation after SCI. However, excitatory input from the periphery seems less focused and distributed to a wider range of motoneuron pools after SCI (Hyngstrom et al., 2008; Johnson et al., 2013) thereby increasing the chance of activating multiple muscles. The uncontrolled movements that can result are one reason why spasms are considered a negative consequence of SCI. Thus, medication is often prescribed to reduce the number and strength of the spasms by reducing the afferent input and/or motoneuron excitability *per se*. Chronic baclofen, however, weakens motor units (Thomas et al., 2010) so non-pharmacological ways to manage spasticity may allow the high motor unit firing rates typical of some spasms to maintain muscle strength and to reduce atrophy to some extent (Kernell et al., 1987; Ditor et al., 2004; Harris et al., 2007). Medication will also impact the function of muscles that remain under voluntary control after SCI so these situations reflect the difficult choices that have to be made to manage muscle spasms.

AUTHOR CONTRIBUTIONS

Zijdewind: Project conception and design, data acquisition, analysis and interpretation, manuscript writing, final approval of the manuscript.

Rob Bakels: Data analysis and interpretation, manuscript writing, final approval of the manuscript.

Christine K. Thomas: Project conception and design, data acquisition, analysis and interpretation, manuscript writing, final approval of the manuscript.

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Motoneuron firing in amyotrophic lateral sclerosis (ALS)

Mamede de Carvalho[1,2]*, Andrew Eisen[3], Charles Krieger[4,5] and Michael Swash[1,2,6]

[1] Institute of Physiology and Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
[2] Department of Neurosciences, Hospital Santa Maria, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
[3] Emeritus Professor of Neurology, University of British Columbia, Vancouver, BC, Canada
[4] Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada
[5] Department of Medicine (Neurology), University of British Columbia, Vancouver, BC, Canada
[6] Institute of Neuroscience, Barts and The London School of Medicine, Queen Mary University of London, London, UK

Edited by:
Maria Piotrkiewicz, Nalecz Institute of Biocybernetics and Biomedical Engineering, Poland

Reviewed by:
C. J. Heckman, Northwestern University, USA
Maria Piotrkiewicz, Nalecz Institute of Biocybernetics and Biomedical Engineering, Poland

***Correspondence:**
Mamede de Carvalho, Institute of Physiology and Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Avenida Professor Egas Moniz, Lisbon 1649-029, Portugal
e-mail: mamedemg@mail.telepac.pt

Amyotrophic lateral sclerosis is an inexorably progressive neurodegenerative disorder involving the classical motor system and the frontal effector brain, causing muscular weakness and atrophy, with variable upper motor neuron signs and often an associated fronto-temporal dementia. The physiological disturbance consequent on the motor system degeneration is beginning to be well understood. In this review we describe aspects of the motor cortical, neuronal, and lower motor neuron dysfunction. We show how studies of the changes in the pattern of motor unit firing help delineate the underlying pathophysiological disturbance as the disease progresses. Such studies are beginning to illuminate the underlying disordered pathophysiological processes in the disease, and are important in designing new approaches to therapy and especially for clinical trials.

Keywords: amyotrophic lateral sclerosis, lower motor neuron, motor cortex, motor units firing, upper motor neuron

INTRODUCTION

Motor unit recruitment and firing is fundamental to voluntary movement and motor control. Adrian and Bronk (1929) studied motor unit recruitment and firing rates in normal human muscle, using their newly fabricated concentric needle electrode. They noted that "force exerted by a muscle during a voluntary contraction was the result of the concurrent recruitment of motor units and modulation of the rate at which they discharged action potentials." Denny-Brown and Pennybacker (1938), observed that in the process of recruitment of motor units in electromyographic (EMG) recordings the first recruited units were always small units, successively larger units being recruited as force increased. Henneman et al. (1965), confirmed a pattern of orderly recruitment of successively larger motor units, as deduced by studies of action potentials recorded from motor nerves, not muscle, and introduced the concept of the "size principle" (Grimby and Hannerz, 1968, 1981; Duchateau and Enoka, 2011), showing that at lower levels of activation only low-threshold slow-fatiguing motor units are recruited. At higher force requirements motor units fire more rapidly (Denny-Brown and Pennybacker, 1938). The technological limitations imposed by electrode recording characteristics in human EMG recordings during recruitment imply that low threshold units are sampled almost exclusively in EMG recordings (Grimby and Hannerz, 1981). Grimby and Hannerz (1981), also suggested that, during phasic activity, motor units are recruited in a different order than in tonic activity. More recently, Stein et al. (1972), and Milner-Brown et al. (1973), confirmed that motor units were recruited in relation to size.

Studies of motor unit firing have been neglected in categorizing lower motor neuron (LMN) and upper motor neuron (UMN) disorders, and in mixed UMN and LMN disorders, for example amyotrophic lateral sclerosis (ALS; de Carvalho et al., 2012).

UPPER MOTOR NEURON IN AMYOTROPHIC LATERAL SCLEROSIS

The modulation of spinal motor neuron firing by the brain and spinal pathways via UMNs, especially by the motor cortex, poses a particular problem in motor physiology, and in understanding the clinical effects of lesions in the motor system (Denny-Brown, 1966). ALS is a motor system disease, although extra-motor areas are also involved (van der Graaff et al., 2009). Magnetic resonance imaging (MRI) analysis of regional volumetric changes in ALS patients, particularly voxel-based morphometry (VBM), has indicated that brain atrophy occurs not only in motor areas, but also in non-motor areas, including frontal, temporal, and parietal lobes of both hemispheres (Agosta et al., 2010; Turner and Modo, 2010; Mezzapesa et al., 2013). Thus ALS is a degenerative brain disease, which is not confined to the motor system. Frontotemporal Dementia (FTD), is a well established, disorder having some features similar to ALS (Lillo and Hodges, 2009; Neary and Snowden, 2013), an observation underscored by the discovery of a hexanucleotide repeat expansion in the first intron of the C9ORF72 gene on chromosome 9p21 associated with both ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). In addition, evolutionary and early life developmental concepts as they relate to the clinical deficits in ALS, also support early cortical involvement in ALS (Eisen et al., 2013).

