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Endurance-exercise training adaptations in spinal motoneurons: potential functional relevance to locomotor output and assessment in humans

^{1,2*} Kevin Power, ^{1,2} Evan J. Lockyer, ^{3,4} Alberto Botter, ^{3,4} Taian Vieira and ^{1,2} Duane Button

¹*Human Neurophysiology Lab, School of Human Kinetics and Recreation;* ²*Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada;* ³*Laboratory for Engineering of the Neuromuscular System (LISiN), Department of Electronics and Telecommunication;* ⁴*PoliToBIOMed Lab, Politecnico di Torino, Turin, Italy*

Running Head: Spinal motoneurone adaptations to endurance-exercise training

Key words: locomotion, task-dependent, high-density surface EMG

Abbreviations

AHP	Afterhyperpolarization
CPG	Central pattern generator
EMG	Electromyogram
F/I	Frequency-current
NMDA	N-Methyl-D-aspartate
PIC	Persistent inward current
SFA	Spike-frequency adaptation
Vth	Voltage threshold

INVITED REVIEW

*Corresponding author:

Kevin E. Power

Professor

Human Neurophysiology Lab, School of Human Kinetics and Recreation

Memorial University of Newfoundland

St. John's, Newfoundland | A1C 5S7

Email: kevin.power@mun.ca

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Abstract

It is clear from non-human animal work that spinal motoneurons undergo endurance training (chronic) and locomotor (acute) related changes in their electrical properties and thus their ability to fire action potentials in response to synaptic input. The functional implications of these changes, however, are speculative. In humans, data suggests that similar chronic and acute changes in motoneuron excitability may occur, though the work is limited due to technical constraints. To examine the potential influence of chronic changes in human motoneuron excitability on the acute changes that occur during locomotor output, we must develop more sophisticated recording techniques or adapt our current methods. In this review, we briefly discuss chronic and acute changes in motoneuron excitability arising from non-human and human work. We then discuss the potential interaction effects of chronic and acute changes in motoneuron excitability and the potential impact on locomotor output. Finally, we discuss the use of high-density surface electromyogram recordings to examine human motor unit firing properties and thus, indirectly, motoneuron excitability. The assessment of single motor units from high-density recording is mainly limited to tonic motor outputs and minimally dynamic motor output such as postural sway. Adapting this technology for use during locomotor outputs would allow us to gain a better understanding of the potential functional implications of endurance training induced changes in human motoneuron excitability on motor output.

Introduction

Sherrington famously referred to the spinal motoneurone as “the final common path,” and further stated that:

“It is the sole path which all impulses, no matter whence they come, must travel if they are to act on the muscle fibres to which it leads.” --- Sir Charles S. Sherrington, “The Integrative Action of the Nervous System,” (1906)

This statement underlies the fact that regardless of the descending drive or afferent feedback associated with motor output, it is ultimately the spinal motoneurone that determines whether or not muscle will be activated to produce movement. It thus reasons that a detailed understanding of spinal motoneurone excitability is imperative to our understanding of movement.

Since the initial intracellular recordings of spinal motoneurons (Hodgkin and Huxley, 1952) much has been learned about their passive and active properties. A large body of evidence now shows that the electrical properties of spinal motoneurons are training- (exercise-endurance vs control)(MacDonell and Gardiner 2018), state- (rest vs locomotion)(Power et al. 2018) and task- (isometric vs locomotor)(Kalmar 2018; Power et al. 2018) dependent. In this review we first provide background information on the neurophysiological mechanisms underpinning spinal motoneurone adaptations to endurance-exercise training followed by a discussion regarding the task-dependent nature of spinal motoneurone excitability. We then briefly discuss the potential implications for studies examining spinal motoneurone adaptations following endurance-exercise training. Finally, we provide a rationale as to why spinal motoneurone excitability following endurance-exercise training should be examined during the motor output used to train the motor system (i.e. training and task-dependent changes) and discuss the emerging use of high density surface electromyogram (EMG) recordings for the study of motor units during dynamic motor output.

Endurance-exercise training induced adaptations in spinal motoneurons

Evidence from non-human animal studies

Due to the complexity of in-vivo intracellular motoneurone recordings, there are only a handful of non-human studies that illustrate the effect of endurance-exercise training on the properties of healthy motoneurons. Typically, these studies were performed using rodents and their endurance-exercise training program included either voluntary exercise such as spontaneous wheel running or forced exercise such as treadmill training, swimming or cycling. Table 1 illustrates the general effect of endurance-exercise on the passive and active electrophysiological properties of motoneurons. The origin of this line of research demonstrated that passive properties were altered by both 12 weeks of spontaneous wheel running and 16 weeks of progressive treadmill training (Beaumont and Gardiner 2003) and active properties were altered by 16-20 weeks of endurance-exercise (MacDonell et al. 2012). The directional changes in these electrophysiological properties illustrate that motoneurons are either becoming, or have become, more excitable and fatigue resistant. These motoneurone adaptations may lead to an optimization and efficiency of force production during prolonged activity. Interestingly, but not surprisingly, these studies also demonstrated the potential for endurance-exercise type-dependent effects on ‘slow vs fast’ motoneurons and these effects have been reviewed elsewhere (Gardiner et al. 2005, 2006; MacDonell and Gardiner 2018). A progressively increasing treadmill exercise intensity (e.g.

increasing the slope of the treadmill) may allow for differential recruitment and activation of slow vs fast motoneurons compared to spontaneous wheel running. For now, it appears that overall the ‘slow’ motoneuron is impacted more by endurance-exercise (Beaumont and Gardiner 2002, 2003). There are several other non-human studies illustrating the effect of endurance-exercise on ‘injured’ motoneurons (i.e. motoneurons below the site of a spinal cord injury or motoneurons with cut axons) but they will not be discussed here other than that the passive and active properties of injured motoneurons typically change in the opposite direction compared to endurance-exercise trained motoneurons and that they are maintained (i.e. the properties revert back towards those of healthy sedentary motoneurons) by endurance-exercise (Beaumont et al. 2004; Gardiner et al. 2005; MacDonell and Gardiner 2018).

The adaptation of passive and active properties of the motoneuron reflect targeted changes in the movement of ions (i.e. ionic conductance) due to the number, location and modulation of ion channels in and around the dendrites and soma. However, the literature is rudimentary on this topic. In an attempt to determine the ionic conductances underlying the changes in passive and active properties due to endurance-exercise (Gardiner et al. 2006) designed a mathematical model using a 5-compartment motoneuron (Dai et al. 2002). They found that some of the specific ion channels that are important for the development of motoneuron action potential generation and firing frequency (e.g. fast-sodium, delayed rectifier potassium and leak conductances) may have a role in the observed endurance-exercise induced changes. Endurance-exercise has also been shown to affect various neurotrophic factors, synaptic inputs, intra-cellular signalling mechanisms, proteins, transcription factors and genes, all of which could alter the ion channels and thus transmembrane conductance (MacDonell and Gardiner 2018). Brain-derived neurotrophic factor, its receptor tyrosine kinase B, and components of its intracellular signaling pathway are increased in the motoneuron following endurance-exercise (Gómez-Pinilla et al. 2001; Gómez-Pinilla et al. 2002). Decreases in mRNA content for serotonin 1A, GABA A, metabotropic 1, and glutamate receptors, and potassium conductance calcium-activated channel protein have also been found in endurance-exercise motoneurons (Woodrow et al. 2013). These receptor and protein changes could alter the excitatory and inhibitory input onto motoneurons and subsequently their passive and active electrophysiological properties. While it still remains unknown, other motoneuron ion channels and their conductances such as persistent inward currents (PICs), may also be altered by endurance-exercise. Once activated, these PICs act to maintain the firing frequency of the motoneuron even when there is reduced excitatory synaptic input or amplify the synaptic excitatory input and significantly enhance its firing frequency (Heckman et al. 2003) and there is evidence to support these currents exist in lumbar motoneurons of the rodent (Button et al. 2006). Recently it was shown that 3 weeks of treadmill exercise hyperpolarized the PIC onset through a sodium channel and increased the PIC amplitude through a calcium channel in dorsal raphe nucleus 5HT neurones in mice (Ge and Dai 2020). Changes in motoneuron PIC due to exercise may also underly some of the changes in the passive and active properties of that shown in Table 1, however, this remains unknown at the moment.

Table 1. Definitions of terms used to describe the active and passive properties of spinal motoneurons and their acute and chronic changes discussed in this review. Endurance-exercise trained = EET.