Classic Charcot-type ALS is characterized by a variable combination of upper and LMN deficits. In about 1% of ALS patients, the UMN component appears in isolation for many years (by

definition for more than 4 years); this is referred to as primary lateral sclerosis (PLS). In about 10% of patients there are isolated LMN features, termed progressive muscular atrophy (PMA). Pathological studies reveal that there is corticospinal (CST) tract degeneration in both PLS and PMA (Ince et al., 2003), an observation that led Gowers (1893) to classify PLS, PMA, and ALS as variants of a single clinical entity, later classified by Brain (1962) as Motor Neuron Disease. PLS usually presents insidiously in the sixth decade of life, with a symmetric, slowly progressive spastic paresis, beginning in the lower extremities, that evolves into a tetrapyramidal syndrome with marked pseudobulbar features (Pringle et al., 1992). Most patients with PLS later develop LMN features. Resting state functional MRI changes in patients with PLS resemble those of ALS (Agosta et al., 2014).

SITE OF ONSET OF ALS

When Charcot (1874) coined the term ALS, he recognized the importance of degeneration of the lateral columns and of cells in the ventral horns of the spinal cord, either occurring together, which he termed deuteropathic change or only involving only the ventral horns, which he termed protopathic. The notion of upper or LMN syndromes was then unknown, although Lockhart Clarke, in London (Turner et al., 2010), had shown that loss of cells in the ventral horns was accompanied by muscular atrophy. No observations were made as to the possible relevance of these observations to causation of ALS. In his Tuesday lectures, Charcot laid stress on "fibrillary twitches" of muscles in ALS as a particular feature of the disease. Gowers (1893), pointed out that ALS seemed to begin focally and then to spread through the motor system. In contemporary studies, the earliest changes in ALS have been studied using transcranial magnetic stimulation (TMS; Vucic et al., 2013; Vucic and Kiernan, 2013), and functional MRI (Foerster et al., 2013; Zhang et al., 2014). Although there remains uncertainty as to whether "sick" corticomotor neurons can induce anterograde death of spinal motoneurons in ALS, or if corticomotoneurons and anterior horn cells degenerate independently, as suggested in PLS or PMA (Eisen and Weber, 2001), the current evidence supports early cortical motor hyperexcitability in ALS. The importance of the degeneration of cortico-motoneuronal cells in the early clinical findings of loss of dexterity in ALS is well recognized. Indeed, a relatively small reduction of these motoneurons will have greater clinical impact than loss of cortical neurons involved in simpler motor functions (Eisen and Weber, 2001).

MOTOR CORTEX (M1) NEURONS IN ALS

Precise control of fine finger movement is a characteristic of human motor behavior. The motor neuron circuitry, which includes neurons and cells located both in the cerebral cortex and the spinal cord, is controlled by a complex neural network (Jara et al., 2014). This initiates precise movement of the legs, arms, and hands, breathing, and vocalization, all of which become severely compromised in ALS. The cortical motoneurons known as Betz cells in humans are located in layer V of the motor cortex. They are also referred to as, CST neurons, or corticomotoneurons. These neurons are characterized by: (1) a large pyramidal cell body, (2) a single apical dendrite that extends toward layer I displaying major branching and arborization, especially within layer II/III, (3) numerous basal dendrites arising from the basolateral surface, and (4) a very long axon that projects toward spinal cord targets (Molnar and Cheung, 2006; Ozdinler and Macklis, 2006; Molyneaux et al., 2007).

Corticospinal neurons are distributed over broad regions of the frontal cortex including premotor areas of the frontal lobe (Maier et al., 2002; Lemon and Griffiths, 2005; Lemon, 2010). The corticomotoneurons in the primary motor cortex (M1) are the major source of descending motor commands for voluntary movement (Lemon, 2010; Quallo et al., 2012; Vigneswaran et al., 2013). These originate, in part, from CST neurons in cortical layer V, whose axons descend to the spinal cord. CST neurons are of two general types. In one type, axons terminate in the intermediate zone of the spinal cord, where they contact spinal interneurons. Some of these interneurons make connections with spinal motoneurons and mediate a component of the descending commands for movement (Maier et al., 2002). The axons of the second type of CST neuron terminate in the ventral horn of the spinal cord, where they make *monosynaptic* connections with spinal motoneurons. These CST neurons are termed cortico-motoneuronal cells, and are thought to have a role in the generation and control of highly skilled movements, including skilled distal movements and the independent use of digits (Muir and Lemon, 1983; Baker et al., 1995). However, spinal motoneurons receive synaptic input from many sources, implying that even direct corticomotoneurons have a variable influence on the muscles they innervate. Single motor tract axons are not a simple "private line" connecting the cells of origin and spinal motoneurons innervating a single muscle, but instead they may exert simultaneous excitatory and inhibitory influences on different groups of spinal interneurons and motoneurons of multiple muscles at widely separated spinal segments (Shinoda et al., 1986, 2006) as part of the requirement for motor control – see **Table 1**.

When ALS symptoms begin in the upper limb, typically it is the pincer (precision) grip that is initially weak. The resulting "split-hand syndrome" in which there is thenar-hand weakness and wasting (thenar complex and first dorsal interosseous), with relative sparing of the hypothenar hand, is characteristic of ALS (Eisen and Kuwabara, 2012; Menon et al., 2013). TMS studies of the size of the excitatory postsynaptic potential (EPSP) recorded from the first dorsal interosseous muscle (FDI) during different hand tasks demonstrate the largest EPSP when the FDI is used in a pincer grip (Flament et al., 1993). Whereas in normal subjects the cortical:peripheral ratio (motor evoked potential/compound muscle action potential amplitude) is larger for the thenar compared with the hypothenar complex, in keeping with a stronger corticomotoneuronal input to the thenar hand, this ratio is reversed in ALS, indicating selectively reduced corticomotoneuronal input to the thenar complex (Weber et al., 2000). It is possible that increased functional connectivity, determined by resting state functional MRI, in both the sensorimotor and frontotemporal systems in ALS may reflect a compensatory process in relation to the structural breakdown of the motor and frontotemporal systems (Agosta et al., 2013; Filippi et al., 2013).

During primate evolution a reduction in the number of synapses between the motor cortex and spinal motoneurons innervating the digits has occurred, with an extension of the

Table 1 | Some characteristics of descending motor tracts.

(1) The lateral descending motor tract group (Cortical spinal and rubrospinal tracts):
Run mainly in the contralateral lateral funiculus of the spinal cord.
Mainly control distal limb muscles rather than axial and proximal muscles.
Exert stronger excitatory effects on flexor muscles and stronger inhibitory effects on extensor muscles.
Excitatory inputs are mediated monosynaptically and inhibitory inputs disynaptically.
(2) Medial descending motor tract group (Vestibular spinal tract, tectospinal tract, reticulospinal tract).
The medial system is phylogenetically and ontogenetically older than the lateral system.
The tracts in the medial system mainly run in the ventral funiculus.
The medial system characteristically steers the body, and integrates limb and body movements as well as developing movement synergisms of individual limbs, involving various parts.
Discrete lesions of the medial system usually produces motor disturbance of the axial and the proximal muscles.

direct neocortical, corticomotoneuronal projections beyond the cervical segments of the spinal cord (Lemon and Griffiths, 2005; Vigneswaran et al., 2013). Magnetic stimulation studies have shown that in humans there are direct monosynaptic connections from the motor cortex to the motoneuron pools of virtually all muscle groups, except those of the extra-ocular muscles and vesical and anal voluntary sphincter muscles, and the abductor muscles of the larynx, which are uniquely less vulnerable in ALS until late in the course of the disease (Eisen and Weber, 2001). Reaching and grasping, actions that are fundamental to prehension and the manipulation of objects and tools in primates are complex coordinated activities that are particularly sensitive to abnormalities in central motor control (Lemon and Griffiths, 2005). For example, in the macaque, corticomotoneuronal cells can be just as active during tool use as during precision grip (Vigneswaran et al., 2013).

In humans and other primates, phylogenetically older, indirect pathways project from the motor cortex onto motoneurons (Lemon and Griffiths, 2004). These pathways include the propriospinal, reticulospinal, and rubrospinal tracts (see **Table 1**). These phylogenetically, older indirect pathways project to segmental interneurons but their contribution to hand movements remains uncertain (Lawrence and Kuypers, 1965, 1968a,b; Isa et al., 2013).

CORTICOMOTONEURONAL HYPEREXCITABILITY

Eisen et al. (1992) suggested that corticomotoneuronal hyperexcitability might be a mechanism causing both UMN and LMN degeneration in ALS through glutamate-induced excitotoxicity. Increased plasma glutamate levels were first recognized by Plaitakis and Caroscio (1987). TMS studies have demonstrated that cortical hyperexcitability is an early feature of sporadic and familial ALS, linked to motoneuron degeneration (Vucic and Kiernan, 2013; Vucic et al., 2013). In addition, longitudinal studies in asymptomatic SOD-1 mutation carriers and in the G93A SOD1 mouse model (Browne et al., 2006), revealed that cortical hyperexcitability develops prior to the clinical disease onset (Vucic et al., 2008). Loss of parvalbumin-positive inhibitory interneurons in the motor cortex of ALS patients probably contributes to the development of cortical hyperexcitability (Nihei et al., 1993). In addition, reduced expression of the astrocytic glutamate transporter, excitatory amino acid transporter 2 (EAAT2), has been reported both in the SOD-1 mouse model and in the motor cortex and spinal cord of ALS patients (Philips and Rothstein, 2014).

An increased expression of glutamate receptors permeable to excessive influx of Na^+ and Ca^{2+} ions has been reported on motoneurons in ALS, thus increasing susceptibility to glutamate toxicity (Williams et al., 1997). There are several molecular features which may render motoneurons vulnerable to glutamate toxicity in ALS. First, motoneurons preferentially express glutamate receptors, such as the AMPA receptors, which are more permeable to influx of Ca^{2+} ions (Kawahara et al., 2004), and motoneurons in ALS patients lack the intracellular expression of Ca^{2+} binding proteins parvalbumin and calbindin D28k, both required to buffer intracellular Ca^{2+} (Alexianu et al., 1994). Aberrant activity of the inositol 1,4,5-triphosphate receptor type 2 receptor has been reported in ALS resulting in higher intracellular concentrations of Ca^{2+} within the motor neurons (Choe and Ehrlich, 2006). Ultimately, an influx of Ca^{2+} ions through the ionotropic glutamate receptors NMDA occurs, resulting in increased intracellular Ca^{2+} concentration and activation of Ca^{2+}-dependent enzymatic pathways that mediate neuronal death (Choi, 1987; Meldrum and Garthwaite, 1990). Glutamate excitotoxicity may also result in production of free radicals and thereby cause cell death (Bondy and LeBel, 1993; Bondy and Lee, 1993).

CORTICOMOTONEURONAL FIRING

Motor maps derived using intracortical microstimulation have suggested that the motor cortex consists of a mosaic of individual columns, each controlling a single muscle (Asanuma, 1975). However, multiple columns of cortical motoneurons, distributed relatively widely in the motor cortex innervate single spinal motoneurons (Rathelot and Strick, 2006); and individual CST neurons target multiple LMNs representing both agonist and antagonist muscles (Cheney et al., 1985). As in sensory maps, motor maps consist of clusters of functionally related neurons, but their topographic arrangement is coarser (Schreiner and Winer, 2007). Movements can most easily be evoked by stimulation of the deep layers of the motor cortex (Young et al., 2011). Recovery after a lesion in the motor cortex reflects re-establishment of patterns of neuronal firing and cortical circuitry. Maps of movement categories, and the corresponding firing patterns of individual neurons in the motor cortex can be related to patterns of movement (Melgari et al., 2008; Zartl et al., 2013), a hypothesis first suggested by Hughlings Jackson, and demonstrated by cortical stimulation in the macaque by Ferrier in the 19th century. Penfield and colleagues in Montreal, later showed this to be true also for the human brain. Individual CST neurons generally influence whether facilitatory or inhibitory LMNs innervating groups of muscles – termed the cell's muscle field (Aflalo and Graziano, 2006a).

Microstimulation studies established the sufficiency of motor cortical activity in directing movement and suggested that semidiscrete subregions control the movement of distinct body parts (Rasmussen and Penfield, 1947; Donoghue and Wise, 1982; Gioanni and Lamarche, 1985). This topographic organization, however, reveals relatively little about the precise operations performed by motor cortical networks (Aflalo and Graziano, 2006b). Studies using longer-duration stimulation in monkeys and mice raise the possibility that the motor cortex may be more accurately subdivided on the basis of their involvement in different categories of behavior – defensive postures or movements of the hand to the mouth are examples (Harrison and Murphy, 2012; Bonazzi et al., 2013; Griffin et al., 2014). Movement tuning is defined by the firing rate of component neurons. It must ultimately arise from the pattern of input that drives a particular neuron to fire. As in other cortical areas, local inhibitory neurons in motor cortex act in concert with excitatory neurons to tune downstream cells. Electrophysiological recordings have found that fast-spiking interneurons in motor cortex contribute to movement tuning by restricting all but the most excited neurons from firing. Inhibitory neurons increase their firing rates throughout movement preparation and execution, so they are probably involved in shaping movements. Dendritic gating, and amplification, may provide additional modulation (Aflalo and Graziano, 2006a). The firing rate of a single motor cortex neuron is coarsely related to the direction of arm movement, but the activity of a population of neurons can be transformed into a vector that governs the speed and direction of arm movement.