Terms	Definition	Δ EET (chronic)	Δ Locomotion (acute)
Resting membrane potential	Membrane potential in the absence of motor output	Hyperpolarized	Unchanged or hyperpolarized in extensor motoneurons
Voltage threshold (V _{th})	The membrane potential at which inward Na ⁺ overcomes outward K ⁺ and an action potential is initiated	Hyperpolarized	Hyperpolarized
Rheobase	Current required to generate an action potential 50% of the time	No Change	Phase-dependent
After-hyperpolarization (AHP) amplitude	Hyperpolarized membrane potential in the soma following an action potential	Increased	Decreased or absent
Threshold current for rhythmic firing	Minimum current intensity evoking firing for 500ms	Decreased	Decreased
Maximum steady state firing frequency	Firing frequency at end of 500ms at threshold current for rhythmic firing	Decreased	Unknown
Frequency-current relationship (F/I slope)	The increase in firing frequency per increase in current intensity over a 500ms current injection	Decreased	Decreased or zero
Spike frequency adaptation	The reduction in firing frequency over a period of constant current injection	Decreased	Decreased
Voltage-dependent excitation	Enhanced firing rate as the membrane potential depolarizes	Unknown	Increased

Evidence from human studies

Similar to the non-human literature, the number of studies examining the effects of endurance-exercise training on motoneurone properties is limited. Endurance-exercise training using a leg cycling intervention there is an increased output of the motoneurone pool (i.e. higher surface EMG) and a reduced motor unit firing rate at submaximal contraction levels (Vila-Chã et al. 2010). Because firing rates decreased but overall EMG increased the authors suggested that additional motor unit recruitment likely occurred. This is certainly plausible if Vth hyperpolarization occurred as it did in the rat model following endurance-exercise training and also given that a clustering of motor unit threshold properties has been demonstrated in the dominant vs non-dominant hand, a model that though not endurance-exercise training specific is use-dependent (Adam et al. 1998).

The caveat with these studies is that the endurance-exercise training induced adaptations in motor unit firing properties were observed during sub maximal isometric contractions. As will be discussed in the next section, motoneurone excitability is state- and task-dependent (Power et al. 2018) raising important questions regarding the impact of training using a locomotor output but assessing motor unit excitability during a isometric contraction. This raises several important questions that must be considered, such as: 1) What would happen if motor unit firing properties were examined during leg cycling, the locomotor output that was used to train the motor system? and 2) Would the observed changes in motor unit firing be present, amplified, reduced or would they simply be the same? We currently lack the technology to adequately address these questions in humans but the following sections will provide a strong rationale as to why we must develop or adapt current technologies to allow us to do so.

State- and task-dependent changes in spinal motoneurone properties during locomotor output

Evidence from non-human animal studies

It is clear that the electrical properties of spinal motoneurons are modulated following endurance-exercise training. There are several important aspects of that work, however, that must be noted when trying to determine whether those changes result in a functional modulation of motor output. Specifically, spinal motoneurons are state- (i.e. rest vs motor output) and task- (i.e. isometric contraction vs locomotion) dependent (Power 2018).

In the quiescent state an increase in the firing rate or the slope of the frequency-current relationship occurs when the AHP amplitude is reduced. As the motor system is activated, however, this relationship is altered. During fictive locomotor outputs the AHP is substantially reduced or absent when compared to AHPs produced via intracellular current injection at rest (Brownstone et al. 1992; Power et al. 2010; MacDonell et al. 2015). The reduced AHP amplitude increases the variability of the firing rates and either increases, or more frequently, abolishes the F/I current relationship. Thus, the AHP does not control the firing frequency of spinal motoneurons during locomotor output (MacDonell et al. 2015). This effect is maintained during locomotor output in a spinal preparation suggesting that the spinal central pattern generator (CPG) is capable of inducing AHP amplitude changes independent of descending influences (Schmidt 1994; Power et al. 2010). A decrease in AHP amplitude would lead to a reduction in the effort required to maintain repetitive firing and thus force output.

Brownstone (1992) also showed that a brief hyperpolarizing current injection eliminated repetitive firing during locomotion indicating the presence of voltage-dependent excitation which

they later confirmed in a follow-up study (Brownstone et al., 1994). This finding explained the previously observed non-linear responses of spinal motoneurone firing properties during locomotor output (Brownstone et al. 1992). It is presently unclear whether PICs and/or NMDA channels carry this voltage-dependent excitatory current. Regardless of the mechanism(s), voltage-dependent excitation during locomotor behaviours that results in non-linear responses of motoneurons to excitatory synaptic input may facilitate motoneurone recruitment via voluntary effort, reflexive input or from the spinal CPG.

Spike frequency adaptation (SFA) has been proposed to be a mechanism associated with neuromuscular fatigue given that as motoneurone firing rate declines so too does muscle force output. Though well-documented, SFA has mainly been examined with non-human animals in the resting state, thus limiting this interpretation (Button et al. 2007). In addition, Brownstone and colleagues (2011) showed that SFA is absent during locomotor output which is perhaps not surprising given that locomotor output can occur for extended periods of time without evidence of fatigue. The influence of SFA on motor output is thus unclear. V_{th} hyperpolarization is another change in motoneurone properties that occurs during fictive locomotor outputs that would reduce the effort required to activate the motoneurone pool (Krawitz et al. 2001; Gilmore and Fedirchuk 2004; Power et al. 2010; MacDonell et al. 2015). Additional, potentially important, findings regarding V_{th} hyperpolarization include: (1) the degree of V_{th} hyperpolarization is not intensity dependent, (2) it occurs in motoneurons that are not actively engaged in the locomotor output and (3) it occurs in all types of motoneurons examined (i.e. fast and slow; flexor, extensor and bi-functionals). Thus, V_{th} hyperpolarization is a robust state-dependent change in motoneurone excitability.

An important caveat with these state-dependent changes is that they are also task-dependent, occurring during CPG-mediated rhythmic, locomotor outputs (Power et al. 2018). For example, Power et al. (2010) examined the firing properties of the same motoneurone during two motor outputs, tonic stance/weight support and fictive scratch. During scratch, a CPG-mediated rhythmic locomotor output, motoneurons were characterized by a hyperpolarized V_{th} and a reduction in AHP amplitude. During tonic stance, however, the same motoneurone demonstrated a depolarized V_{th} and either a reduction or no change in AHP amplitude, suggesting not only task-dependent changes in motoneurone excitability but also that the modulation of V_{th} and AHP amplitude are via different neuromodulatory mechanisms. Extensor motoneurons are also tonically inhibited during *scratch* and depolarized during *stance*, suggesting task-dependent changes in synaptic input to the motoneurone pool in addition to task-dependent differences in motoneurone membrane properties (Perreault, 2002).

Task-dependent differences in motoneurone properties are vitally important when one considers that the motor output itself may dictate, to a certain degree, motoneurone excitability. The implication is twofold: that the mode of motor output used to ‘train’ or examine motoneurone properties may be task-dependent, potentially influencing the interpretation of findings. Thus, it seems reasonable to suggest that motoneurone excitability should be examined using the same motor output that was used to train the motor system. At a minimum, the predictions of the influence of changes in motoneurone properties during locomotor outputs based on their properties in the resting state or using a different task, should be made with caution. In other words, the task-dependency of motoneurone excitability questions the appropriateness of assessing motoneurons during a tonic contraction following a training program that involved a rhythmic motor output.

Evidence from human studies

Given the inability to directly isolate and record from spinal motoneurons in humans, it is impossible to discern changes in specific intrinsic spinal motoneurone properties in a manner similar to non-human animal studies. In human work, researchers rely on the use of non-invasive measurement techniques to gain surrogate insights into the activity of the motoneurone pool during different tasks. Using motor unit recordings, task-dependent differences in motoneurone excitability have been reported using isometric or isotonic contractions. Motor unit recruitment thresholds and firing patterns depend not only on the task (Tax et al. 1989), but also on the direction (Thomas et al. 1987) and velocity (Desmedt and Godaux 1979) of the motor output. Research having used locomotor outputs to examine motoneurone excitability is scarce, mainly due to technical constraints.

Studies that have been performed to examine motoneurone excitability during locomotor output in humans use leg and arm cycling as a model of locomotion given they are partially controlled via spinal CPGs (Zehr and Duysens 2004). In these paradigms, non-invasive stimulation of the corticospinal tract produces motor evoked potentials recorded from the surface EMG as an indirect measure of motoneurone excitability (McNeil et al. 2013; Lockyer et al. 2018, 2021). Thus, arm and leg cycling are a model of the locomotor outputs used to examine motoneurone properties in non-human animals while non-invasive stimulation is the measure of motoneurone pool excitability, as detailed in our recent reviews (Power et al. 2018; Lockyer et al. 2021). During both leg and arm cycling motoneurone excitability is modulated throughout the movement (Sidhu et al. 2012; Forman et al. 2014) though it is presently unclear whether these changes are specific to the locomotor output (i.e. task-dependent). Recently, we have published several studies investigating whether spinal motoneurone excitability during arm cycling is task-dependent by comparing responses evoked during cycling to those evoked during position- and intensity-matched isometric contractions (Copithorne et al. 2015; Forman et al. 2014; Forman et al. 2018; Power and Copithorne 2013). We have shown that task-dependency in spinal motoneurone excitability is present but depends on the position of the arm during arm cycling (Forman et al. 2014). Our interpretation of the changes in motoneurone pool output, and thus excitability, is limited in that we cannot speak to recruitment threshold or firing properties. Thus, more precise mechanisms underlying task-dependent changes in spinal motoneurone excitability are not known. At present, the findings from human work suggest that similarities in the task-dependent firing of spinal motoneurons may be similar to those observed during locomotor output in non-human animals (Power et al. 2018).

Endurance-exercise training induced adaptations in motoneurons and potential implications for locomotor output

A recent, detailed review, applied a mathematical model that included known changes in motoneurone and muscle properties following endurance-exercise training to gain insight into the functional relevance of these adaptations (MacDonell and Gardiner 2018). The model was designed to assess a single output from the motor unit pool in response to relatively simple synaptic input. Importantly, the authors acknowledged that the model did not include factors such as PICs or rhythmic bouts of excitatory and inhibitory input as would occur with locomotor outputs, thus limiting the applicability of the model to non-locomotor outputs. The present review does not employ a modelling approach, but it is interesting to speculate about the functional importance of endurance-exercise training induced adaptations in spinal motoneurons *during* locomotor output. For example, consider V_{th} hyperpolarization which occurs following endurance-exercise training and is also an acute change that occurs during a locomotor output. A question then becomes, would

these changes be cumulative? If this were the case the implication would be that substantially less synaptic input to trained motoneurons would be required to initiate and maintain firing during locomotion, thus reducing effort levels and potentially reducing fatigue effects. Recent evidence showed that PICs were enhanced in spinal interneurons following endurance-exercise training (Ge and Dai 2020) suggesting that 1) the synaptic input to motoneurons may be enhanced following training and 2) a similar adaptation may also occur in spinal motoneurons. The input from the interneurons may be both excitatory and inhibitory, making the implications unclear for motoneurone excitation during locomotor output. A potential increase in PICs, however, suggests another means to increase motoneurone excitability by reducing the effort required to maintain firing during ongoing locomotor output and by reducing SFA, which is reduced following endurance-exercise training. It is also possible that changes in SFA following endurance-exercise training have no effect on motoneurone firing during locomotion given that it may not occur (Brownstone 2006).

It is also important to consider that enhanced motoneurone excitability following endurance-exercise training may be accompanied by changes in motoneurone properties that would act to reduce excitation in an attempt to ensure fine control of firing rates and thus muscle force output. Evidence to support this idea comes from the increase in AHP amplitude and hyperpolarization of the membrane potential in motoneurons following endurance-exercise training. These adaptations would counter increases in firing rates by increasing the difficulty in activating voltage-dependent excitation thus reducing amplification of synaptic input and also by countering the reduction in AHP amplitude that occurs during locomotor output.

Future directions/perspective

We presented evidence in support of the need to assess motoneurons during conditions reproducing the training protocol if functional inferences are to be drawn on endurance-exercise training induced adaptations in spinal motoneurons. As mentioned, the absence of human studies that have done this is due to the technical difficulties associated with the assessment of motoneurons, particularly during limb movement. In humans, the properties of spinal motoneurons may be assessed indirectly, based on the firing instants identified from action potentials represented in the EMG. When EMG is sampled from within the muscle during steady contractions, with intramuscular electrodes, action potentials of single motor units, and thus the instants at which they were discharged from the spinal motoneurons, may be discriminated with ease: the process of identifying the firing instants from EMG has been termed decomposition. During non-stationary conditions, wherein muscle shape changes and so the relative position between electrodes and fibers, the discriminative power of decomposition of intramuscular EMG may be not sufficient for tracking the instants at which motoneurons discharge. Moreover, discomfort arising from the wires may be not tolerable or alter how subjects perform the movement. Most importantly, only a few motoneurons may be assessed at a given time due to the limited pick-up volume of the intramuscular recording system. The decomposition of surface EMG sampled from multiple skin locations covering the same target muscle appears, however, to help in contending with these issues, at least during stationary conditions (e.g. isometric contractions). In the next section we present arguments supporting the potential of this advanced recording technique for the assessment of spinal motoneurons during non-stationary conditions in humans, while considering the necessity of using it with care. Even if this advanced technology can be adapted to assess motoneurone/motor unit excitability during dynamic motor output in humans, details regarding the firing properties of motoneurons will be limited compared to those

described in non-human animals. For example, examining voltage-dependent excitation or estimating PICs may not be possible during a rhythmic motor output such as cycling, at least not based on current methods (Gorassini et al. 2002).

High-density surface EMG

In this section we propose that motoneurone (motor unit) adaptations to endurance-exercise training are best appreciated when assessed by surface EMGs detected from multiple locations over the whole, target muscle; the high-density surface EMG (Vieira and Botter 2021). Our reasoning is motivated by the same view that often discourages the use of surface EMG to study single motor units: that surface EMGs are generally sensitive to a broader number of motor units, and therefore more interferential, than intramuscular recordings (Farina and Holobar 2016). While the applicable relevance of longitudinal studies is out of dispute, longitudinal designs demand assessing *variables of interest* in response to a given *intervention*, over a given period and for a *fixed set of participants*. In the context of Figure 1, interest lies in how endurance-exercise training (intervention) may change the properties (variables of interest) of motor units (set of participants). Both the intervention and the variables of interest are under direct control of the experimenter. Participants, conversely, are not. Experimenters can only expect that motor units exposed at baseline and in follow-up assessments are the same and, most crucially, that the properties of motor units assessed at different time points genuinely reflect the training adaptations. Limiting assessments to conditions mirroring those imposed by the intervention protocol would appear to be a necessity, as discussed in previous sections, though not sufficient to assess training-induced adaptation in motor unit properties. Being able to show a representative fraction of motor units that is indeed recruited during the training period is equally important, otherwise inferences on training adaptations would be inaccurate. It is then that the presence of a relatively high number of action potentials of different motor units, with fibers residing in different muscle locations, would make of the high-density surface EMG the most suitable means for studying motor unit responses to training.

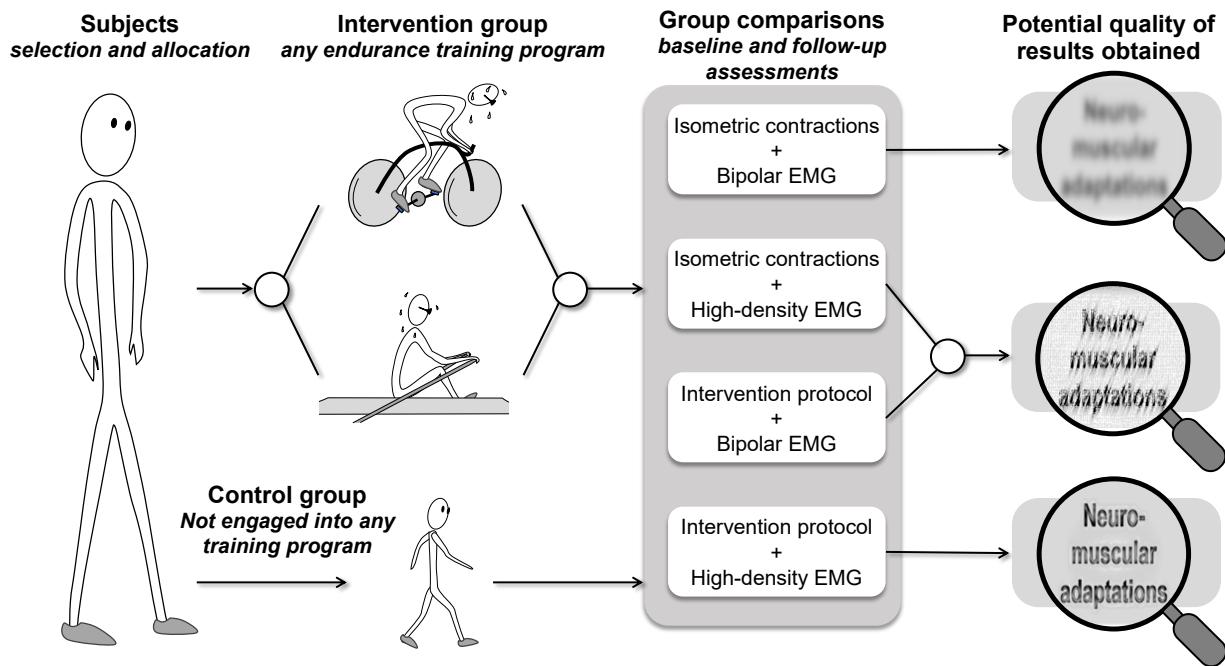


Figure 1: Illustration highlighting the importance of the assessment technique and motor output used to gain a more detailed understanding of exercise-endurance training and task-dependent modulation of motor unit excitability in humans.

In the following sections we deepen the concepts at the base of our reasoning. We first provide evidence favoring the potential of high-density surface EMG in revealing a bigger number of representative motor units when compared to intramuscular EMGs. We then briefly comment on the different means proposed for assessing single motor units from surface EMGs. After that we provide a balanced view of limitations and perspectives associated with the use of this technique in dynamic motor outputs.

Assessing a representative population of motor units with high-density surface EMG.