In ALS, positron emission tomography studies indicate greater cerebral blood flow during motor activation in the contralateral primary sensorimotor cortex and adjacent ventral premotor and parietal association cortex, lateral premotor cortex, the supplementary motor area, the anterior cingulate cortex, the paracentral lobule and the superior and inferior and inferior parietal cortex (Kew et al., 1993). The enhanced motor activation with more marked involvement of the premotor cortex was later documented by functional resonance magnetic studies (Konrad et al., 2002). This observation probably results from cortical reorganization in response to Betz cell loss (Kew et al., 1993).

LOWER MOTOR NEURON AND MOTOR UNIT RECRUITMENT

The LMN consists of the spinal or brainstem motoneurons that receive input from the descending CST system, as well as from other descending and local segmental connections, many of them interneuronal or conveying afferent input, together with their axonal connections to the cluster of muscle fibers innervated by each motoneuron. The number of muscles fibers making up a motor unit varies in different muscles, with the smallest number in muscles such as finger intrinsic muscles that are engaged in delicate coordinated movement. They show homogeneous histochemical patterns in each motor unit related to their contraction and relaxation characteristics, broadly classified as type I, Type IIA or type IIB motor units (Burke et al., 1971).

In each muscle, force may be graded by varying the firing rate of the activated motor units. The relation between force and firing rate follows a sigmoid curve, force gradation takes place within the middle steep portion of this curve (Kernell et al., 1999).

MOTONEURONS AND THEIR CONNECTIONS

The control of movement can be evaluated at several levels of the motor control hierarchy, but it is ultimately the spinal motoneuron that is the "lens" through which all motor circuitry must focus its activity (Miri et al., 2013). In general, afferent sensory, efferent motor and interneuronal inputs will dictate LMN firing and so influence movement.

SPINAL MOTONEURON FIRING

The conventional view regarding LMN firing is that during maintained low intensity motor activities, spinal motoneurons fire at a rate that in healthy humans is greater than 5–6 Hz, with interspike latencies which are not fixed, but which fluctuate to a limited degree. From extensive work on feline and rat LMNs and modeling studies in humans, it is believed that during motor activity voltage trajectories of mammalian motoneurons, including human LMNs, display predictable changes in firing frequency (Kernell, 1965; Schwindt and Calvin, 1972). Kernell (1965) claimed that LMN firing rates in cat increased linearly with stimulating current until the firing rate reaches approximately 25 Hz (so called "primary range" firing). With stronger stimulation, firing rate increases linearly in many LMNs, but with a greater slope in the relation of stimulating current and firing frequency (so called, "secondary range" firing; Kernell, 1965). In cat LMNs, secondary range firing can reach rates in excess of 125 Hz (Kernell, 1965). A major determinant of the firing rate is the change in membrane voltage following the spike, the after-hyperpolarization potential (AHP). This waveform has both a descending component ("the scoop"), and an ascending, gradually depolarizing component ("the ramp"), when the membrane potential rises to spike threshold. Slow motoneurons exhibit AHP half-decay times longer than 20 ms (Gossen et al., 2003; Button et al., 2006). However, in humans at low-firing rates ("subprimary range") LMN depolarization is driven by the noisy input (Matthews, 1996; Kudina, 1999; Macdonell et al., 2008). Adaptation and steady-state firing in motoneurons are assumed to be governed by summation of AHP conductance, the amount of adaptation and the shape and slopes of the steady-state frequency-to-current relation can be explained by non-linear summation of successive spikes (Baldissera et al., 1978).

Multiple potassium conductances contribute to repolarization and therefore the AHP of the LMN action potential, some with short duration "fast" components mediated by IA (4-amino-pyridine-sensitive) and IK (tetraethylammonium-sensitive) potassium channels. "Medium"-duration components of the AHP are mediated by Ca^{2+}-dependent K^+ conductances (medium afterhyperpolarization; e.g., apamin-sensitive conductance; Zhang and Krnjevic, 1987; Viana et al., 1993), which are generated by small-conductance calcium-activated potassium channels (Sah and Faber, 2002; Miles et al., 2005; Deardorff et al., 2013). Differences between the AHP in LMNs of different sizes (small and large) and between LMN types (fast, F also called type II; and slow, S, also called type I; there are also fatigue-resistant type IIa and fast-fatigable type IIb MNs) are partially responsible for

the different firing characteristics of these LMNs. The medium-duration AHP is mediated by the apamin-sensitive, SK channel subunits SK2 and SK3, where SK2 is present in all rat alpha-MNs and SK3 is expressed only in a population of S-type rat MNs with longer duration, larger amplitude AHPs (Deardorff et al., 2013). SK2 and SK3 channels are present in feline LMNs in which the peak of the AHP amplitudes appears to be related to the proportion of SK3 and SK2 channels (Deardorff et al., 2013). These SK2 and SK3 channels are prominently associated with cholinergic C-type synapses, a potential site of modulation of AHP duration and hence, LMN firing rate (Deardorff et al., 2013). Computer simulations of the voltage trajectories of LMNs have been used to derive similar biophysical properties of cat and human LMNs (Jones and Bawa, 1997). Sites of modulation of LMN firing rate include inputs from CST inputs, muscarinic input from interneurons, monoaminergic projections and others especially afferent projections.

Although there is a limited understanding of the relation between LMN firing and the biochemical properties of LMNs recent studies have begun to identify biochemical profiles of different LMN populations. For instance, the notch protein signaling pathway appears to be relevant for LMN specification. In vertebrates and invertebrates neuronal fate is influenced by this pathway which simplistically includes a receptor termed "notch" and a ligand, often called "delta," where both delta and notch can be transmembrane proteins which influence cell–cell interactions. The notch ligand, delta-like homolog 1 (Dlk1) is expressed more highly by large LMNs, compared to small LMNs and co-relates with the fast firing IIb LMN phenotype both by LMN size and firing frequency (Muller et al., 2014). Although Dlk1 may "promote a fast LMN biophysical signature," the variability in expression of Dlk1 is also consistent with previous views that LMN properties may be distributed continuously within the LMN population (Heckman and Enoka, 2012), perhaps related to the variability in expression of specific biochemical "markers" such as Dlk1. Dlk1 could modify LMN properties by changing the profiles of genes expressed in the Dlk1+ expressing MN population. One of the genes modified by Dlk1 expression is a subunit of the delayed rectifier K+ conductance in LMNs called Kcng4 which may be responsible for some but not all of the changes in firing frequency seen in the type IIb LMNs (Muller et al., 2014).

The properties of LMNs profoundly influence their susceptibility to development of ALS. It has been known from studies in mice over-expressing mutant human SOD1 that fast fatiguing (type IIb) LMNs are much more susceptible to LMN axon loss from muscle targets than type I LMNs (Frey et al., 2000; Pun et al., 2006; Hegedus et al., 2008; Saxena et al., 2013). In longitudinal studies of isometric twitch forces and immunocytochemical assessment of muscle innervation in mouse it has been determined that there is impaired sprouting ability of the larger, fast fatiguing IIb LMNs and that axonal die-back of LMNs precedes a significant decline in LMN number (Kennel et al., 1996; Fischer et al., 2004).

A consequence of the stereotyped voltage trajectories of LMNs is the relatively regular firing pattern of normal LMNs and the belief that some specific impairment of LMN firing might characterize ALS. Previous studies have reported irregular firing in single motor unit potentials (MUPs) in ALS patients (Piotrkiewicz and Hausmanowa-Petrusewicz, 2013), when other simultaneously recorded MUPs from the same muscle fired regularly. These observations were interpreted as suggesting that disease involvement occurred disproportionately in some LMNs, compared to others. The irregularities of firing are attributed variably to ectopic discharges, two or more sequential firings of a MUP (double discharges or "doublets"), or the blocking of neurotransmission in small axonal branches (Piotrkiewicz and Hausmanowa-Petrusewicz, 2013). The loss of LMNs typically results in an increase in the MUP firing rate with an "incomplete" recruitment pattern during routine EMG recordings. However, there is also often a reduction in the firing rate of MUPs with sustained contractions which has often been labeled as "fatigue" and is poorly understood.

The characterization of changes to LMN excitability in ALS has proved challenging. One approach to defining altered LMN excitability is to employ a technique called "threshold tracking" (Burke et al., 2001), which examines nerve excitability by applying subthreshold current pulses and monitoring voltage responses to track changes in the threshold by which modulation of the underlying ionic conductances can be inferred. This method is described elsewhere and has led to claims that ALS patients have altered Na^+ and K^+ conductances depending on the stage of disease (Kanai et al., 2006). Early in ALS, patients may have increased persistent Na+ conductances which could lead to the appearance of fasciculations. Later in the disease, K+ conductances decline (Kanai et al., 2006). However, electrophysiological changes found in ALS patients are not replicated with similar protocols applied to mice over-expressing human mutant SOD indicating differences between ALS and the mouse model (Boerio et al., 2010).

Clinically or electrophysiologically detectable fasciculations are almost universal in ALS, but are not requisite for diagnosis. Fasciculation refers to the spontaneous, intermittent activation of some or all of the muscle fibers of one or more motor unit. Isolated fasciculation potentials, without other neurogenic changes are observed in early affected muscles of ALS patients, before increased "jitter" and denervation signs are observed (de Carvalho and Swash, 2013a). The relatively early onset of fasciculation in ALS has led to the view that it results from MN excitability and is a marker or consequence of the MN dysfunction (Denny-Brown and Pennybacker, 1938; de Carvalho and Swash, 2013b). However, fasciculations are not specific in that they can be recorded in many diseases that affect MNs and are often observed in healthy individuals. There is also evidence that some fasciculations are generated at supraspinal sites (de Carvalho et al., 2000). Specific ion channel conductances that might be impaired in ALS include those related to mAHP duration and have long been believed to be a potential factor modulating LMN firing rate. For instance, in experiments using axotomized MNs, Kuno et al. (1974), found changes in mAHP durations which they postulated might be related to a "trophic" signal associated with the activity of the innervated muscle retrogradely transported to the LMN soma (Czeh et al., 1978) or to increased activation of "C"-type synapses on LMN somata as a compensatory response to LMN de-afferentation (Pullen and Sears, 1983). How LMNs function as part of neuron circuits or as members of a more widely distributed "connectome" is a key issue for future research.

CORTICOSPINAL INPUT TO MNs

The differing patterns of corticospinal tract projections in different species may correlate with the susceptibility to LMN dysfunction in some species and not others. The relation between CST projections and LMN dysfunction is discussed above – see **Tables 1** and **2**.

CHOLINERGIC INPUTS: THE C-TYPE SYNAPSE

The distinctive C-type synapses on LMNs exhibit presynaptic, pleomorphic vesicles, and a post-synaptic submembranous cisternal structure (Pullen and Sears, 1983; Pullen and Athanasiou, 2009). These synapses comprise 5–8.5% of the total synaptic content on LMNs and are particularly abundant on soma and proximal dendrities. C-type synapses are associated with muscarinic Ach receptors (mAchR; m2), as well as with K channels (Kv2.1) and SK channels. They are resistant to de-afferentation and spinal hemisection, suggesting that they represent a specific population of spinal cholinergic interneurons. Zagoraiou et al. (2009) have shown that the sole source of C-type synapses is a small group of spinal interneurons (VOc neurons) that express the transcription factor Pitx2. The somata of these cholinergic neurons are located around the central canal and are particularly associated with innervation of LMNs supplying proximal than distal muscles. These interneurons are associated with locomotion (Zagoraiou et al., 2009). They modulate LMN excitability by regulating the mAHP, presumably through their association with SK channels.

These C-type inputs may be particularly important for our understanding of ALS. Examination of LMNs in a transgenic mouse model of ALS and in patients dying with ALS, have shown an increase in C-terminal coverage of spinal LMN with disease progression (Pullen and Athanasiou, 2009). It has been suggested that this is a compensatory response to loss of descending inputs in ALS. Moreover, it is likely that increased C-terminal input will modify LMN firing properties. The relevance of this adaptive response may explain, in part, the clinical observation as to why there are not more prominent UMN signs in ALS patients, even in the presence of substantial UMN loss (Swash, 2012).

MONOAMINERGIC DESCENDING PATHWAYS

Reticulospinal projections probably contribute to LMN excitability through monoaminergic pathways, including serotonergic and nor-adrenergic fibers (Heckman and Enoka, 2012). These monoaminergic projections largely synapse with LMN dendrites and their activation results in large, persistent inward currents.