When aiming to assess single motor units from a single recording site, intramuscular EMGs are preferred over surface EMGs. Two reasons often motivate the preference, owing to the filtering effect introduced by the tissue interposed between electrodes and muscle fibers (Stashuk 2001; Farina and Holobar 2016). First, intramuscular recordings are in general more selective than surface recordings, providing a less interferential signal. Second, the degree of overlapping of action potentials of different units is smaller in intramuscular than in surface EMGs, collectively granting intramuscular recordings a greater discriminative power. In the past twenty years, the possibility of sampling EMGs with grids of electrodes has offset however the preference for surface recording. In addition to being non-invasive, high-density surface EMGs are expected to expose a greater number of units than intramuscular EMGs.

It is not the absolute number of electrodes per se to favor the use of multiple, surface recordings, though the notion that a greater number of recording channels increases the potential for discriminating single motor units holds as a whole (Farina et al. 2008; Nawab et al. 2010). The point here is the number of sufficiently selective detection points taken from different regions of the same, target muscle. While intramuscular electrodes sample EMGs from a small, limited muscle volume, high-density surface grids sample EMGs from a large muscle volume. This

increased spatial sampling offers an attractive advantage for the identification of single motor units in surface EMGs, as illustrated in Figure 2. Action potentials of single motor units can be readily appreciated in multiple surface EMGs, collected along the proximo-distal region of medial gastrocnemius, and in the intramuscular EMG sampled centrally from the muscle, below the ninth surface electrode (Figure 2A; (Vieira et al. 2019)). The correspondence that appears to exist between the timing of action potentials in both recordings is questionable when inspecting signals over an expanded time scale (Figure 2B). For every action potential observed in the intramuscular EMG there is a time-matched representation in the surface EMG. The opposite however is not true. Only a fraction of the action potentials present in the fourth bipolar EMG is represented in the intramuscular EMGs (cf. first 50 ms in Figure 2B). If we accept that muscle fibers of different motor units may be spatially localized within the muscle, as evinced for muscles with different architectures (Vieira et al. 2011; Gallina and Vieira 2015a; Borzelli et al. 2020), the benefit of assessing single motor units from high-density surface recordings is immediate. High-density surface EMGs provide access to motor units which fibers reside in different muscle regions, likely outside the pick-up volume of a localized, intramuscular recording.

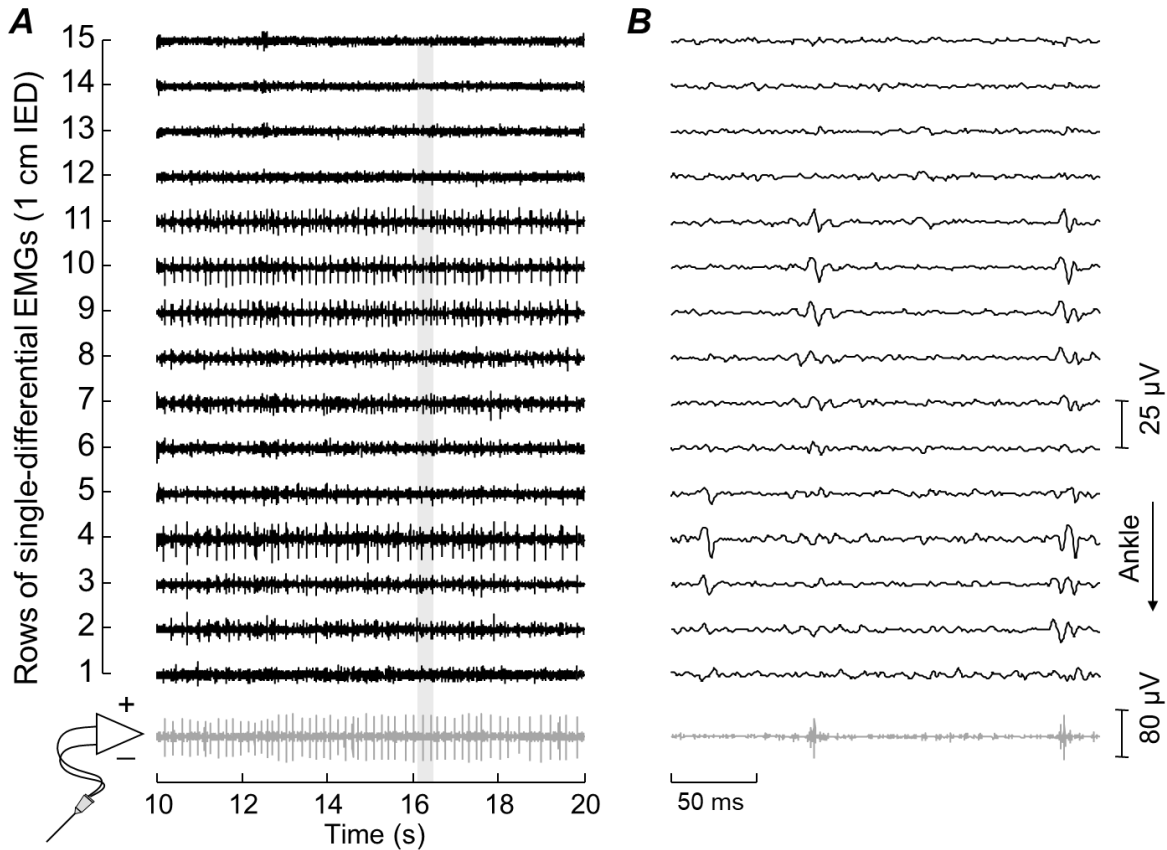


Figure 2: Surface and intramuscular EMGs collected from the medial gastrocnemius muscle during 10 s of upright standing (**A**). Single-differential EMGs (black traces) were collected with an array of 16 electrodes (1 cm inter-electrode distance; IED), positioned along the muscle proximo-distal axis. The intramuscular EMG (grey trace) was collected with a pair of wire electrodes inserted roughly below the ninth surface electrode. Panel **B** shows an expanded view (250 ms) of a portion (shaded rectangle) of signals shown in **A**. Reproduced from Vieira et al 2019 (Vieira et al. 2019).

High-density surface EMGs do not just yield a potentially large number of motor units for analysis. By being sensitive to motor units in different muscle regions, high-density EMGs likely provide a representative view of whatever neuromuscular adaptations are to be studied at the muscle or motor unit level. When sampling a single EMG, from a localized muscle region, there is the possibility that the detected signal does not reflect the whole muscle (Vieira and Botter 2021). The necessity of collecting EMGs representative of the whole muscle, and thus of a potentially greater fraction of its motor unit population, is particularly important when there are reasons to expect that neuromuscular adaptations may manifest locally within the muscle. If endurance training leads to structural adaptations localized within the muscle, as suggested in response to resistance training (Wakahara et al. 2013; Illera-Domínguez et al. 2018), high-density EMGs would not only maximize the potential of assessing a greater number of motor units but also of revealing muscle sites where motor units are most likely to respond to the training stimulus.

As inferences on changes in muscle excitation are more likely valid if limited to the muscle region from which EMGs are sampled, we propose that inferences on the adaptation of motor unit properties in response to interventions should be limited to the muscle region from which units are assessed. While detecting EMGs from multiple muscle regions maximizes the potential for unveiling motor unit adaptations to training, this is the first step of a painstaking process, which demands additional efforts to extract single motor units from the high-density EMGs.

Exposing single motor units from surface recording: the decomposition of surface EMGs.

The signal detected by an EMG electrode is the sum of the action potentials of single motor units combined over time and space, and giving rise to an interference signal. Decomposition algorithms allow one to invert this process, breaking down the interference EMG signal into its constituent trains of motor unit action potentials (Stashuk 2001). Decomposition is complicated by the fact that action potentials of different units overlap. This generates superimposed potentials that the decomposition algorithm must resolve to accurately provide the firing pattern of individual motor units. For highly selective detection systems, such as indwelling electrodes, the superimposition is less likely to occur given the duration of isolated action potentials is as short as a few units of milliseconds. In this case, the identification of single motor unit firings can be performed with simple approaches based on amplitude thresholds or with segmentation and classification algorithms, followed by supervised or automatic resolution of superimposition occurrences (Stashuk 2001). When EMG is detected with surface electrodes, the detection selectivity is lower, implying a higher number of motor units contributing to the recorded signal. Moreover, the filtering effect of the muscle and subcutaneous tissues removes the high frequency components, broadening the waveform of action potentials (Farina et al. 2002a; Lowery et al. 2004). Collectively, these factors increase both the similarity of action potentials of different units and the probability that they overlap, thus reducing the possibility to discriminate individual motor units (Farina and Holobar 2016). Although these issues make the decomposition of a single surface EMG impractical, they can be mitigated with high-density detection. Indeed, multiple recordings from the same muscle increase the chances of detecting action potentials of different motor units, a necessary condition for decomposition (Farina et al. 2008): high-density surface EMG coalesces high sensitivity and high discriminative power. These aspects make it a key tool to characterize the activity of populations of motor units from a large muscle volume. Several algorithms for decomposition of high-density signals have been proposed and the interested reader is referred to technical documents of relevance in this field (Holobar and Zazula 2007; Holobar et al. 2009,

2010; Chen and Zhou 2016; Negro et al. 2016). Here, we focus attention on the assessment of decomposition results and on its application to dynamic motor output.