THE DOUBLE DISCHARGE (DOUBLET)

During the evaluation of patients by concentric needle EMG, MUPs are sometimes seen to fire in groups of two or three. Double discharges are rarely found in normal individuals, although they can observed in some muscles during slow voluntary contractions in trained subjects (Bawa and Calancie, 1983; Kudina and Andreeva, 2013). In healthy subjects, the motoneurons with early excitability recovery are capable of firing double discharges

Table 2 | Some descending and segmental inputs on motor neurons.

Tract or Input	Origin	Target	Significance	Reference
Corticospinal tract	Widespread cortical regions – especially frontal cortex	Direct contact with dendrites of motor neurons	Single CST axons project to different motor neuron pools	Maier et al. (2002), Lemon and Griffiths (2005), Lemon (2010)
Rubrospinal tract	Magnocellular red nucleus	Motor neurons of forelimbs	Significance unknown, possible role in early motor development	Humphrey et al. (1984), Cheney et al. (1985)
Reticulospinal tract	Arises from pontine and medullary reticular formation	Direct and indirect inputs to motor neurons	May influence motor behavior especially after damage to CST. Regulate tonic motor neurons	Wilson and Yoshida (1969), Riddle et al. (2009), Baker (2011)
Propriospinal tract	Cervical spinal cord	May be indirect	Reaching and grasp behavior, may mediate some CST activation	Kinoshita et al. (2012)
Locus coeruleus	Fibers arise from locus coeruleus, but also adjacent brainstem sites	Widespread projections in spinal cord including motor neurons	Important source for adrenergic inputs to motor neurons, may regulate persistent inward currents (PIC)	Kuypers (1981), Lee and Heckman (1998), Heckman and Enoka (2012)
Raphe nucleus	Raphe nucleus	Widespread projections	Likely provide serotonergic input to motor neurons	Kuypers (1981)
Voc neurons	Small group of spinal interneurons (transcription factor Pitx2)	Direct input to motor neuron soma and proximal dendrites (∼5%)	Responsible for cholinergic input to motoneurons and "C-type" synapses	Zagoraiou et al. (2009)
Other interneuron populations	Other small interneuron populations (V1–V3)	Segmental inputs	Modulation of motor neuron firing	Zagoraiou et al. (2009)

with a 5–15 ms interspike interval (Kudina and Alexeeva, 1992). The incidence of double discharges is significantly higher in ALS patients, but doublet interval durations and firing patterns of doubling motor units did not differ between ALS and healthy subjects (Piotrkiewicz et al., 2008). It might represent dysfunction of the LMN pool in ALS (Zalewska et al., 1998). The underlying mechanism should involve delayed depolarization (Weber et al., 2009; Kudina and Andreeva, 2010).

THE PRESYNAPTIC TERMINAL OF THE MOTONEURON

Most neurodegenerative diseases are characterized by extensive neuronal dysfunction and death within the central nervous system. The functional deficit in ALS has therefore been assumed to follow neuronal death. However, there is a recent literature claiming that neuron pathology in ALS is due to a degenerative process that begins in the presynaptic terminal, the neuromuscular junction, or the distal axon (Fischer et al., 2004), which is described in normal aging (Valdez et al., 2012). This degenerative process may initially lead to dying back of the distal axon ("distal axonopathy"), with relative histological preservation of the neuronal cell body and proximal axon. This "dying back" process causes dysfunction in the longest and largest motor axons first. Subsequently, more proximal portions of the axon become affected. This view is based on neuropathological evaluation of some types of toxin-induced peripheral nerve damage (peripheral neuropathy), in which the small intramuscular nerve branches may be affected initially but more proximal portions of axons are spared. This phenomenon probably results from metabolic changes in distal parts of axons associated with reduced axonal transport.

In ALS patients, and in a rodent model of ALS based on over-expression of mutant human superoxide dismutase (mSOD), derived from familial human ALS, pathological changes consistent with distal axonopathy have been reported. Similar changes have been recognized in human familial mutant SOD1 ALS (Fischer et al., 2004). In addition, changes in neuromuscular transmission, presumptively related to changes in pre-synaptic Ca^{2+} concentration, or altered calcium channel function have been observed (Appel et al., 2001). In the transgenic mouse model a large proportion of neuromuscular junctions show denervation change at a stage when there are only slight reductions in in the numbers of spinal LMNs or ventral root fibers, as determined by electrophysiological (Kennel et al., 1996), or morphological assessment (Fischer et al., 2004; Parkhouse et al., 2008). Furthermore, there are differences in the susceptibility to degeneration of various synaptic subtypes. Synapses of fast fatigable LMNs are vulnerable to synaptic loss, whereas the synapses of slow LMNs are relatively resistant (Frey et al., 2000; Pun et al., 2006; Hegedus et al., 2008). These progressive changes in specific synapses with progressive disease suggest that synapse-specific mechanisms are important. This view is supported to some degree by the observation that the WldS gene, which protects against axonal injury, modestly prolongs survival in the mSOD mouse (Fischer et al., 2005). Synaptic terminals that are less susceptible to denervation also demonstrate greater ability to generate stimulus-induced synaptic sprouting. This might arise from differences in the regulation of the actin cytoskeleton of the synapse. It is also possible that modulation of presynaptic autoreceptors on LMNs might influence its function. For instance, protein kinase alterations influence LMN neurotransmitter release (Santafe et al., 2006). Collectively, these observations suggest that the physiological properties of motor neurons influence firing and that these firing properties can, in turn, influence motor neuron survival, likely by modulating cell excitability (Saxena et al., 2013).

FUNDAMENTAL CURRENT ISSUES

There has been long standing interest in the possibility that the ionic, or ligand-gated channels expressed by motor neurons might be associated with vulnerability to dysfunction or death in ALS. Considerable attention has been paid to the role of ligand-gated excitatory amino acid receptors, with regard to excitotoxicity, and this topic has been reviewed extensively. Although the mechanisms that lead to aberrant firing of motor neurons, such as the generation of fasciculations in ALS, are unclear, it is clear that motor neuron action potential generation remains critical even as the disorder progresses. As described above, the AHP and conductances underlying this phase of the action potential are modulated by cholinergic inputs (Heckman and Enoka, 2012), and these synaptic inputs are abnormal in a murine model of ALS (Fischer et al., 2004). Nonetheless, involvement of descending inputs from cortex, or from subcortical structures such as monoaminergic axons from locus coeruleus (noradrenergic), or the median raphe (serotonergic), which facilitate persistent inward currents could underlie hyper-excitability and cell dysfunction, and cell survival. Axonal and presynaptic dysfunction is also relevant to the development of ALS. Furthermore, although it is beyond the scope of this review, it is important to recognize that cell dysfunction is not restricted to motor neurons, since other cells in the vicinity of UMNs or LMNs, such as astrocytes, microglia, or glia in the axon periphery may contribute to the development of ALS.

MOTOR UNIT FIRING IN NEUROLOGICAL DISEASE

Motor unit recruitment and firing patterns determine the characteristics of individualized muscle and limb functions; this requires organization at cortical, basal ganglia, cerebellar, and spinal level in a complex and interwoven output, based on hardware (connections and pathways) and software (learned patterns and combinations of activities). Much remains to be learned about the mechanisms that determine functional capacity.

The main EMG feature in ALS is the abnormal MUP recruitment derived from LMN loss, in particular we observe a reduced number of recruited motor units and an increased firing rate of the recruited motor units. The recruitment order is probably not much altered in ALS (Milner-Brown et al., 1973), but it has been suggested that the recruitment order can be abnormal in neurogenic atrophy, with motor units generating high-twitch tensions sometimes recruited first (Herdmann et al., 1988). Physiologically, the firing rate of new recruited motor units tend to stabilize when other units are added to the recruitment pattern. Indeed, a slight increase in the firing rate is observed in the initially recruited units, but the primary mechanism for increasing force output is the spatial recruitment of more motor units (Petajan, 1991). When no more motor units can be recruited firing rate is very increased, up to 50 Hz. ALS combines both lower and upper motoneuron involvement in the same segment. The impact of UMN lesion

on the normal fluctuation of LMN discharge rate (Rosenfalck and Andreassen, 1980) is not well known in ALS, but can disturb dexterity and maximal strength.

MOTOR UNIT ACTIVATION AND ITS MODULATION

The force generated by voluntary striated muscle contraction is regulated by two main mechanisms: recruitment of motor units and modulation of their firing rate. Both recruitment and firing rate modulation arise in response to a common excitatory drive (De Luca et al., 1982). The upper limit of motor unit recruitment differs between muscles, as well as during increasing force. In some hand muscles most motor units are recruited with muscle force less than 40–80% maximum, but in biceps brachii and tibialis anterior are recruited up to approximately 90% maximal voluntary contraction force (Heckman and Enoka, 2012). There are a number of sensory feedback loops that modulate LMN firing rates. The regular discharge of motor units at low rates of firing is modulated by muscle spindle activity, as shown in cat experiments by Burke (1968), and, in human recordings by Hagbarth and Vallbo (1969) and Vallbo (1974). These investigators considered that the spindle discharge reinforced alpha motor neurons activity and was important in sensitizing the motor system to sensory input and therefore in modulating motor activity. Cutaneous afferent input feedback also influences motor unit recruitment (Garnett and Stephens, 1981; Kanda and Desmedt, 1983). After cutaneous stimulation higher threshold units show lower recruitment thresholds, thus altering recruitment order (Kanda and Desmedt, 1983). Low threshold motor unit activity is never entirely regular (Rosenfalck and Andreassen, 1980), especially when a unit is firing very slowly, a feature attributed by Stålberg et al. (1973) to spontaneous fluctuations in the trigger level of the motoneuron membrane, variable presynaptic inflow, and short term irregularity in the neuronal depolarization curve, due to "synaptic noise." In general, motor units in muscles innervated by lumbar roots show a slower and more regular firing pattern than motor units in upper limb muscles (Stålberg et al., 1973). The variability of low threshold motor unit discharge is higher in old than in young adults, a feature consistent with the greater force fluctuation observed at low muscle forces in older people (Tracy et al., 2005), this probably derives from longer AHP associated with aging (Piotrkiewicz et al., 2007). In addition, discharge rate variability decreases with an increase in muscle force. Adding this change in discharge rate variability to a motor unit model dramatically improved the ability of the model to produce simulated force fluctuations that mirrored those observed experimentally (Moritz et al., 2005). Motor units with a low threshold for maintained voluntary activity show longer contraction times, lower twitch tension, greater fatigue resistance, smaller MUP amplitudes and lower axonal conduction velocity than motor units of higher threshold (Grimby and Hannerz, 1968).

In ALS, the reduced number of motor units is the major determinant of fatigue, but several mechanisms are involved (Thomas and Zijdewind, 2006). The impact of fatigue on the recruitment properties of motor units in UMN lesions, LMN lesions, and mixed disorders is incompletely understood (Bigland-Ritchie et al., 1986). In general, motor unit discharge rate is reduced and is more regular during fatigue (Gandevia, 1998). Several authors have reported silencing of motor units during prolonged contractions (Kato et al., 1981; Fallentin et al., 1993). Several explanations have been proposed: motoneuron adaptation to a constant excitatory input (Kernell and Monster, 1982); reflex dis-facilitation by a decline in the group Ia excitatory input from muscle spindle afferents (Macefield et al., 1991); reflex inhibition from group III and IV muscle afferents (Duchateau and Hainaut, 1993) and modulation of AHP (Person and Kudina, 1972). It has been suggested that a reduction in motor unit discharge rate matches the slowing of motor unit contractile speed during fatigue, optimizes force, and prevents energy loss (Marsden et al., 1983). However, such adaptation might not occur in high-threshold motor units (Carpentier et al., 2001), representing intrinsic differences in motoneuron adaptation to peripheral feedback. Diverse properties of proximal and distal muscles and relative differences in type I and type II muscle fiber proportions in a muscle might explain some physiological differences. In proximal muscles, monotonic decline in the recruitment threshold of the motor units and the progressive recruitment of new motor units without change in the recruitment order was observed during submaximal fatiguing contractions (Adam and De Luca, 2003), but in distal muscles low-threshold motor units show either no change or an increase in recruitment threshold, as opposed to high-threshold ones that tended to show a progressively reduced threshold (Carpentier et al., 2001). However, these dissimilarities could result, in part, from different experimental protocols.

Central drive is intensified during a fatigue task, as shown by the observation of additional higher threshold motor unit recruitment during fatigue tests (Bigland-Ritchie et al., 1986; Garland et al., 1994). However, there is a complex mechanism of motoneuron adaptation and feedback influences (Peters and Fuglevand, 1999).