Prior to processing motor unit data, a key question to be aware of is: how confident should we be about the decomposition results? In literature, the answer to this question is mainly based on the properties of the firing patterns of decomposed units. A-priori knowledge of the expected, firing rate variability during the considered motor task is often used to infer on the quality of decomposed motor units. Highly variable firing rates in isometric, steady-force contractions, for example, may be regarded as result of missing or false-positive firings (Nawab et al. 2010). During force-varying contractions, conversely, the firing rate of motor units is expected to vary with changes in force demand, to extents depending on when individual units are recruited. When decomposing high-density EMGs, inspection of firing pattern statistics is not sufficient to assess the quality of decomposition results; inspection of the decomposed waveforms is necessary. And this is a grueling process requiring experience and careful consideration on the relative arrangement between fibers and the surface electrodes, as illustrated in Figure 3. When fibers are parallel to the skin, the propagation of the potential and the presence of phase opposition in correspondence of the skin location where the potential arises can be regarded as quality indicators (Figure 3a,b). These characteristics, however, cannot be observed, nor used as quality factors, in cases where electrodes and fibers do not reside in parallel planes. For instance, localized, non-propagating potentials are uniquely expected for in-depth pennate muscles (e.g. gastrocnemius, soleus; Figure 3c). Decomposed waveforms are not always as clear as those shown in Figure 3a-c. Figure 4 shows examples of action potentials of dubious validity, detected from the biceps brachii muscle during an isometric, constant force contraction. While the firing pattern of these units meet the quality criteria for decomposition, their action potentials do not (caption of Figure 4). Figures 3 and 4 should be regarded as a non-exhaustive reference to acceptable and questionable outputs of the decomposition algorithm, hopefully assisting in the assessment of decomposition results. It is expected to stimulate the exercising of careful inspection of both firing pattern and waveforms resulting from the decomposition of high-density surface EMGs, prior to drawing inferences of basic or applied relevance.

The issues just discussed are aggravated in the applied context of this review, wherein we advance the notion that assessing adaptations to endurance-exercise training demands the exposure of motor units recruited in dynamic motor outputs. The association between changes in waveform and changes in muscle geometry is a crucial aspect to deal with when decomposing high-density signals during dynamic motor output. When decomposing high-density surface EMGs during constant-force, isometric contractions, in first approximation, muscle geometry can be considered stable, and so the shape of motor unit action potentials. In the course of a dynamic motor output, conversely, changes in muscle shape lead to significant alterations in the arrangement of fibers relative to electrodes and thus in the shape of the action potentials, as documented by studies using the high-density and the bipolar techniques (Potvin 1997; Vieira et al. 2017b; Collins and Button 2018). Consequently, any algorithm attempting to decompose EMGs in dynamic motor output must account for the influence of anatomical changes on the shape of action potentials of individual motor units. At least during slow, cyclic contractions, initial successful attempts to validate the decomposition of surface EMGs have been achieved (De Luca et al. 2015; Glaser and Holobar 2019). We envisage a progressive increase in the number of technical studies aimed at improving these promising results, furthering the potential for the assessment of motor units responding to endurance-exercise training.

Examples of results obtained for the decomposition of high-density surface EMG

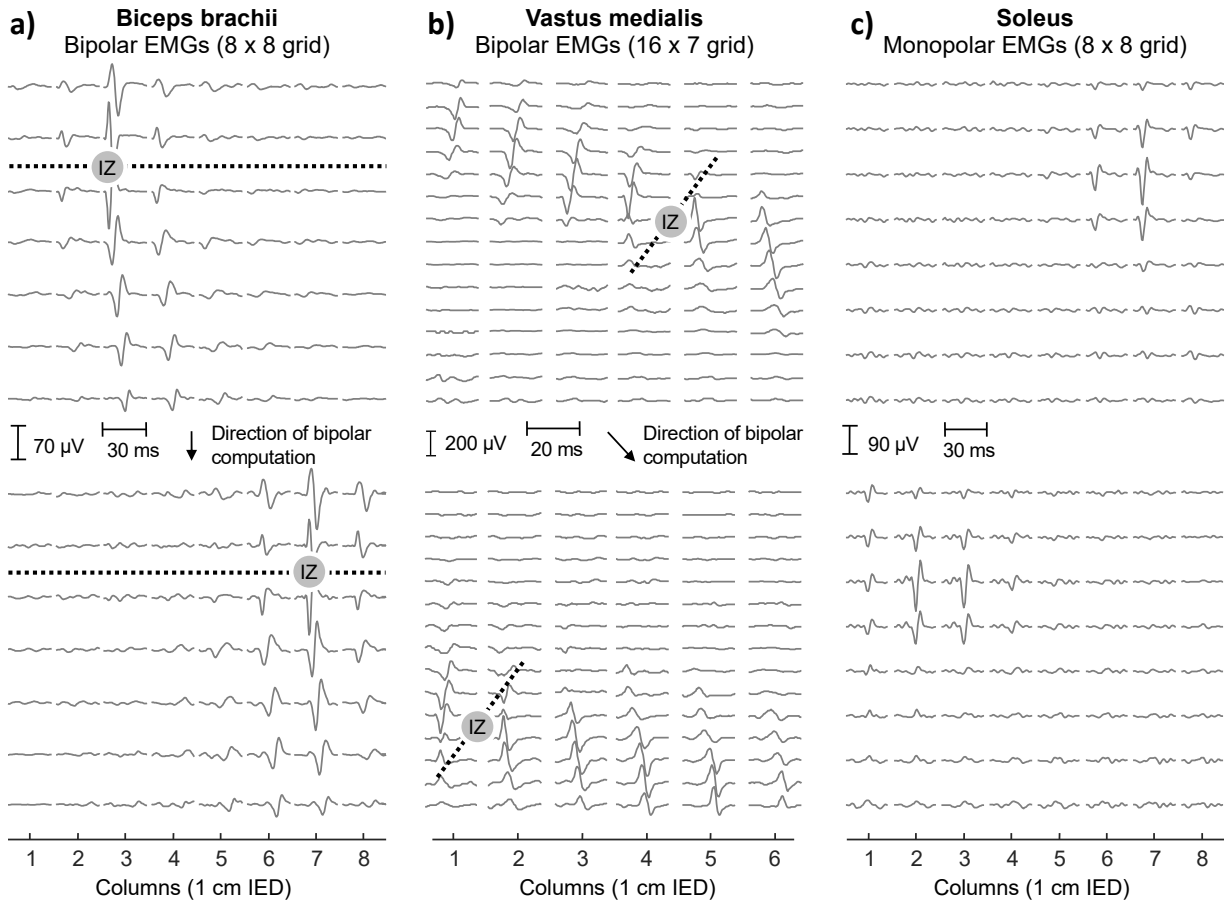


Figure 3: Examples of action potentials obtained after triggering and averaging epochs (20-30 ms) centered at the firing instants resulting from the decomposition of high-density surface EMGs; each set of signals in each panel illustrates the average, surface representation of one motor unit, as identified by the decomposition technique. For biceps brachii (a; (Hamilton et al. 2018)), innervation zone and propagation can be seen for two motor units mainly represented in two different columns. For vastus lateralis (b; (Gallina and Vieira 2015b)) propagation is not readily appreciated, as the fibers were not aligned parallel to columns in the grid; propagation takes place along an oblique direction. Neither innervation zones nor propagation can be observed for motor units from the in-depth pinnate, soleus muscle (Cohen et al. 2021). The representation of action potentials in a localized cluster of channels is possibly the most appropriate quality criterion for assessing decomposition results in this case.

The potential of combining representativeness and exposure to assess motor unit adaptation to training

Exposing the firing instants of individual motor units out of surface EMGs collected from multiple muscle regions is a necessary, though not sufficient, condition for assessing adaptations to training. Experimenters should assert the units exposed are indeed those engaged during the training period. Otherwise, the validity of inferences on motor unit adaptation to training would be questionable. Based on the knowledge matured in the field of surface EMG, here we propose that inferences on motor unit adaptation would require the assessment of a representative fraction of the population of motor units recruited during the training sessions.

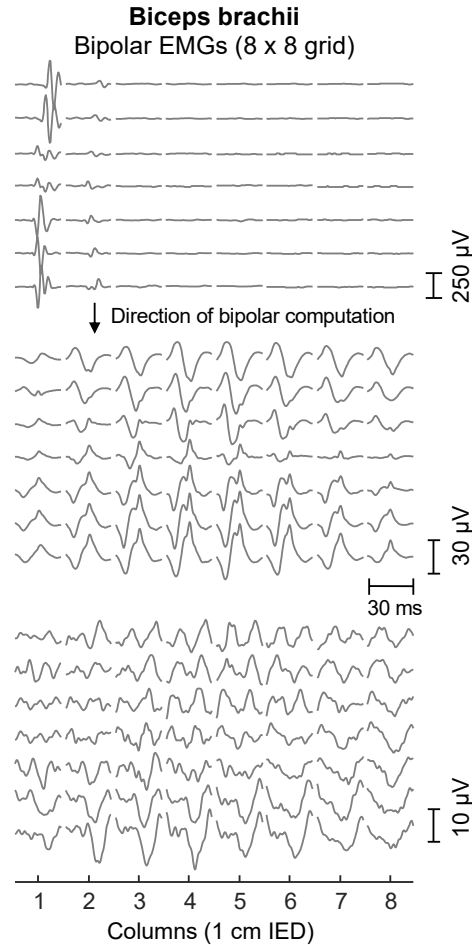


Figure 4: Often, decomposed firing patterns lead to questionable, surface representations. On top, multiple changes in polarity and changes in the waveform suggest the decomposed firing pattern does not belong to a single motor unit. On bottom, the lack of clear waveform resembling an action potential, the remarkably low amplitude of signals and the presence of power line interference (20 ms periodic changes in the top rows), similarly question the validity of the decomposed firing pattern. These decomposition results should be disregarded from analysis. In the middle panel, one may at first deem the unit unacceptable, based on the low amplitude of the action potentials shown. Considering the consistent change in polarity, the expanded duration of single action potentials and the large end-of-fiber effect contribution (narrow peaks overlapping middle-bottom potentials), this is likely a motor unit with most fibers located deeply within biceps. Our experience would suggest retaining this unit.

While we may confidently assess the same subject at different time points from the baseline of a training program, we cannot be equally confident to assess the same motor units at different time points. In a rigorous simulation study, Farina and colleagues (2008) observed that less than 50% of the different motor units simulated could be distinguished from bipolar EMGs provided by a 9x9 grid of electrodes. Distinction between units was made according to a stringent, conservative criterion, considering both the shape similarity and amplitude of the waveforms obtained for the different channels. In 2017, a method based on the similarity of multi-channel action potentials has been proposed for the longitudinal tracking of single motor units decomposed during isometric contractions (Martinez-Valdes et al 2017). This method has been applied to assess adaptations over short-training periods (REF), presuming the changes in the waveform of action potentials induced by training adaptations are marginal. When evidence favoring the immutable representation of single motor units in surface EMG is not available, and this may be

expected for training periods longer than those considered in the above-mentioned studies, the possibility of tracking motor units with training may be complicated; we would not be just attempting to follow-up the same motor units in different sessions but to follow-up the same motor units within a muscle that is expected to have responded to the training stimulus. The literature documents, indeed, a number of significant muscle adaptations to training (Hamilton and Booth 2000; Yeo et al. 2008; Hughes et al. 2018).

Moreover, as discussed by Timmons (Timmons 2011), endurance-exercise and resistance training phenotypes may not occupy the opposing ends of what is often considered to be a broad spectrum. Although to a lesser extent, when compared to resistance training, endurance-exercise training may trigger changes in muscle size and architecture (Farup et al. 2012). Logically, the longer the training period is the higher the potential for the muscle to respond. We therefore encourage readers to consider the effect of these possible adaptations on the fragile representation of action potentials of single motor units in the surface EMG when attempting to assess long-term training responses by following motor units. To make our point clearer, we illustrate how physiological and anatomical changes affect the representation of action potentials of single motor units in the surface EMGs. Figure 5 shows how changes in conduction velocity, subcutaneous thickness and misalignment between fibers and electrodes affect the action potentials of a single and the same motor unit, simulated with a thorough, analytical model (Farina and Merletti 2001). These are three out of multiple factors that may affect measurements taken at different time points of a training program. The extent to which action potentials change depends on the magnitude of the combined effect of all the factors affecting measurements. While we may anticipate how specific factors may independently affect the representation of action potentials (Figure 5), predicting how the action potentials of a single motor unit would change after a given period of training appears not possible. Attempts to track motor units with training would therefore require limiting the application of tracking algorithms over periods during which the effect of training-induced adaptations on the surface representation of single motor units is marginal. Future studies are necessary to identify the optimal duration, which arguably depends on the muscle, training type, exercise intensity and subject phenotype.

We understand Figure 5 shows a qualitative example for a single, simulated motor unit. Nevertheless, the influence of the physiological and anatomical factors considered in Figure 5, as well as that of other sources on the surface representation of action potentials, is well acknowledged in the literature (Roeleveld et al. 1997; Farina et al. 2002b; Vieira et al. 2017a)(Glaser and Holobar 2019). It seems therefore prudent to consider the possibility that the same motor unit, assessed with equal means and equal procedures across a sufficiently long training period, may yield action potentials with different waveforms. Legitimate questions arising from this observation would be: How similar should the surface representation be for paired action potentials to be classified as belonging to the same motor unit? On the opposite extreme, how confidently can we state that different surface representations of action potentials obtained at different time points do not posit the same motor unit? The concerns we advance in this section are expected to be taken constructively, as they require the need of validating any methodological attempt based on the similarity of waveforms to accurately track motor units across training periods. Future studies are therefore necessary to address: i) how accurately the same motor units may be tracked across time points between which changes in the surface representation of their action potentials took place; ii) how much the changes in action potentials induced by training adaptations affect tracking accuracy; iii) the effect of different muscle architectures on tracking

accuracy. Once these three points have been addressed, an issue of more general concern would still be open: how much do the changes in the properties of the motor units that may be accurately tracked, as well as of the whole set of decomposed units, reflect those in the population of units responding to training? In our view, at the moment, the most appropriate approach for assessing how motor units may adapt to training demands maximizing the potential for exposing a representative fraction of motor units recruited during the training sessions. By detecting high-density surface EMGs from a broad muscle region we augment the number of single motor units that may be exposed. By decomposing these high-density EMGs during conditions most likely loading the motor units recruited during the training sessions we increase the possibility that the exposed units constitute a representative fraction of those responding to training.

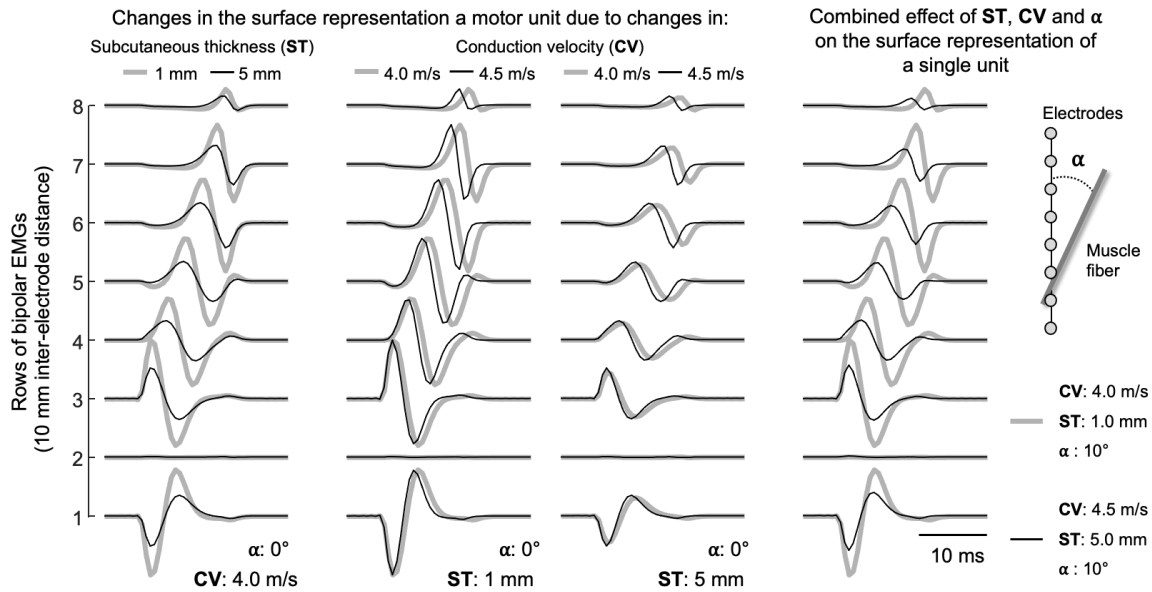


Figure 5. Action potentials of the same motor unit simulated with a planar model (Farina and Merletti 2001) for different conditions, indicated on the top of each plot. Simulation parameters were selected as in (Gallina and Vieira 2015a), with the center of the elliptic, motor unit territory located 5 mm below the fat-muscle interface. Fibers were dispersed uniformly from the territory center, extending over 10 mm in the depth direction and 25 mm in the transverse direction (territory eccentricity: 0.9). Variations in subcutaneous thickness, conduction velocity and inclination were selected to reproduce realistic changes from training; 10° is a reasonable misalignment error, considering there is no specific criterion that could be used to precisely quantify the inclination of fibers relative to electrodes from experimental data. The changes in shape shown here may be sufficiently small to allow for following up the same motor units across training sessions. Alternatively, these changes could increase the similarity of two different motor units after training. The size of the effect of training adaptations on the waveform of action potentials of single units and the discrimination power in presence of training adaptations remain to be addressed.