IMPACT OF UPPER MOTOR NEURON AND LOWER MOTOR NEURON LESION ON MU RECRUITMENT

In patients with UMN lesions, without LMN involvement, motor units fire more slowly than normal, probably dependent on longer AHPs in LMNs in affected segments. This has been described in stroke (Liang et al., 2010; Ivanova et al., 2014). In ALS, the impact of UMN lesion on AHP of LMN is certainly dependent on LMN dysfunction, it has been described a AHP shortening in early stages of muscle involvement, increasing with higher relative force deficit (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011). This could be explained by the increased susceptibility of the fast motor units to degeneration in ALS (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011; Piotrkiewicz et al., 2012). This explains that in ALS firing rates have been reported as higher than normal in strong muscles but slower in weak muscles (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011; Piotrkiewicz et al., 2012). Moreover, in ALS patients is observed a higher variability of the interspike-interval of the firing motor units, this finding is consistent with increased excitability of the LMN in this disease (Piotrkiewicz et al., 2012).

UMN lesion causes a consequent reduction in force (Rosenfalck and Andreassen, 1980), and the normal physiological variability of the motor unit firing rate is reduced (Rosenfalck and Andreassen, 1980; de Carvalho et al., 2012) – **Figure 1**. In patients with ALS there is a complex interplay of different physiological

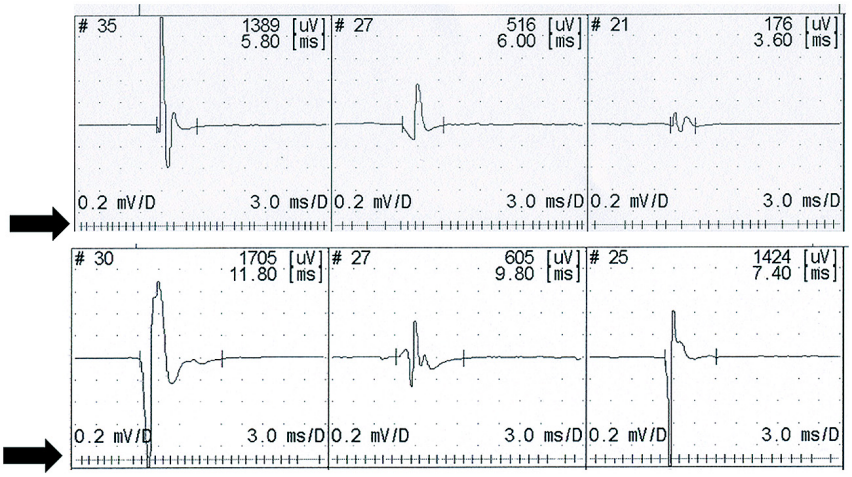

FIGURE 1 | Recruitment of three motor units on slight contraction. The upper recording from a control subject shows physiological slight variability in the firing rate. Lower trace shows a stable firing rate recorded in a patient with upper motor neuron lesion (primary lateral sclerosis). Motor unit potentials are displayed above a raster showing the firing rate.

abnormalities within the spinal motor neurons, presumably reflecting the combination of UMN and LMN features found in this disease (Lawyer and Netsky, 1953; Brownell et al., 1970; Chou, 1995), and possibly the involvement of other systems, such as spinocerebellar afferent pathways (Brownell et al., 1970; Swash et al., 1988). Disruption of interneuronal function in the dorsomedial segment of the ventral horn in the spinal cord (Stephens et al., 2006), dysfunction of Renshaw cells (Raynor and Shefner, 1994; Stephens et al., 2006), and loss of gamma motor neurons (Swash and Fox, 1974; Swash et al., 1986), results in loss of local control mechanisms governing motor unit firing patterns at spinal segmental level (Swash, 2012). In ALS patients with marked clinical signs of UMN lesion, the firing rate behavior approaches that found in clinical conditions only affecting UMNs, in muscle with preserved strength (Rosenfalck and Andreassen, 1980). Reduced modulation of LMN firing by peripheral afferents has been reported in PLS (Floeter et al., 2005).

In UMN lesion with spasticity the α-motoneurone is hyperexcitable, so that the membrane potential is closer to threshold than normal, causing facilitation of the effect of voluntary activation on motoneurons, and reduced variability in firing rate. This decreased variability in motor unit firing in UMN syndromes has been explained by activation of persistent inward currents producing stable plateau, which resist changes in response to small inputs (Lee and Heckman, 1998).

In conditions with LMN dysfunction there is a relative failure of the cell's ability to maintain the membrane potential as close to threshold as in pure UMN disorders. Indeed, in patients with LMN dysfunction, as in polyneuropathy and PMA, there is a trend to increased variability in the mean motor unit firing rate as compared with control subjects (de Carvalho et al., 2012).

CONCLUDING REMARKS

The complex synaptic relationships between the motor cortex, the descending pyramidal and extrapyramidal motor pathways, including the propriospinal system, and the organization of the spinal segmental motor system itself, are progressively destroyed during the course of ALS (Swash, 2012). This process also involves afferent connections at segmental level. This cascade of degeneration determines the clinical phenotype, but the factors leading to the relative severity of involvement, for example, of cortical neurons, UMN pathways, and LMN systems remain unknown. ALS often begins relatively focally, in that weakness may present in localized fashion in one limb before it spreads, in an orderly mode, to become more diffuse (Ravits and La Spada, 2009). However, there is increasingly convincing evidence that ALS has a long pre-clinical phase, before the disease becomes clinically manifest (Eisen et al., 2014). This phase, however, is currently undetectable except insofar as cortical motor physiological studies, using threshold tracking, have revealed early reduction in cortical inhibition, leading to the concept that motor cortex hyperexcitability may be the earliest detectable effect of the disease, perhaps even preceding LMN dysfunction. The critical abnormality in synaptic input to cortical motor system neurons leading to this effect is uncertain.

A major feature of ALS is the inexorable clinical course once the disease has commenced. There are few, if any, incontrovertible reports of survival, or arrest of disease progression. In this respect, ALS seems particularly malignant in its course. Although ALS has been traditionally regarded as a motor degeneration, there are more widespread changes in the brain; for example, there is early degeneration of the spinocerebellar pathways, a spinal afferent system that synapses in Clarke's nucleus in the upper cervical region (Swash et al., 1988). Furthermore, involvement of frontal and prefrontal cortex and its connections is a major feature manifesting as fronto-temporal dementia. This is an evident clinical feature affecting more than half of people with ALS. This clinical manifestation can be regarded as functionally part of the effector system of the brain. Posterior brain systems, related to visual, somatic, and auditory sensory functions, and olfaction, and their

integrating systems in parietal lobes and spinal cord, are spared. The underlying factors conferring resistance of these pathways to the disease, as is the case also for ocular muscles and the ano-vesical sphincter system, are unknown. With regard to the kinetics of the degenerative process in the motor system, therefore, there is much to be learned.

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