Conclusion

The electrical properties of spinal motoneurons are altered following endurance-exercise training in non-human animals. If, or how, these chronic changes in excitability interact with the acute changes that coincide with the onset of locomotor output results in functional implications is unclear. Importantly, and central to the thesis of this review, future work should attempt to examine motoneurons during the motor output in which the endurance-exercise training occurred to take into account the task-dependency of motoneurone excitability. As discussed in our review, the traditional decomposition of intramuscular EMGs does not appear to meet the technical

requirement for exposing motor units during such dynamic, locomotor conditions. Even if it was appropriate for this purpose, its validity would be questionable for two main reasons. First the structural changes possibly induced by the training may undermine the discriminative power of motor unit decomposition. Second, and most importantly, only a small and possibly unrepresentative fraction of units would be assessed. The recent advances made in the field of decomposition of surface EMG in dynamic motor outputs, combined with the possibility of sampling EMGs from multiple muscle regions, suggests the high-density EMG technology is sufficiently mature to further our understanding on motoneurone, or more specifically motor unit, excitability during a locomotor output following endurance-exercise training.

References

- Adam A, Luca CJD, Erim Z (1998) Hand Dominance and Motor Unit Firing Behavior. *Journal of Neurophysiology* 80:1373–1382. <https://doi.org/10.1152/jn.1998.80.3.1373>
- Beaumont E, Gardiner P (2002) Effects of daily spontaneous running on the electrophysiological properties of hindlimb motoneurons in rats. *J Physiol* 540:129–138. <https://doi.org/10.1113/jphysiol.2001.013084>
- Beaumont E, Gardiner PF (2003) Endurance training alters the biophysical properties of hindlimb motoneurons in rats. *Muscle & Nerve* 27:228–236. <https://doi.org/10.1002/mus.10308>
- Beaumont E, Houle JD, Peterson CA, Gardiner PF (2004) Passive exercise and fetal spinal cord transplant both help to restore motoneuronal properties after spinal cord transection in rats. *Muscle & Nerve* 29:234–242. <https://doi.org/10.1002/mus.10539>
- Borzelli D, Gazzoni M, Botter A, et al (2020) Contraction level, but not force direction or wrist position, affects the spatial distribution of motor unit recruitment in the biceps brachii muscle. *European journal of applied physiology* 120:853–860. <https://doi.org/10.1007/s00421-020-04324-6>
- Brownstone RM, Jordan LM, Kriellaars DJ, et al (1992) On the regulation of repetitive firing in lumbar motoneurons during fictive locomotion in the cat. *Exp Brain Res* 90:. <https://doi.org/10.1007/BF00230927>
- Button DC, Gardiner K, Marqueste T, Gardiner PF (2006) Frequency–current relationships of rat hindlimb α -motoneurons. *The Journal of Physiology* 573:663–677. <https://doi.org/10.1113/jphysiol.2006.107292>
- Button DC, Kalmar JM, Gardiner K, et al (2007) Spike frequency adaptation of rat hindlimb motoneurons. *Journal of Applied Physiology* 102:1041–1050. <https://doi.org/10.1152/japplphysiol.01148.2006>
- Chen M, Zhou P (2016) A Novel Framework Based on FastICA for High Density Surface EMG Decomposition. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* 24:117–127. <https://doi.org/10.1109/TNSRE.2015.2412038>

- Cohen JW, Vieira T, Ivanova TD, et al (2021) Maintenance of standing posture during multi-directional leaning demands the recruitment of task-specific motor units in the ankle plantarflexors. *Exp Brain Res* 239:2569–2581. <https://doi.org/10.1007/s00221-021-06154-0>
- Collins BW, Button DC (2018) The effect of shoulder position on motor evoked and maximal muscle compound action potentials of the biceps brachii. *Neuroscience Letters* 665:206–211. <https://doi.org/10.1016/j.neulet.2017.12.019>
- Dai Y, Jones KE, Fedirchuk B, et al (2002) A modelling study of locomotion-induced hyperpolarization of voltage threshold in cat lumbar motoneurons. *J Physiol* 544:521–536. <https://doi.org/10.1113/jphysiol.2002.026005>
- De Luca CJ, Chang SS, Roy SH, et al (2015) Decomposition of surface EMG signals from cyclic dynamic contractions. *Journal of Neurophysiology* 113:1941–1951. <https://doi.org/10.1152/jn.00555.2014>
- Desmedt JE, Godaux E (1979) Voluntary motor commands in human ballistic movements. *Ann Neurol* 5:415–421. <https://doi.org/10.1002/ana.410050503>
- Enoka RM (2019) Physiological validation of the decomposition of surface EMG signals. *Journal of Electromyography and Kinesiology* 46:70–83. <https://doi.org/10.1016/j.jelekin.2019.03.010>
- Farina D, Cescon C, Merletti R (2002a) Influence of anatomical, physical, and detection-system parameters on surface EMG. *Biological cybernetics* 86:445–456. <https://doi.org/10.1007/s00422-002-0309-2>
- Farina D, Cescon C, Merletti R (2002b) Influence of anatomical, physical, and detection-system parameters on surface EMG. *Biological cybernetics* 86:445–456. <https://doi.org/10.1007/s00422-002-0309-2>
- Farina D, Holobar A (2016) Characterization of Human Motor Units from Surface EMG Decomposition. *Proceedings of the IEEE* 104:353–373. <https://doi.org/10.1109/JPROC.2015.2498665>
- Farina D, Merletti R (2001) A novel approach for precise simulation of the EMG signal detected by surface electrodes. *IEEE Transactions on Biomedical Engineering* 48:637–646. <https://doi.org/10.1109/10.923782>
- Farina D, Negro F, Gazzoni M, Enoka RM (2008) Detecting the unique representation of motor-unit action potentials in the surface electromyogram. *Journal of Neurophysiology* 100:1223–1233. <https://doi.org/10.1152/jn.90219.2008>
- Farup J, Kjølhede T, Sørensen H, et al (2012) Muscle Morphological and Strength Adaptations to Endurance Vs. Resistance Training. *Journal of Strength and Conditioning Research* 26:398–407. <https://doi.org/10.1519/JSC.0b013e318225a26f>

- Forman D, Raj A, Button DC, Power KE (2014) Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. *Journal of Neurophysiology* 112:1142–1151. <https://doi.org/10.1152/jn.00210.2014>
- Gallina A, Vieira T (2015a) Territory and fiber orientation of vastus medialis motor units: A Surface electromyography investigation. *Muscle and Nerve* 52:1057–1065. <https://doi.org/10.1002/mus.24662>
- Gallina A, Vieira T (2015b) Territory and fiber orientation of vastus medialis motor units: A Surface electromyography investigation. *Muscle and Nerve* 52:1057–1065. <https://doi.org/10.1002/mus.24662>
- Gardiner P, Beaumont E, Cormery B (2005) Motoneurons “learn” and “forget” physical activity. *Can J Appl Physiol* 30:352–370. <https://doi.org/10.1139/h05-127>
- Gardiner P, Dai Y, Heckman CJ (2006) Effects of exercise training on α -motoneurons. *Journal of Applied Physiology* 101:1228–1236. <https://doi.org/10.1152/jappphysiol.00482.2006>
- Ge R, Dai Y (2020) Three-Week Treadmill Exercise Enhances Persistent Inward Currents, Facilitates Dendritic Plasticity, and Upregulates the Excitability of Dorsal Raphe Serotonin Neurons in ePet-EYFP Mice. *Front Cell Neurosci* 14:. <https://doi.org/10.3389/fncel.2020.575626>
- Gilmore J, Fedirchuk B (2004) The excitability of lumbar motoneurons in the neonatal rat is increased by a hyperpolarization of their voltage threshold for activation by descending serotonergic fibres: Descending 5-HT fibres hyperpolarize V_{th} . *The Journal of Physiology* 558:213–224. <https://doi.org/10.1113/jphysiol.2004.064717>
- Glaser V, Holobar A (2019) Motor unit identification from high-density surface electromyograms in repeated dynamic muscle contractions. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* 27:66–75. <https://doi.org/10.1109/TNSRE.2018.2885283>
- Gómez-Pinilla F, Ying Z, Opazo P, et al (2001) Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle. *European Journal of Neuroscience* 13:1078–1084. <https://doi.org/10.1046/j.0953-816x.2001.01484.x>
- Gómez-Pinilla F, Ying Z, Roy RR, et al (2002) Voluntary Exercise Induces a BDNF-Mediated Mechanism That Promotes Neuroplasticity. *Journal of Neurophysiology* 88:2187–2195. <https://doi.org/10.1152/jn.00152.2002>
- Gorassini M, Yang JF, Siu M, Bennett DJ (2002) Intrinsic Activation of Human Motoneurons: Possible Contribution to Motor Unit Excitation. *Journal of Neurophysiology* 87:1850–1858. <https://doi.org/10.1152/jn.00024.2001>
- Hamilton LD, Mani D, Almuklass AM, et al (2018) Electrical nerve stimulation modulates motor unit activity in contralateral biceps brachii during steady isometric contractions. *Journal of Neurophysiology* 120:2603–2613. <https://doi.org/10.1152/jn.00235.2018>

- Hamilton MT, Booth FW (2000) Skeletal muscle adaptation to exercise: a century of progress. *Journal of Applied Physiology* 88:327–331. <https://doi.org/10.1152/jappl.2000.88.1.327>
- Heckman CJ, Lee RH, Brownstone RM (2003) Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *Trends in Neurosciences* 26:688–695. <https://doi.org/10.1016/j.tins.2003.10.002>
- Holobar A, Farina D, Gazzoni M, et al (2009) Estimating motor unit discharge patterns from high-density surface electromyogram. *Clinical Neurophysiology* 120:551–562. <https://doi.org/10.1016/j.clinph.2008.10.160>
- Holobar A, Minetto MA, Botter A, et al (2010) Experimental analysis of accuracy in the identification of motor unit spike trains from high-density surface EMG. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* 18:. <https://doi.org/10.1109/TNSRE.2010.2041593>
- Holobar A, Zazula D (2007) Multichannel Blind Source Separation Using Convolution Kernel Compensation. *IEEE Transactions on Signal Processing* 55:4487–4496. <https://doi.org/10.1109/TSP.2007.896108>
- Hughes DC, Ellefsen S, Baar K (2018) Adaptations to endurance and strength training. *Cold Spring Harbor Perspectives in Medicine* 8:1–17. <https://doi.org/10.1101/cshperspect.a029769>
- Illera-Domínguez V, Nuell S, Carmona G, et al (2018) Early functional and morphological muscle adaptations during short-term inertial-squat training. *Frontiers in Physiology* 9:1–12. <https://doi.org/10.3389/fphys.2018.01265>
- Kalmar JM (2018) On task: Considerations and future directions for studies of corticospinal excitability in exercise neuroscience and related disciplines. *Appl Physiol Nutr Metab* 43:1113–1121. <https://doi.org/10.1139/apnm-2018-0123>
- Krawitz S, Fedirchuk B, Dai Y, et al (2001) State-dependent hyperpolarization of voltage threshold enhances motoneurone excitability during fictive locomotion in the cat. *The Journal of Physiology* 532:271–281. <https://doi.org/10.1111/j.1469-7793.2001.0271g.x>
- Lockyer EJ, Benson RJ, Hynes AP, et al (2018) Intensity matters: effects of cadence and power output on corticospinal excitability during arm cycling are phase and muscle dependent. *Journal of Neurophysiology* 120:2908–2921. <https://doi.org/10.1152/jn.00358.2018>
- Lockyer EJ, Compton CT, Forman DA, et al (2021) Moving forward: methodological considerations when assessing corticospinal excitability during rhythmic motor output in humans. *Journal of Neurophysiology* jn.00027.2021. <https://doi.org/10.1152/jn.00027.2021>
- Lowery MM, Stoykov NS, Dewald JPA, Kuiken TA (2004) Volume conduction in an anatomically based surface EMG model. *IEEE Transactions on Biomedical Engineering* 51:2138–2147. <https://doi.org/10.1109/TBME.2004.836494>

- MacDonell CW, Button DC, Beaumont E, et al (2012) Plasticity of rat motoneuron rhythmic firing properties with varying levels of afferent and descending inputs. *Journal of Neurophysiology* 107:265–272. <https://doi.org/10.1152/jn.00122.2011>
- MacDonell CW, Gardiner PF (2018) Mechanisms and functional implications of motoneuron adaptations to increased physical activity. *Appl Physiol Nutr Metab* 43:1186–1193. <https://doi.org/10.1139/apnm-2018-0185>
- MacDonell CW, Power KE, Chopek JW, et al (2015) Extensor motoneurone properties are altered immediately before and during fictive locomotion in the adult decerebrate rat: Motoneurone properties during fictive locomotion. *J Physiol* 593:2327–2342. <https://doi.org/10.1113/JP270239>
- McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. *Front Hum Neurosci* 7:. <https://doi.org/10.3389/fnhum.2013.00152>
- Nawab SH, Chang SS, De Luca CJ (2010) High-yield decomposition of surface EMG signals. *Clinical Neurophysiology* 121:1602–1615. <https://doi.org/10.1016/j.clinph.2009.11.092>
- Negro F, Muceli S, Castronovo AM, et al (2016) Multi-channel intramuscular and surface {EMG} decomposition by convolutive blind source separation. *Journal of Neural Engineering* 13:26027. <https://doi.org/10.1088/1741-2560/13/2/026027>
- Potvin JR (1997) Effects of muscle kinematics on surface EMG amplitude and frequency during fatiguing dynamic contractions. *J Appl Physiol* (1985) 82:144–151. <https://doi.org/10.1152/jappl.1997.82.1.144>
- Power KE, Lockyer EJ, Forman DA, Button DC (2018) Modulation of motoneurone excitability during rhythmic motor outputs. *Appl Physiol Nutr Metab* 43:1176–1185. <https://doi.org/10.1139/apnm-2018-0077>
- Power KE, McCrea DA, Fedirchuk B (2010) Intraspinal mediated state-dependent enhancement of motoneurone excitability during fictive scratch in the adult decerebrate cat: Intraspinal regulation of motoneurone excitability during fictive scratch. *The Journal of Physiology* 588:2839–2857. <https://doi.org/10.1113/jphysiol.2010.188722>
- Roeleveld K, Stegeman DF, Vingerhoets HM, Van Oosterom A (1997) The motor unit potential distribution over the skin surface and its use in estimating the motor unit location. *Acta Physiologica Scandinavica* 161:465–472. <https://doi.org/10.1046/j.1365-201X.1997.00247.x>
- Schmidt BJ (1994) Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord in vitro. *Exp Brain Res* 99:. <https://doi.org/10.1007/BF00239588>
- Sidhu SK, Cresswell AG, Carroll TJ (2012) Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. *Journal of Applied Physiology* 113:401–409. <https://doi.org/10.1152/jappphysiol.00486.2012>

- Stashuk D (2001) EMG signal decomposition: How can it be accomplished and used? *Journal of Electromyography and Kinesiology* 11:151–173. [https://doi.org/10.1016/S1050-6411\(00\)00050-X](https://doi.org/10.1016/S1050-6411(00)00050-X)
- Tax AAM, van der Gon JJD, Gielen CCAM, van den Tempel CMM (1989) Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. *Exp Brain Res* 76:55–63. <https://doi.org/10.1007/BF00253623>
- Thomas CK, Ross BH, Calancie B (1987) Human motor-unit recruitment during isometric contractions and repeated dynamic movements. *Journal of Neurophysiology* 57:311–324. <https://doi.org/10.1152/jn.1987.57.1.311>
- Timmons JA (2011) Variability in training-induced skeletal muscle adaptation. *Journal of Applied Physiology* 110:846–853. <https://doi.org/10.1152/japplphysiol.00934.2010>
- Vieira TM, Bisi MC, Stagni R, Botter A (2017a) Changes in tibialis anterior architecture affect the amplitude of surface electromyograms. *Journal of NeuroEngineering and Rehabilitation* 14:81. <https://doi.org/10.1186/s12984-017-0291-5>
- Vieira TM, Bisi MC, Stagni R, Botter A (2017b) Changes in tibialis anterior architecture affect the amplitude of surface electromyograms. *Journal of NeuroEngineering and Rehabilitation* 14:. <https://doi.org/10.1186/s12984-017-0291-5>
- Vieira TM, Botter A (2021) The Accurate Assessment of Muscle Excitation Requires the Detection of Multiple Surface Electromyograms. *Exercise and Sport Sciences Reviews* 49:23–34. <https://doi.org/10.1249/JES.0000000000000240>
- Vieira TM, Lemos T, Oliveira LAS, et al (2019) Postural Muscle Unit Plasticity in Stroke Survivors: Altered Distribution of Gastrocnemius' Action Potentials. *Frontiers in Neurology* 10:1–10. <https://doi.org/10.3389/fneur.2019.00686>
- Vieira TMM, Loram ID, Muceli S, et al (2011) Postural activation of the human medial gastrocnemius muscle: are the muscle units spatially localised? *Journal of Physiology-London* 589:431–443. <https://doi.org/10.1113/jphysiol.2010.201806>
- Vila-Chã C, Falla D, Farina D (2010) Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training. *Journal of Applied Physiology* 109:1455–1466. <https://doi.org/10.1152/japplphysiol.01213.2009>
- Wakahara T, Fukutani A, Kawakami Y, Yanai T (2013) Nonuniform muscle hypertrophy: Its relation to muscle activation in training session. *Medicine and Science in Sports and Exercise* 45:2158–2165. <https://doi.org/10.1249/MSS.0b013e3182995349>
- Woodrow L, Sheppard P, Gardiner PF (2013) Transcriptional changes in rat α -motoneurons resulting from increased physical activity. *Neuroscience* 255:45–54. <https://doi.org/10.1016/j.neuroscience.2013.09.038>

Yeo WK, Paton CD, Garnham AP, et al (2008) Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *Journal of Applied Physiology* 105:1462–1470. <https://doi.org/10.1152/japplphysiol.90882.2008>

Zehr EP, Duysens J (2004) Regulation of Arm and Leg Movement during Human Locomotion. *Neuroscientist* 10:347–361. <https://doi.org/10.1177/1073858404264680>